Pennsylvania Department of Health
Final Performance Summary Report
Formula Grants

Overview of the Health Research Project Performance Review Process and Criteria

An applicant that receives a health research grant under Tobacco Settlement Act / Act 77 of 2001, Chapter 9, is subject to a performance review by the Department of Health upon completion of the research project. The performance review is based on requirements specified by Act 77 and criteria developed by the Department in consultation with the Health Research Advisory Committee.

As part of the performance review process, each research project contained in a grant is reviewed by at least three experts who are physicians, scientists or researchers. Reviewers are from the same or similar discipline as the research grant/project under review and are not from Pennsylvania. Reviewers use the applicant's proposed research plan (strategic plan), the annual progress report and final progress reports to conduct the review. A grant that receives an unfavorable performance review by the Department may be subject to a reduction in funding or become ineligible for health research funding in the future. The overall grant evaluation rating is based on the ratings for the individual research projects contained in the grant.

This performance review report contains the outcome of the review for the grant as a whole (outstanding, favorable, or unfavorable), strengths and weaknesses of each research project, as well as recommendations for future improvement.

The following criteria were applied to information submitted by research grant recipients:

- **Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?**
  - Did the project meet the stated objectives?
  - Were the research design and methods adequate in light of the project objectives?
  - Consider these questions about data and empirical results: Were the data developed sufficiently to answer the research questions posed? Were the data developed in line with the original research protocol?
  - If changes were made to the research protocol, was an explanation given, and, if so, is it reasonable?
  - Consider (only for clinical research projects) the extent of laboratory and clinical activities initiated and completed and the number of subjects relative to the target goal.
  - Were sufficient data and information provided to indicate or support the fact that the project met its objectives or made acceptable progress?
  - Were the data and information provided applicable to the project objectives listed in the strategic research plan?

- **Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?**
  - What is the significance of this project for improving health?
  - Consider the value of the research completed towards eventual improvement in health outcomes.
  - Consider any changes in risk factors, services provided, incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of impact and effectiveness of the research being conducted.
  - Consider any major discoveries, new drugs and new approaches for prevention, diagnosis and treatment, which are attributable to the completed research project.
  - What are the future plans for this research project?

- **Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?**
  - If leveraging of funds were expected, did these materialize?
  - Are the researchers planning to apply for additional funding in the future to continue or expand the research?

- **Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted/filed?**
  - If any of the above listed were expected, did these materialize?
  - Are the researchers planning to submit articles to peer-reviewed publications, file for any licenses, or patents or begin any commercial development opportunities in the future?
  - Consider the number/quality of each.

- **Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?**
  - Were there improvements made to infrastructure?
  - Were any new investigators added or were any researchers brought into the institution to help carry out this research?
  - Were funds used to pay for research performed by pre- or post-doctoral students?

- **Criterion 6 - Did the project lead to collaboration with research partners outside the institution, or new involvement with the community?**
  - Are the researchers planning to begin any collaborations as a result of the research?
  - For clinical research only: consider the number of hospitals and health care professionals involved and the extent of penetration of the studies throughout the region or the Commonwealth.

Overall Evaluation Rating

An overall evaluation rating is assigned to each research project. The rating reflects the overall progress the project attained in meeting the stated goals and objectives. The rating is based on a scale of 1–3, with 1 being the highest. An average rating is obtained from all the reviews (minimum of 3) of each project and is the basis for the determination of the final overall rating for each project as follows:

1.00 – 1.33 = *Outstanding*
1.34 – 2.66 = *Favorable*
2.67 – 3.00 = *Unfavorable*

The grant level rating is an average rating from all projects as above. The numerical rating appears in parentheses for the grant and each project in the ***Overall Grant Performance Review Rating*** section of the report.

Overall Grant Performance Review Rating

Grant Rating: Favorable (1.71)

Project Rating:

Project	Title	Average Score
0864501	Vitamin D and Crohn's Disease: From the Bench to the Bedside	Favorable (1.67)
0864502	Epigenetic Regulation of Inactive X Chromosome Expression	Favorable (2.00)
0864503	Role of UGT2B7 Genotype in Patient Response to Tamoxifen	Favorable (2.00)
0864504	Functional Brain Imaging of Memory and Language for Epilepsy Surgery	Favorable (1.67)
0864505	Molecular Targets for Preventing Loss of Skeletal Muscle Mass	Outstanding (1.00)
0864506	Research Infrastructure - Biological Research Laboratory Construction	Outstanding (1.33)
0864507	Regulation of Nutrient Sensing and Muscle Wasting by Alcohol	Favorable (2.33)
0864508	Murine Induced Pluripotent Stem Cells: Differentiation and Bone Formation	Outstanding (1.33)
0864509	Evaluation of mTOR as a Chemoprevention Target in Skin Cancer"	Favorable (1.67)
0864510	IRES-mediated Synthesis of Proteins Integral to Adaptation to Hyperoxia	Favorable (2.00)
0864511	Stroke Recovery in Type II Diabetes	Favorable (1.67)
0864512	Modulation of Basal Ganglia Electrophysiology by Dopaminergic Cell Transplant	Outstanding (1.33)
0864513	Identification and Analysis of Arterial Blood Pressure Noise in Baroreceptor Denervated Rats	Favorable (2.33)
0864514	Myocardial Protein Synthesis After Alcohol Intoxication	Outstanding (1.00)
0864515	Development of Nanoliposomal Therapeutics for Leukemia	Favorable (1.67)
0864516	P16 Alteration and BRAF Mutation and Patient Outcomes in Papillary Thyroid Cancer	Favorable (2.33)
0864517	The Interaction of Environmental Agents and LDL-Cholesterol in Parkinson's Disease	Outstanding (1.33)
0864518	Moving Experimental Cancer Therapeutics from the Research Bench to the Clinic	Outstanding (1.00)

Project	Title	Average Score
0864519	Changes in Oxygen-induced Proliferative Retinopathy in 4E-BP1/2 Knockout Mice	Favorable (2.00)
0864520	Molecular Mechanisms of Uninfected Red Cell Phagocytosis in Severe Malarial Anemia	Favorable (2.33)
0864521	In Vivo Anti Tumoral Properties of Ceramide Nano Liposomes in a Murine Hepatocellular Cancer	Favorable (1.67)
0864522	The Use of Biomarkers to Predict the Onset of Vasospasm in Aneurysmal Subarachnoid Hemorrhage	Favorable (2.00)
0864523	Tim2 Expression on Oligodendrocytes: A new Immune System Target	Favorable (2.33)
0864524	Mechanisms of Microsatellite Mutagenesis in Human Cells	Favorable (1.67)
0864525	Epigenetic Therapy of Human B Cell Malignancies	Favorable (1.67)
0864526	Novel Multielectrode Recording Techniques for Assessment of Taste Functions in the Brain	Outstanding (1.33)
0864527	Glycosphingolipids and Diabetic Retinopathy	Outstanding (1.33)
0864528	Epithelial/Dendritic Cell Cross-Talk in Acute Kidney Injury	Outstanding (1.00)
0864529	Synergistically Acting Targeted Therapeutics for Melanoma	Outstanding (1.33)
0864530	Diabetic Changes in Contractile Proteins and Contractility in Arterial versus Venous Grafts	Unfavorable (2.67)
0864531	Autism Indicators: Erythrocyte Membrane Fluidity and/or Lipid Composition	Favorable (2.33)
0864532	Efficacy of Gemcitabine for Pancreatic Cancer: Role of DNA Polymerases	Favorable (2.00)
0864533	Embedded Rural Clinical Research Infrastructure: Utilization of Community-Based Nurses and Paramedics	Favorable (2.00)
0864534	Cytoadherence in Maternal Malaria	Favorable (2.00)
0864535	Impact of iPS Cell-derived Highly Reactive Immune Cells on Cancer	Outstanding (1.33)
0864536	Cannula Development and In Vivo Testing for Pediatric VAD Development	Outstanding (1.33)
0864537	Role of Leucine Metabolism in Leucine Signaling	Outstanding (1.33)

Project Number: 0864501
Project Title: Vitamin D and Crohn's Disease: From the Bench to the Bedside
Investigator: Cantorna, Margherita

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The project met the stated objectives.

The research design was adequate but a less-than-expected patient enrollment forced a modification of the approach to increase recruitment into the study.

The data obtained were sufficient in number to obtain statistically significant results that were in line with the stated objectives.

Changes were made to the original research protocol and a very reasonable explanation was provided. Pragmatic difficulties with patient visits to the clinic limited participation in the study. However, changes were made to the procedure for phlebotomy and to the exclusion criteria that resulted in increased patient recruitment.

The patients enrolled in the project were sufficient to generate meaningful data that ultimately passed scrutiny of peer review in a reputable journal. The laboratory and clinical data obtained during the tenure of the project were in line with the proposed objectives.

There are several strengths to this proposal. The first concerns the questions posed and the central hypothesis, which are significant, particularly in light of the need by patients with IBD. Secondly, the team assembled to do the work is very capable. Lastly, the approach proposed has tested the hypothesis and the objectives of the proposed study were obtained.

Weakness inherent in the project was the inability to recruit the 50 patients as proposed. This was a significant error in the protocol that might have been better anticipated considering the experience of the PI's with such clinical trials. They were initially only able to attract 13 patients and even after modifying some of the parameters of the study they still only recruited 18 patients. While this was sufficient to meet the overall objective, a larger population might have been able to provide insight into various groupings observed in their data (e.g., correlation with age, disease index, medication/treatment).

Another weakness is the narrow breadth of the study. The PIs are experts on vitamin D regulation of immune cell function and limiting the readouts to blood Vit D levels is ostensibly missing out on opportunity to extract some mechanistic data from the samples.

Reviewer 2:
The guiding hypothesis for this proposal was that because of low dietary vitamin D intakes and malabsorption issues, Crohn's patients will have low circulating vitamin D levels that will be detrimental for their health. The objective was to conduct a phase I trial of vitamin D supplementation in 50 adult patients with mild-to-moderate Crohn's disease. Because the study was a feasibility study, it was designed with no placebo controls, rather, the patients served as their own controls. The investigators planned to identify vitamin D deficient Crohn's patients and give them 1000IU vitamin for 24 weeks and do baseline and 6-month data collection for diet, serum vitamin D levels, inflammation, bone mineral density, Crohn's activity scores and quality-of-life surveys. The specific stated objectives were: 1. Evaluate whether providing a vitamin D supplement of 1000 IU/d is well-tolerated, 2. Assess whether a daily supplementation with vitamin D (1000 IU/d) will lead to increased circulating vitamin D, 3. Determine whether the aforementioned treatment will lead to favorable changes in a clinical tool used to describe and characterize Crohn's disease (Crohn's Disease Activity Index),and 4. Evaluate whether inflammatory markers measured in blood (C-reactive protein, erythrocyte sedimentation rate) show improvements following a six-month trial with vitamin D. They also planned to evaluate two secondary objectives; to assess the effects of the vitamin D intervention on changes in quality-of-life measures and to explore whether vitamin D intervention will lead to improvements in markers of bone health and bone density among Crohn's disease patients.

The project did evaluate the stated objectives with necessary changes in the design to achieve the objectives. The vitamin D supplement was well-tolerated. However, the investigators recognized (as a weakness) that they were able to recruit only 18 of the proposed 50 subjects throughout the study period. All had low vitamin D status. On those 18 subjects they did measure baseline and 6-month data collection for diet, serum vitamin D levels, inflammation, bone mineral density, Crohn's activity scores and quality-of-life surveys. The investigators learned early on that the 1000IU was not sufficient to raise the vitamin D status to the target range. Therefore, they had to increase the dose of vitamin D up to 5000IU for 13 of the 18 patients. In addition, they changed recruiting and sample collection strategies to try to get and keep more subjects in the study. They lost 3 early in the process. The initial research design was adequate to meet the objective except that the investigators learned that far more than 1000IU of vitamin D ended up being required to raise the levels. In half the patients they were not able to get the vitamin D levels above the target 40ng/ml in the blood. However, this is an important point highlighting the difficulty in raising vitamin D levels in this population.

The data were developed sufficiently to answer the research questions posed with the exception that the investigators were not able to recruit the proposed 50 subjects. So, for several of the measures they were underpowered for statistical analysis. The data were developed in line with the original research protocol. As previously described, the dosage of vitamin D had to be changed in order to raise vitamin D levels in the serum in these subjects. This highlights the difficulty in getting vitamin D increases in Crohn's patients. All the changes to the protocols were necessary and reasonable. The investigators measured all of the initially-proposed

collections. There was sufficient data to support the fact that the project met its objectives even with the small population, and they made progress towards their goals. The data and information provided were applicable to the project objectives listed in the strategic research plan.

Strengths were stated above but briefly summarized. They met all the objectives with the minor weakness of recruiting only 18 of the proposed 50 subjects. However, they were still able to show an increase in blood vitamin D, identify a dose and improved scores for symptoms and quality-of-life after supplementation with vitamin D. They showed no effect on serum markers of inflammation and bone health with vitamin D supplementation.

Reviewer 3:
The objective of this project is to conduct a phase I trial of vitamin D supplementation for 6 months in 50 adult patients with mild-to-moderate Crohn's disease. No placebo control arm was designed. The primary objective is to assess whether the patients will tolerate 1000 IU daily vitamin D supplementation, and whether this dose is able to raise patients' serum vitamin D status. It also will assess whether this supplementation will have a favorable impact on the patients in terms of disease index, quality-of-life and serum inflammation markers. The secondary objective is to assess the effect of this supplementation on skeletal health and bone-related factors such as serum PTH, calcium and alkaline phosphatase.

The anticipated duration of the project was from Jan. 1, 2009 to June 30, 2010, but the study was extended to December 31, 2012 (2.5 years longer). Instead of recruiting 50 patients, the study was actually completed with only 18 patients (only 36% of the original recruitment). The extension and the low patient recruitment reflect the inability of the investigators to recruit sufficient patients for their study. For a clinical trial, small sample size decreases the value and credibility of the observed outcome.

The original exclusion criteria of the study design excluded patients who use vitamin D supplements in excess of 200 IU/d; but, in the trial, patients who take 120-800 IU/d were actually included, again reflecting the inability to recruit sufficient patients. As the investigators pointed out, high vitamin D intake could interfere with the interpretation of the data.

Because the 1000 IU dose failed to substantially change the patients' serum vitamin D status in the first two weeks, the investigators raised the dose to 5000 IU/d for the majority of the patients. After 24 weeks, most of the patients showed increases in serum vitamin D status, with about half of the patients reaching the target level of 40 ng/ml or higher. Patient compliance was excellent. After supplementation, the patients showed significant improvement in disease index scores and in quality-of-life scores. Adverse effects were reported but limited, and the effect on serum calcium and PTH was minimal. These are strengths. However, due to the small sample size and the lack of a placebo control arm in the trial, the real implication of these observations is difficult to assess at this time.

The trial is based on the hypothesis/assumption that vitamin D suppresses inflammation/immune response, but after vitamin D supplementation there were absolutely no changes in the serum inflammatory status in these patients. So there is a big gap that needs to be filled between the theory and the outcome. In other words, the hypothesis is not consistent with the experimental

outcome, suggesting that the investigators lack, or have not identified, the mechanistic insight into the clinical outcome.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The results from the current study have significant potential for improving the health outcomes of Crohn's patients. The present study has demonstrated that Crohn's patients are deficient in vitamin D and have identified the minimal oral dosing needed to restore serum levels to that of non-Crohn's individuals. It has also demonstrated that restoring vitamin D levels can reduce disease symptoms.

The present study has provided the necessary proof-of-principal to drive the therapeutic approach further in terms of larger populations, disease severity, and increase our understanding of the mechanism. The PIs have published the results from the project and plan to submit larger grant applications to this end.

The strength of the proposal is that despite shortcomings in patient enrollment they were still able to generate sufficient data to meet their objectives. Their published conclusions should have some impact in the acquisition of funds to continue the line of work.

The shortcoming is that the lack of mechanistic insight generated by the present work may preclude their ability to design an adequate protocol to convince reviewers of future proposals. As a single example, if the Vit D deficit is due to compromised absorption resulting from active disease, this would imply that the treatment would not be effective at maintaining remission. It is not clear, however, whether the Vit D levels re-stabilize in Crohn's patients in remission.

Reviewer 2:
This project had important benefit and impact even though it was a small study with the weakness of not recruiting enough subjects and having no controls. In this small feasibility clinical trial in central PA vitamin D3 supplementation for 24wks effectively improved vitamin D status, decreased severity of symptoms scores (CDAI) and improved quality-of-life scores (IBDQ) in 18 patients. This is a highly significant finding for these subjects and should be repeated in a larger study with controls. By raising serum vitamin D levels, Crohn's patients with mild-to-moderate symptoms exhibited a decrease in CDAI scores such that the majority of patients achieved remission. The effect of vitamin D3 on the CDAI scores was remarkable with all but one patient having a decrease in CDAI scores and 78 % of the patients had a drop of 70 points or more, which is considered a treatment response in the clinic. The vitamin D3 effect was also reflected in the improved IBDQ scores, which is a second validated assessment tool used to monitor therapeutic efficacy in clinical trials of IBD.

The primary goal of the study was to determine what dose of vitamin D3 would be required to raise serum 25(OH)D3 levels into a target range of over 40 ng/ml. Consistent with others, they

found that Crohn's patients did in fact have low serum 25(OH)D3 levels. Even though the majority of the patients were on vitamin D supplements at baseline, that level of supplementation was insufficient to sustain serum 25(OH)D3 above 40 ng/ml. In fact, half of the Crohn's patients screened for the study had frank vitamin deficiency with serum levels of 25(OH)D3 suggesting malabsorption. The maximal amount of vitamin D3 (5000 IU/d) was needed to raise serum 25(OH)D3 above 40 ng/ml. The serum levels of 25(OH)D3 were slow to respond to the vitamin D3 supplement and even between 12 weeks and 24 weeks serum 25(OH)D3 levels continued to go up even though the dose of vitamin D3 was not changed. It seems likely that the increased level of 25(OH)D3 between the 12-week and 24-week time point reflects improvement in CDAI scores, a healthier gut, and better absorption of the vitamin D supplement. There were no signs of vitamin D toxicity.

There was no effect of vitamin D3 on any of the serum proteins measured, and there was no correlation between these inflammatory biomarkers and the improvement in CDAI scores seen. At this time, it is unclear by what mechanisms vitamin D might be improving the outcomes. Also, there was not enough statistical power for a significant effect of vitamin D on bone health. Another limitation is that these data are only applicable to the mild-to-moderate patients and those that match the inclusion/exclusion criteria and therefore not generalizable. However, this was designed as a feasibility study.

Based on the results, the investigators propose a placebo-controlled and longer term study for efficacy in a large population of patients. In addition, they want to determine the mechanisms by which vitamin D regulates the inflammation and immune function in Crohn's patients in particular.

Reviewer 3:
The outcome of the project appears to suggest a beneficial impact of vitamin D supplementation on patients with Crohn's disease; but, because of the very small sample size, the lack of placebo control arm in the trial, and the lack of mechanistic insight to explain the outcome, the real impact of this project is difficult to evaluate. That is, the beneficial impact of vitamin D supplementation on patients with Crohn's disease, if any, remains to be determined, and this trial has not completely addressed this issue. In fact, trials of vitamin D supplementation in IBD patients have been reported elsewhere, which decreases the novelty of this project. The value of this project is thus limited. Generally speaking, for a small trial like this one with a limited number of patients recruited after a long extension of the project and no placebo controls, the large dollar amount budgeted for this project is hard to be justified.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The investigators claim to have submitted a grant to the Crohn's & Colitis Foundation, which presumably scored sufficiently favorable to support a revision, which they stated is in progress. The PI also states intent to submit to the NIH.

The strategy to submit to the foundation as a prelude to the NIH is a sound one. Reviewers' comments "may" be useful in developing the project to a competitive level. No weaknesses are evident in this approach, and it is not expected during the tenure of funding that the PIs obtain data, manuscript or additional funding, particularly in the present competitive environment.

Reviewer 2:
There was no stated objective to leverage funds or that they had any other funding. This project was not funded by any other means during the reporting period. The investigator did apply for a Crohn's and Colitis Foundation of America grant in the fall of 2012. They are in the process of revising that application to resubmit it. In addition, they are planning on submitting a grant to the National Institutes of Health within the next six months. They are determining the applicable program announcements and research funding announcements at the NIH for this submission. The investigators are moving forward with seeking external funding based upon these data. The funding climate is very difficult at this time. However, these results are intriguing and should result in a competitive application. This is a strength that they have already applied for funding using these data and continue to revise an application.

Reviewer 3:
There is no indication of additional funding.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The results from this study were submitted and accepted (April 18, 2013) in the relatively new "Clinical and Translational Gastroenterology" journal published by NPG.

This is a respected peer-reviewed journal and is entirely appropriate for the results and conclusions obtained.

Reviewer 2:
The project did not result in any licenses, patents or commercial development opportunities. This was not the stated objective of this application.

The project did result in 1 peer-reviewed publication titled the Therapeutic effect of vitamin D supplementation in a pilot study of Crohn's patients. Yang L, Weaver V, Smith JP, Bingaman S, Hartman TJ, Cantorna MT. Clin Transl Gastroenterol. 2013 Apr 18;4:e33. The journal is a new journal that does not have an impact factor yet but is indexed in PubMed and other search engines. One assumes that other high impact journals did not give this manuscript a high priority score or felt there were weaknesses. While there are known weaknesses that the authors state, the research could lead to a clinical observation of importance and future studies and has value for publication as identified by the reviewers at Clin Transl Gastroenterol. Regarding the number of publications, given that all these data belong together in one manuscript, it is appropriate that the investigators have one just manuscript.

Reviewer 3:
The original plan was to publish the work in the journal of Inflammatory Bowel Disease, a very prestigious journal in the field of IBD research. Instead, the work was published in 2013 in Clinical and Translational Gastroenterology, an online open access journal with much less prestige. This change of plan might be due to the quality of the work. Only one publication has resulted from this work.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
No improvements were made to infrastructure, or new recruitment, but several students were stated to be engaged in the project.

The infrastructure at the PI's institution is excellent and therefore enhancement was not expected to be an issue. However, based upon the small sample size (18), it is difficult to envision the need for 10% effort for 3 co-investigators, and 5% effort for 5 additional students/techs/post-docs.

Reviewer 2:
There were no infrastructure improvements proposed. There were no new investigators added to the proposal.

There were three graduate students paid from these funds for 5% of their time. In addition, the money used for research supported a total of 1 undergraduate, 4 pre-docs and 1 post-doc for research involvement in the project. Yang, the post-doc, ended up being the first author on the publication.

It is a significant strength that there was strong involvement of students in this project. There appeared to be a significant amount of training of students that likely occurred as a result of this project both in clinical human study design and conduct.

Reviewer 3:
This is a very small conventional phase I clinical trial without placebo controls. The trial was conducted using the personnel (PI and two co-PIs) and established infrastructure of the University. No new investigators were added for the study. No outside researchers were brought into the institution to help the research. It was unclear whether the fund was used to pay for students or post-doctoral fellows to carry out the research. Overall, this project does not seem to enhance the quality and capacity for research at the grantee's institution.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The proposal was successful in demonstrating a productive internal collaboration between Dr. Smith, M.D., and Dr. Cantorna, PhD.

Establishing this collaboration and demonstrating productivity is essential for a competitive grant submission to expand the project. No weakness noted.

Reviewer 2:
The research that resulted from this project did not lead to collaborations outside of the institution or community directly. However, two of the three PIs have recently moved to Emory (Hartman) and the NIH extramural program (Smith). Therefore, the future collaborative research and proposals will include collaborations with Dr. Hartman at Emory and possibly intramural researchers at the NIH that collaborate with Dr. Smith.

The research and recruiting was conducted at two locations both at the Pennsylvania State University, the College of Medicine (Hershey, Pennsylvania) and the main campus (University Park, Pennsylvania).

Reviewer 3:
This is a small clinical study carried out within a single institution. No collaboration with outside investigators or with other hospitals was noticed. No new collaborations as a result of this research were noticed. No new involvement of research with the community was noticed.

Section B. Recommendations

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.

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.

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.

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.

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.

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.

ADDITIONAL COMMENTS

Reviewer 1:
The project proposed a study with significant potential impact for a devastating disease with considerable morbidity in the USA. The results were obtained that supported their hypothesis, met their objectives, and led to the publication of their work in a respectable journal. Based upon the implications of their findings, they are actively engaged in attracting further funding to expand the project, which has a good chance of funding due to the established productivity of the team that was a direct result of this project.

Reviewer 3:
This is a clinical trial with only a small number of patients far below the target number even after a long and extended recruitment time. For clinical trials, small sample size decreases the value,

credibility and thus the impact of the outcome. Moreover, this trial lacks a placebo control arm, which further devaluates the clinical findings. Thirdly, the outcome of this trial does not support the hypothesis that is supposed to serve as the intellectual foundation of this trial. Therefore, the mechanistic basis of the observed outcome remains unclear, which also reduces the value of the data.

Generic Recommendations for Pennsylvania State University

Reviewer 3:
Because of the numerous major weaknesses identified in this trial, the real value of this project should be reassessed. Whether this trial should be continued needs to be reconsidered. The overall recommendation is unfavorable.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Did the project meet the stated objectives? The project addressed all of the 3 aims in the original proposal. The aims were linear, so Aim 3 was dependent on the results of Aim 1 and Aim 2. Aim 3 was not feasible based on findings from Aims 1 and 2, so the PI used the remaining resources to conduct an alternative set of experiments building on the findings from Aim 1 and Aim 2. These new experiments were innovative and produced important results that will increase the impact of the project.

The project generated precisely the data that were proposed in the original application, and these were sufficient to conclude that methylation state does not predict expression levels from the inactive X. Furthermore, the project generated data not proposed in the original application, which demonstrated that expression from the inactive X is not completely stochastic, but must be reproducibly directed by some epigenetic mark set during development.

Strengths: The project addressed an important question in human genetics. Although Aim 3 was not feasible following completion of Aims 1 and 2, the PI resourcefully redirected research to perform a clever and informative experiment to understand the dynamics of inactive-X expression at a single cell level.

Weaknesses: There doesn't appear to be a control assay for Aim 1. Did the PI interrogate a gene thought not to escape X-inactivation? One gene is not labeled on Figure 1.

Reviewer 2:
The project aims to address epigenetic gene regulation on X chromosome. Three Aims: (1) to measure levels of inactive X expression for seven genes that cluster in Xp11.3 in a panel of 40 primary cell lines that have previously been used to effectively monitor inactive X expression; (2) to correlate inactive X expression with epigenetic modifications by measuring DNA methylation at the CpG islands of these genes in multiple female cell lines, and (3) to establish a methylation assay that could be used to effectively predict expression levels from the inactive X that could evaluate the role that inactive X genes contribute to traits that show gender differences.

The PI is a pioneer in defining X-linked genes that are either subject to X-inactivation or escape X-inactivation. Dosage compensation of X-chromosome is tightly linked to normal physiology and the perturbation of X-linked gene expression leads to disease. The research teams examined the relationship between DNA methylation and X-linked gene expression in a number of established cell lines. The results showed that most of CpG islands are unmethylated in the several genes they examined. This is a surprising result, implicating that other epigenetic mechanisms are involved in regulating gene silencing or escape of X-inactivation. Overall, the progress of the project meets the stated objective of this grant proposal.

Reviewer 3:
Major strength: The investigators completed the first aim ahead of schedule, which was to develop the SNP expression assays for the seven genes of interest in the region. They were able to assay all 40 fibroblast cell lines for inactive X expression. While the overall aim was straightforward, there are always difficulties in identifying SNPs that can be used as well as developing and troubleshooting assays. The completion of this aim is a major strength.

Strengths: The results from the original experiments were unexpected, since only one gene, DDX3X, in the inactive X region examined showed significant expression. One other gene, USP9X, showed modest expression and these two were used to evaluate DNA methylation of CpG sites in the region. Again, unexpected results revealed that the sites were completely hypomethylated, and therefore uncorrelated with gene expression. While these results were unexpected and perhaps disappointing, they do indicate potentially that other mechanisms contribute to the regulation of inactive X expression.

Since Aim 3 was no longer relevant, the investigators used their resources to study the heritability of inactive X expression with two CEPH pedigrees. The conclusions from these studies were that inactive X expression could not be explained by simple inheritance patterns, and that the expression patterns were not stochastic. The final conclusion was that other epigenetic mechanisms played a role in stably establishing the expression of the inactive X.

Weakness: In the original proposal, the investigators stated that "If results are not concordant…we will develop additional primer sets to extend our pyrosequencing to cover each complete CpG island." It is not apparent in the final report if this was done. One potential reason for the lack of correlation with gene expression is that the investigators are not looking at the region that actually controls gene expression. Completion of the original alternative plan would have attempted to address that.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
A major focus of genomic medicine is to predict the functional impact of rare genetic variants. The mapping of rare genetic variants to functional space is exceptionally complex. Although we can, today, do a decent job of predicting the functional impact of an amino acid substitution on

protein function, there are many layers of additional data that are needed in most cases to actually predict the impact of a coding nucleotide substitution on organismal phenotype. Is the exon contained in transcripts that are expressed in the tissue(s) of interest? Are both maternal and paternal copies of the gene expressed at equal levels? In the case of the X chromosome, this is further complicated by the process of X-inactivation. The project described here has produced fundamental data that will be used to improve models for how genetic variants on the X-chromosome are expressed in women and what their relevance may be for human health.

The research is focused on a very specific fraction of the genome, less than 3% of the total, but the complexity of the problem warrants this special focus. I have no doubt that a model of how X-linked genes are expressed across female tissues throughout development and life will become a part of clinical pathogenicity prediction programs used in our lifetime.

The data produced here are pilot data, really – more work will be needed to codify these findings into a formal tool for improving our interpretation of disease-causing variation on the X. But they are an important early step in this direction.

Before this project, it has not been appreciated that genes that can escape X-inactivation show variable expression across individuals. Furthermore, it is novel and interesting to show that this variability is apparently clonally inherited, but not specified by local CpG methylation; therefore, there may be an epigenetic code to be deciphered for us to determine the rules of X-linked expression in women.

The PI plans to follow up on these results by asking whether other features at the investigated loci support a permissive state or which other epigenetic features do indeed directly reflect inactive X expression levels.

Reviewer 2:
Because variations in the expression of X-linked genes are associated with gender differences and genetic variations that are associated with health and disease, this research has important implications for X-linked diseases. The results from this project will lead to additional grant applications. Of course, because of the limited budget of this grant, the gain of knowledge in the competitive field of X-inactivation is incremental at this stage.

Reviewer 3:
Strengths: Even though the results were unexpected, the likely benefit from this study is the incremental gain in knowledge typical of basic science research. Understanding the mechanisms that regulate gene expression on the inactive X chromosome has clinical significance, as well as the overall importance in the understanding of epigenetic regulation of gene expression. While the impact may not be large, it is well justified by the funds that were allocated for this project.

The future plans based on this data are consistent with basic science research. The investigators plan to publish their findings (two manuscripts were in preparation at the time of the final report) and use their data as preliminary findings for extramural funding (two March of Dimes grants had been applied for).

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Grants have been submitted but none funded yet.

Strengths: The PI has applied for multiple grants based on this project and will continue to apply for grants. I am optimistic that further work will be funded once the story has become mature enough.

Reviewer 2:
The PI has submitted one NIH grant application and another proposal to the March of Dimes Foundation.

Reviewer 3:
Two grant applications were listed that used data from this project. These were both March of Dimes grants. One, submitted 9/2011, was not funded and a second, submitted 9/2012, was listed as pending at the time of the report. The investigators stated that if the second grant was not funded they would apply to the NIH with the R21 mechanism. These applications seem reasonable based on the data generated.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strengths: The project produced publishable data. Due to a medical leave of absence by a trainee, no manuscripts have been submitted, but I fully expect to see one paper materialize from this work.

Weaknesses: None.

Reviewer 2:
One manuscript is completed and to be submitted. Another one is in preparation.

Reviewer 3:
Weakness: No manuscripts have been published based on the findings from this project. Two were listed as in preparation, but they do not appear to have been published yet.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strengths: Funds were used to pay for research by a pre-doctoral student.

Reviewer 2:
The funding was used to support a graduate student to complete a PhD research project. It is very timely under the current federal funding atmosphere.

Reviewer 3:
Strength: Funds were used to pay for one student for a year.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Weaknesses: 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.

Reviewer 2:
Because of the limited budget of this grant, it was only used for supporting of one graduate student. No new collaboration was borne out this project.

Reviewer 3:
Apparently no new collaborations were made.

Section B. Recommendations

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.

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.

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.

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

ADDITIONAL COMMENTS

Reviewer 3:
This project was somewhat hindered by the unexpected results identified with the gene expression and DNA methylation data. The investigators did a good job of pursuing other avenues of research (the CEPH cell lines), but also seemed to have missed an opportunity by not following up on the alternative approach described in the original proposal. Specifically, they apparently did not examine alternate CpG islands once the original ones did not provide the expected results.

Other than that, the lack of publications and funding from this project are disappointing, but not entirely unexpected given the results.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The goal of this study was to examine the effects of the UGT2B7 variant on TAM metabolite profiles and toxicity in women taking TAM. The ultimate goal was to establish a patient cohort to test the contribution of pertinent UGT variants to variability in TAM pharmacokinetics and response in breast cancer patients treated with TAM and to provide preliminary results for an NIH application. The PI proposed testing their hypothesis by measuring levels of TAM metabolites in patients on TAM and correlating levels with UGT2B7 genotype. They used a clinical questionnaire to capture patient toxicity and side effects (hot flashes, arthralgias, deep vein thrombosis and uterine cancer) with the goal of correlating these with patient UGT2B7 genotype. The long-term goals of this project were to determine whether UGT2B7 genotype impacts breast cancer recurrence and metastasis. It was hoped that this project would lead to significant publications. In addition, the data/samples garnered from the Penn State patients would be combined with those in the Collaborative Breast Cancer (COBRA) Pharmacogenetics Consortia, a consortium of groups at a number of academic institutions that has collected data on over 300 women with breast cancer treated with TAM.

The goal was to recruit a total of 45-50 eligible patients per year over 3 years, obtain pretreatment medical histories, and blood at baseline (i.e., before TAM therapy is initiated) for genotype analysis and biochemical phenotypes (e.g., TAM metabolites). TAM (20 mg/day orally) was to be prescribed for all subjects as part of adjuvant therapy and will be followed up on an outpatient basis at 1 day, and 4, 8, and 12 months after the start of TAM therapy. TAM and its metabolites as well as TAM-induced pharmacodynamic changes were to be obtained at the different time points.

In the first year of the grant, Dr Lazarus's group established the UPLC/MS/MS methodology for measuring tamoxifen and its metabolites. A total of 47 patients were enrolled in this study. Some preliminary analyses were done correlating CYP2D6 and UGT2B7 genotype with tamoxifen metabolite levels and rate of tamoxifen-associated hot flashes in these patients. Therefore, the study team achieved one of their primary-stated objectives.

However, the number of patients analyzed was too small to make any statistically-sound conclusions. It is not clear why the project was dropped after the first year and why the data

resulting from the Penn State study were not combined with COBRA data. It does not appear that any future research, publications or funding will come from the small pilot project.

Reviewer 2:
Strengths: The investigators were able to meet the stated objectives that include the following:
1. Recruitment of 47 breast cancer patients within eligibility criteria.
2. Collection of hot flash diaries.
3. Collection of blood samples at stated time points following TAM treatment.
4. Development of assay to determine TAM metabolites.
5. Determination of UGT2B7 and CYP2D6 genotypes.
6. Correlated TAM metabolite data with genotype data.
7. Correlated genotype data with hot flash frequency and intensity.

The research design appeared to be straightforward and appropriate to address the stated objectives.

A total of 47 patients were recruited on the study, which is only 3 less than the intended accrual of 50.

The data and information provided supported the finding that the project met the objectives.

Weaknesses: While the strategic plan did not propose determining the CYP2D6 genotype, it proved to be an important control. Unfortunately the rationale for performing this additional analysis was not well-developed in the final progress report.

Although the data and information provided supported the finding that the project met the objectives, 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.

Reviewer 3:
The stated objectives were judiciously pursued using the originally-proposed clinical activities (patients and samples) and laboratory experimentation (using the approaches as proposed). However, the outcome of the project was "negative data," the data that did not favor the proposed hypothesis.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The study was very small and stopped after the first year. Dr. Lazarus has left Penn State and it appears that no further work will come out of this study. Therefore, no major discoveries or advances came from this study.

Reviewer 2:
Strengths: The proposed studies clearly and correctly state that the completion of the proposed objective could have led to individualized drug dosing and selection of appropriate endocrine therapies for breast cancer patients. Further, such information could reduce toxic side effects associated with tamoxifen therapy. Since a majority of 200,000 breast cancer patients diagnosed yearly require endocrine therapy, the impact of these studies could have been significant.

Weaknesses: Unfortunately, despite the fact that the objectives were met, the hypothesis that UGTB27 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.

Major new discoveries, new approaches, diagnoses, or new drugs were not attributed to the completion of this research.

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

Reviewer 3:
There is no apparent beneficial impact of the project outcome. The study revealed that the proposed hypothesis is not true and hence, there is no direct significance or impact of the study in improving the health of the breast cancer patients. The impact is too little in light of the dollars spent.

The listed future plans for this project include testing of the same hypothesis on a larger cohort of breast cancer patients receiving Tamoxifen therapy. The PI also proposes to adopt alternative/divergent approaches involving the use of aromatase inhibitors. However, the lab Investigator of this project, Dr. Lazarus, has moved to another institution and this leaves a void to fill for any future studies of this nature by the PI who is a clinician.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
No additional funds came as a result of this work.

Reviewer 2:
Strengths: None

Weaknesses: The intent of the PI as stated in the research strategy was to apply for funding from the NIH/NCI; however, this was not done. No plan was stated to apply for funding in the future.

Reviewer 3:
There was no plan for leveraging the funds. There are no specific plans to apply for additional funding although the PI does mention the intent to adopt an alternative approach to study a larger cohort of this group of patients.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
No publications came from or are likely to come from this small pilot project.

Reviewer 2:
Strengths: None.

Weaknesses: This project did not result in any publications, even though manuscript preparation was listed as a milestone. No licenses or patents were filed. There are no plans to submit any publications, licenses or patents.

Reviewer 3:
Though expected, no publications/patents/licenses and commercial developments resulted from the study because of the "negative data."

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Nothing came as a result of this study.

Reviewer 2:
Strengths: The PI states that because of this study, more breast cancer patients were enrolled in clinical trials than previously.

Weaknesses: Funds did not result in improvement to infrastructure, new investigators brought to the university or training of pre- or post-docs.

Reviewer 3:
There was no infrastructure improvement or addition of new investigators/researchers. No pre- or post-doc researchers were funded. Notably, a substantial chunk of funding was spent on two "gene expression analysts" in their core facility, which is not consistent with the proposed activities. There were no gene expression activities proposed in the strategic plan, nor were there any data generated on gene expression.

The only way the project might be deemed to have enhanced the research capacity at Penn State is that more patients (47 patients) got added to their breast cancer registry.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The project did not lead to any new collaborations, discoveries of potential new research projects.

Reviewer 2:
Strengths: The strategic research plan discussed participation within the Collaborative Breast Cancer (COBRA) Pharmacogenetics Consortia headed by Dr. David Flockhart.

Weaknesses: This collaboration did not materialize nor is it discussed as a future direction in the progress report.

Reviewer 3:
The project did not result in any new collaborations or involvement of community.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:
The grant has ended and nothing else is planned for the future.

Reviewer 2:

1. Weakness: Despite the fact that the objectives were met, the hypothesis that UGTB27 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.

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.

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.

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^{268Tyr} genotype.

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.

ADDITIONAL COMMENTS

Reviewer 1:

The study team started the project in good faith. And they made a good faith effort to complete the study as planned. They enrolled the proposed number of patients within the first year, measured the tamoxifen levels, completed the genotyping and did some preliminary analyses. Then the study was dropped and nothing more came out as a result. This is not at all uncommon for small pilot projects of this nature.

Reviewer 2:

Strengths: The investigators were able to meet the stated objectives that include the following:

1. Recruitment of 47 breast cancer patients within eligibility criteria.
2. Collection of hot flash diaries.
3. Collection of blood samples at stated time points following TAM treatment.
4. Development of assay to determine TAM metabolites.
5. Determination of UGT2B7 and CYP2D6 genotypes.
6. Correlated TAM metabolite data with genotype data.
7. Correlated genotype data with hot flash frequency and intensity.

The research design appeared to be straightforward and appropriate to address the stated objectives.

A total of 47 patients were recruited on the study, which is only 3 less than the intended accrual of 50.

The proposed study clearly and correctly states that the completion of the proposed objectives could lead to individualized drug dosing and selection of appropriate endocrine therapies for breast cancer patients.

Weaknesses: Although the data and information provided supported the finding that the project met the objectives, 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. Unfortunately, despite the fact that the objectives were met, the hypothesis that UGTB27 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.

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

Reviewer 3:
The major strength of the project performance is that the project was executed by following the original objectives and research plan.

Weakness of the project performance is that it did not yield a favorable outcome because it disproved the proposed hypothesis. Hence, the project is not expected to have any beneficial impact on management of breast cancer or human health in general.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
This is a 4-year project funded from 1/1/2009 to 12/31/2012. The aim of this project is to replace the traditional invasive diagnosis procedure (Wada procedure) with a noninvasive fMRI method before temporal lobe epilepsy patients receive temporal lobectomy for the relief of intractable epilepsy. It is anticipated that the new diagnosis procedure, which involves the use of fMRI and language tasks, will improve the accuracy in diagnosis and prediction of surgical outcome.

Strengths: The research protocol was well-designed and the results provide a strong impact to the current clinical diagnosis procedure for the anterior temporal lobe resection. The results from the basic science research work using fMRI and memory tasks in 10 healthy subjects demonstrated that fMRI activations are reliable indicators of language and memory localization. The results were presented as abstracts in the 2011 RSNA meeting and the 2012 ISMRM meeting (we believe it may be the RSNA not ISMRM meeting). The study subject numbers in healthy group were completed and reached the study numbers proposed in the strategic plan. The progress in healthy subject studies is completed and satisfied. There were no changes in research protocol made during the funding period.

Weaknesses: The proposed pre- and post-surgical studies for 10 temporal lobe epilepsy patients were not completed. The final report shows six patients completed pre-surgical studies and two of them completed post-surgical studies. The final report indicated the incompleteness of 10 proposed patients' pre- and post-surgical studies was due to the departure of faculty members and some other variables such as insurance, clinical setup and coordination between patients and the clinical research group. A statement on the final report indicated the Epilepsy Surgery Team is committed to completing the post-surgical studies at no additional cost to the program. It also mentioned a new faculty member, Dr. Frank Gilliam, has been recruited and will join in the research. We are concerned about the coordination between the clinical research team member and patients for the post-surgical studies. The post-surgical results are the most important factor in this translational research project. Increased coordination and a more structured plan focused on acquiring post-surgical results will enhance the success of this project.

Reviewer 2:
The project's specific aims were:
"(i) Determine the fMRI characteristics most associated with typical left hemisphere language dominance. We hypothesize that areas where there is no fMRI activity detected across three language tasks can be safely resected during anterior temporal lobectomy for intractable epilepsy.
(ii) Test the hypothesis that an asymmetry index of medial temporal lobe activity during memory retention tasks will be concordant with Wada memory test result asymmetry and of greater predictive value than the Wada for post-surgical memory function.
(iii)Test the hypothesis that pre-operative fMRI language and memory asymmetries will be predictive of 6-month post-operative re-organization of function."

The project failed to meet its specific aims - its stated objectives - for several reasons. By specific aim:
1) fMRI is an activation procedure; it reveals the cortex that receives increased blood flow, and therefore is assumed to be more active during a task. This cortex is not necessarily critical to task performance and, hence, the simple extent of fMRI activation is not a great predictor of cortex that must be avoided during resection. fMRI can serve as a language lateralization tool, but for localization sufficient to tailor resections, it is not a good candidate. To prove its suitability, a head-to-head comparison with a gold standard technique such as cortical stimulation mapping would be needed in such a small subject group.

2) The intracarotid amobarbital procedure (IAP or "Wada") is not a great gold standard for memory lateralization for many reasons, including its own lack of reproducibility (seen in the progress reports for this project). Many investigators have found that mesial temporal sclerosis itself is a better predictor of memory performance than is the IAP. Presurgical memory performance is also a very strong predictor of postoperative memory performance. Simply comparing to the IAP is not enough.

3) The third specific aim is not specific enough. I am not sure exactly how the inference is supposed to run or why.

Over the grant period, the investigators also failed to recruit enough subjects or to complete analysis or dissemination of their work, other than a textbook chapter that is not clearly related to the grant and two posters. It is unfortunate that recruitment was slow, but I wonder if control scans could have been accomplished during the first six months when, "The project has just begun. There is nothing substantive to report yet."

Reviewer 3:
The stated goal of the proposed research project was to design and implement language and memory paradigms for localization of function in 10 TLE patients and 10 controls. The investigators have been successful in designing an impressive number of different tasks and implementing these in the scanner. They convincingly show preliminary data in 10 controls that their tasks 'work" in that they activate the appropriate language and memory regions in the brain on the group level. They also demonstrate, crucially, that this is achievable on the single subject level in 8 patients. That brings them 8 patients short of the target number and they cite clinical

reasons beyond their control for not reaching the recruitment goal of 10 patients. This is an important study.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strength: Although the proposed studies for 10 TLE patients were not completed, the completed pre-surgical fMRI data from six patients' studies are supportive to the hypothesis and will bring significant beneficial impact to the current pre-surgical diagnosis procedure for temporal lobectomy.

Weakness: Only two patients completed post-surgical studies and one has been reported in the final report. There were data from one patient's post-surgical study represented in the final report. The single patient's results showed the data is promising to provide reliable biomarkers for surgical outcome. The study numbers in this group need to be increased dramatically in order to better demonstrate the beneficial impact of this project.

No risk factor changes, incidence of diseases, and death from disease were reported during the grant period.

Reviewer 2:
This is a very important area of study. Noninvasive presurgical evaluation of cognitive function would allow us to offer surgery to people with intractable epilepsy more rapidly and more safely. As this group is at risk of dying at a rate of over one in 100 per year from sudden unexpected death, the sooner we can offer surgery (with a rate of cure of up to 90% in straightforward temporal lobe epilepsy), the better. Projects like this are a key step toward our understanding of noninvasive protocols for such evaluation. Perhaps even more importantly, such projects help to forge multidisciplinary teams at epilepsy centers who gain experience in evaluation of candidates for surgery using multimodal evaluation. However, it is very important to plan projects very carefully using the most appropriate metrics. Also, as the researchers themselves mention, it may be wise to pursue multicenter collaboration in order to assure that there are sufficient subjects and/or to take advantage of particular expertise available at other centers (or to share expertise available at one's own center). It is unclear what the future of this particular project is.

Reviewer 3:
The impact of this study for local Pennsylvania epilepsy care is high if the paradigm is adopted by the epilepsy surgery team, as is suggested by the investigators. It will allow non/invasive lateralization of language and memory function which is a vast improvement in safety and standard of care over the Wada test.

The wider scientific and clinical community would also benefit if the results from this study were published in the future after target enrollment is reached.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
We did not find other funds to be involved in this work. The final report mentioned that there is interest and a plan for future grant submission with the data obtained from this work, but no submission was made at the time of final report.

Reviewer 2:
No leveraging is apparent and though the report mentions that additional funds will be sought it is unclear what the exact plan will be, as no publications based on this work are in press, it is unclear if recruitment is improved, and the data presented in the final progress report were very similar to those presented in the last FY progress report.

Reviewer 3:
No.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
There was one book chapter published in 2010, and one abstract in 2011 and 2012. No manuscript, peer-reviewed publications, licenses or patents are reported. The quality of the book chapter and the two abstracts are considered very good.

Reviewer 2:
The project resulted in a book chapter (presumably reviewed by an editor), but it does not reflect study results, since it is a review of pediatric neuroanatomy. Several potential papers are mentioned but it is not clear at what stage of preparation these are; only one is based on the current dataset and the others would require further recruitment. Two posters were presented as a result of the project.

Reviewer 3:
The investigator cited one conference publication in 2011, which is directly relevant. They also cite a review chapter, which is less relevant. The investigators are strongly encouraged to publish their main findings, especially once outcome data are available.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
This project brought no significant improvement to the infrastructure at the grantee's institution. There were two faculty members left, which negatively affected the research progress in patient studies. There is one new faculty, Dr. Frank Gilliam, who has been recruited and is anticipated to help complete the patient post-surgical studies. One pre-doctoral student was reported as participating in this project with support from this project.

Reviewer 2:
As mentioned above, perhaps the most notable direct improvement to patient care comes from the fact that there is now a group of individuals who are accustomed to acquiring and discussing functional images as part of the pre-surgical evaluation at their institution. Even if this is not disseminated as a generalizable result, this is not to be underestimated. It also appears that a pre-doctoral student was very involved in this research.

Reviewer 3:
Yes, the project clearly strengthens the care of epilepsy patients and improved the local neuroimaging infrastructure.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
This project did not lead to any collaboration with other research groups outside Pennsylvania State University and the Milton Hershey Medical Center during the grant period. The final report mentioned two potential future research directions, which include a multi-center project based on the current data in this project.

Reviewer 2:
A potential multicenter trial was mentioned as a way of increasing recruitment but concrete plans were not described.

Reviewer 3:
No.

Section B. Recommendations

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.

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.

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.

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.

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.

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?

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.

ADDITIONAL COMMENTS

Reviewer 1:
Strength: The fMRI results from the healthy group were completed and provided good fundamentals for the project moving into patient studies. Part of the data from patient pre-surgical and postsurgical studies show the project protocol to be promising in improving the current diagnosis procedure for the temporal lobe epilepsy patients who are going to receive temporal lobectomy. In conclusion, the data in the healthy group and part of the data from the patient studies are very supportive to the hypothesis.

Generic Recommendations for Pennsylvania State University

Reviewer 1:
Patient recruitment plays a key role and also is the most challenging procedure in this type of translational research. A more structured plan for the coordination between clinical group and patient or patient's family is needed. This problem may also be partially solved by recruiting patients from multi sites.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strengths: Not all objectives were met, but the PI followed the science and important new information resulted.

The objectives of Aim 1 were not met because of errors made in sample preparation. While this is unfortunate, this was not the fault of the PI or his lab, as human samples were being obtained from external sources, which is where the mistake was made.

The research design and methods were appropriate based on the stated objectives.

New information, and a large amount of data, was gleaned from this work with regards to the regulation of muscle protein synthesis.

The lab group ran into some issues maintaining expression of their target protein in rat skeletal muscle, but regrouped to use a sepsis model showing that over expression of eIF2B reduced sepsis-induced reductions in protein synthesis.

The objective to understand the role of microRNAs on translational control of eIF2B was not met, but not due to lack of well designed experiments. This is a complex question. The series of experiments were well thought out with no conclusive results.

Weaknesses: None noted.

Reviewer 2:
The overall goal of this very strong project, the identification of molecules involved in the response of muscle to loading and unloading, was achieved. In a series of elegantly-designed experiments, important hypotheses were tested. The methodology used for the individual studies was state-of-the-art and the collected data/interpretations are convincing. That the original specific aims, which were somewhat ambitious, did not entirely work out as planned is not a weakness of this project but highlights the in part exploratory nature of the project. Most importantly, the investigators tried to execute the original plans and opted for smart alternatives

when it became clear that further pursuing the original path would not be wise. Overall, excellent progress was made on this project without significant weaknesses.

Reviewer 3:
This project was well-designed, performed with expertise, and accurately reported in (at least) 4 published manuscripts. A significant strength of this project was the use of human, animal, and cell culture models to pinpoint specific involvement of effector mechanisms (i.e., eIF2/mTOR/miR-133).

It appears that the proposed work was adequately and sufficiently performed. Importantly, experiments described and performed in this project have led to 2 other funded grants.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The project advanced our understanding of the regulation and degradation of eIF2B, a protein important to the process of protein synthesis.

Studies highlighted a possibility of eIF2B inhibition as a mechanism for statin mediated reductions in protein synthesis.

The study highlighted the complexity of studying how microRNAs modulated protein translation.

Reviewer 2:
The development of pharmacologic interventions of muscle wasting and low muscle mass requires a detailed understanding of the molecular pathways by which loading becomes anabolic. Thus, data from this project are critical towards designing experiments that will test specific compounds to prevent muscle loss (and perhaps even raise its mass). This is an intermediate-to long-term target and conclusions from this project cannot be translated directly to the clinic at this point.

Reviewer 3:
The objectives of this project were: (1) to examine the role of eIF2 in skeletal muscle hypertrophy with particular attention devoted to miR-133 activity, (2) to identify the relationship between eIF2 and canonical mTOR signaling, and (3) to quantify the role of eIF2 in human, animal, and cell culture models of muscle growth.

The results of this project greatly add to the literature concerning skeletal muscle hypertrophy. While no new drugs or treatment paradigms directly evolved from the study, significant framework has been established that will likely lead to future physiological or pharmacological interventions for the treatment of skeletal muscle wasting disorders. As such, this reviewer

strongly believes the budgeted dollars were well-spent and will contribute to the improved skeletal muscle health of future generations.

No major discoveries, new drugs, or approaches are attributable to this project.

Work will continue to elucidate effector mechanisms that drive skeletal muscle atrophy with the aim of identifying specific molecular targets to combat wasting due to disuse.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The PI had an RO1 from the NIH that also funded the project. The PI was successful in renewing his RO1, in part, because of this funding. The PI plans additional grants pending and planned for submission, also in part, as a result of these funds.

Reviewer 2:
This project was leveraged both by a NIH RO1 award as well as by a NIH NRSA. During the funding period, the investigators were able to renew their NIH grant, an outstanding accomplishment in the current funding environment. The PI has also used the momentum gained from this grant to apply for further grants.

Reviewer 3:
This work was used to leverage additional funds from the NIH. Namely, an NIH grant (regulation of skeletal muscle metabolism) has been funded and a second NIH grant (macronutrient regulation of alternative pre-mRNA splicing) is pending funding.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The PI has published two papers, two are accepted, and one additional paper will be submitted. All the manuscripts are in good journals, and move the field forward. The PI was quite productive with the use of these funds.

Reviewer 2:
Four manuscripts directly related to this grant have already been published and it is clear from the miRNA data presented in the progress report that more publications will result from this project. Even though it is impossible to know exactly which part of the data were produced as part of the NIH grant versus the PA DOH grant, it is obvious that the productivity of the investigators was excellent.

Reviewer 3:
Four publications were published based on experiments supported by this project. These works appear in the American Journal of Physiology – Endocrinology and Metabolism (3) and Biochimica et Biophysica Acta. Each journal is highly-respected, often cited, and visibility of these papers should be high.

In addition, future papers that explore and elucidate the role of eIF2 should be forth coming. No inventions, new drugs, major discoveries, or patents have been attributed to this project.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The PI purchased a new cage washer with funds. This purchase benefited the larger research community greatly, as the unit that was replaced was 25 years old. A functional cage washer is a necessity to maintain quality animal husbandry at a research facility.

Reviewer 2:
This project participated in the purchase of a new cage washer, an important improvement in infrastructure that will benefit a large number of users. Funds were also used as stipends for graduate students, thus contributing to training the next generation of scientists. No new collaborations were formed but it does not appear that such new collaborations were needed to successfully accomplish the goals of this project.

Reviewer 3:
The lab contributed to shared costs of animal cage washing equipment. This is a significant investment and supports numerous researchers (~65) affiliated with the Penn State Animal Research and Hershey Center for Applied Research Facility.

In addition, this project fully supported two graduate assistants (Tuckow and Kelleher), partially supported two research technicians (Kutzler and Lacko), partially supported the work of a mass spectrometrist (Stanley), and partially supported the PI, Leonard Jefferson.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The project did not lead to collaborations with research partners outside the institution, but this is not a weakness.

Reviewer 2:
No evidence of new collaborations is presented. While not required for this project, the addition of community outreach would have strengthened the impact of the work. The involvement of high school students or presentations at high schools might have been particularly effective.

Reviewer 3:
No new collaborations have been established, nor has commercial development of any research products been created.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:
None.

Reviewer 2:
None.

Reviewer 3:
None.

ADDITIONAL COMMENTS

Reviewer 3:
As stated above, this reviewer appreciates the use of multiple models - both in vivo and in vitro - to define the role of eIF2 and mTOR in the regulation of skeletal muscle hypertrophy. The experiments in these model systems are complimentary and the results add to the growing knowledge base on anabolic signaling mechanisms that improve muscle health.

This work also allowed for procurement of 2 new NIH grants (to date) and is a testament to the quality of the Jefferson Lab.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The overall project resulted in a great expansion and improvement in research infrastructure for housing research animals. The only qualification for this statement is that the facility was not fully operational at the time of the final report, but was on schedule to be commissioned by July 2013 and fully operational by October 2013. Further investigation by this reviewer has found that the building is open and operational as scheduled.

The scope of the project grew dramatically with the awarding of $14.8M by the NIH for Facility Construction and the expenditure of ~$7M of Pennsylvania State University funds. The majority of Health Research Grant funds were used to purchase animal cages.

Changes were all related to a major increase in the scope of the project, but no objectives were abandoned.

The project met all of its stated objectives and was completed satisfactorily on time. Further, funds were obtained from the NIH (ARRA) = $14,800,000 and internal university funds = $7,000,000 with total co-funding = $21,800,000, as compared to health research grant funds used of $311,524.

Reviewer 2:
The stated goal of the project - to design and build a BSL-3 laboratory- has been met. Indeed, the applicants took advantage of ARRA funding to expand the scope of their initial design and should be commended on their efforts.

No weaknesses noted.

Reviewer 3:
The project met its objectives broadly in the sense that the project funding ($311,524) was spent for equipping the ABSL-3 biocontainment facility of the university; this done by purchasing the Individualized Ventillator Cage (IVC) cage systems for rodents, and meeting the FTE in part (<1%) of the PI. However, the original strategic plan was more broadly stated and did not specify these expense items.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
It was not possible to perform BSL-3 research at Pennsylvania State University prior to the construction of this facility. The ABSL-3 facility was a key missing resource, and now that it is available will enable the development of a multi-disciplinary infectious disease research program not previously possible.

The project contributes essential infrastructure required for research on BSL3 level pathogens. Vaccines and novel therapies are needed for many infectious disease agents of this class.

The project is complete until further expansion or renovation of the animal facilities is required. The research to be done will be a myriad of peer-reviewed projects designed and performed by research scientists and clinicians housing their research animals in the facility.

This project has met the rare standard of attracting major funding from multiple sources, being completed on time, and meeting all objectives. The building of this facility will have a huge impact on infectious disease research at Penn State University for a generation. The funding invested in infrastructure improvements will be returned many fold through successful completion of extramurally funded research projects which will be conducted there.

Reviewer 2:
Strength: The impact will be huge for Penn State; this is a transformational facility.

Weakness: The Penn State researchers have benefited from using off site BSL facilities prior to the completion of the new facility. Some mechanisms to return the favor, i.e., a program for non-PSU investigators to utilize the facility would be appropriate.

Reviewer 3:
The project has a clear beneficial impact in that the ABSL-3 related Research Infrastructure build using in part this project funding will enable Penn State's BSL3 research in infectious diseases. This will also favorably impact the competitiveness of the Penn State researchers to earn grants involving animal research on BSL3-level microbial agents.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Yes, large amounts of funding were obtained from two other sources for this project.

If leveraging of funds were expected, did these materialize? The funds were expended from all

sources and the project was completed on time.

Are the researchers planning to apply for additional funding in the future to continue or expand the research? Not at this time. The project is complete until further expansion or renovation of the animal facilities is required.

The need for the project was clearly established and the project itself was well planned, which greatly aided in attracting funding to build this facility from both Pennsylvania State University and the NIH in addition to Health Research Grant funds.

Reviewer 2:
Yes, the PI did a fantastic job leveraging ARRA funds to expand the facility.

Reviewer 3:
The overall BSL3 construction project did leverage funds from other sources including the NIH, but these funds were independently awarded and did not result from this formula grant. Nonetheless, the user group investigators are planning to submit new grants involving the use of this BSL3 facility in the future.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
This criterion is not directly applicable to this infrastructure project.

Reviewer 2:
No, but the facility is just now completed. I have no doubt that the academic productivity will follow.

Reviewer 3:
None. There are no publications, licenses, patents, or commercial products emanating from this grant because of the nature of this project, which is infrastructure building rather than funding a particular research plan.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The Biological Research Laboratory is an Animal Biosafety Level 3 (ABSL-3) facility which will be used for infectious disease research. It will greatly enhance the research capabilities on the Penn State campus.

A facility manager from Battelle Research Labs was hired, and Girish Kirimanjeswara, MVSc, Ph.D., was hired as the scientific director for the facility. It must be understood that such a new modern facility will be critical for recruiting new research faculty for many years to come.

The completed ABSL3 facility will contribute to an expanded number of research grants and publications from Penn State University in the field of infectious disease research for decades to come.

Reviewer 2:
Resoundingly, yes. This is a first-class facility that was not available prior to the current project.

Reviewer 3:
Yes, the project enhanced the capacity for research at Penn State by contributing in part to the building of infrastructure for an ABSL3 biocontainment facility to be used by the ID investigators institution-wide.
The project did not directly add new researchers, though new investigators and a facility manager were added for the ABSL3 facility with funding from other sources.

No pre- or post-doctoral students were paid from this project funding.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Yes, the project was funded in large part from NIH (ARRA) funds.

The importance of the project is highlighted by NIH providing nearly $15M to build the facility. It is clearly viewed as a national research facility resource.

Reviewer 2:
No, and as indicated above, this is an area of weakness. Some mechanism for collaboration outside PSU should be established. These BSL3 facilities are precious resources that should be shared, if possible, with outside researchers.

Reviewer 3:
None.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:
None.

Reviewer 2:
Develop an outreach system for external investigators to gain appropriate access to BSL3 facility.

Reviewer 3:
The strategic plan should have more unambiguously stated the specific activities performed under this formula grant funding.

ADDITIONAL COMMENTS

Reviewer 3:
The project funding supported in part the building/equipping of an ABSL-3 biocontainment facility at Penn State which is critical to conduct research on BSL3 level microbial agents.

The funds were used mainly to purchase IVC cage systems for the facility which are critical for conducting animal experiments in this facility in a safe and contained manner.

The ABSL3 facility has been a much-needed infrastructure at Penn State considering that several researchers are engaged in research in the area of infectious diseases and immunology/pathogenomics and will be directly benefited by becoming more competitive in obtaining grants from the national funding agencies.

One minor weakness noted was that the strategic plan was open-ended and did not quite specify the specific nature of expenses incurred under this project.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The project better defined the role of PRAS40 in protein synthesis and associated signaling in C2C12 cells but did not test the hypothesis that alcohol affects protein synthesis.

PRAS40 was mentioned in the aims as a read-out measure, but not the primary target of the proposed studies.

Studies were not completed as outlined, and no explanation of deviation is provided.

Reviewer 2:
This project intended to identify the mechanisms that regulate skeletal muscle protein synthesis impairments due to chronic alcohol ingestion. Through *in vivo* and *in vitro* models, the research objectives were: (1) to identify the role of 4E-BP1 and S6K1 interactions with raptor on mTOR-dependent signaling in alcoholic muscle; (2) to identify the effects of chronic alcohol ingestion on the eIF3 scaffolding complex, a central component to protein initiation; and (3) to quantify the subsequent effects of chronic alcohol ingestion on PDCD4:eIF4A in determining protein translation and synthesis. Further, the influence of leucine, a BCAA implicated in protein synthesis, will be used to combat alcohol-induced defects.

The experiments have been correctly designed and performed in order to answer these research questions. Significant strides have been made towards the stated objectives; however, it does not appear that experiments using leucine have been performed to date.

In addition, the strategic research plan does not describe the potential role of Proline Rich Akt Substrate 40kD (PRAS40). While exploring the impact of PRAS40 was not explained, PRAS40 is a phospho-substrate associated with mTOR-Akt signaling and is related to the central theme of the project (i.e., identifying mechanisms the drive decreased protein synthesis in alcoholic muscle). Moreover, investigating the role of PRAS40 in alcoholic myopathy was novel.

Unfortunately, the role of PRAS40 appears to play a small, inconsequential role (if any) in the development of alcoholic myopathy.

Reviewer 3:
The project did not meet, or appear to examine, any of the stated objectives/aims. Late in the final report the "goal of better defining the role of PRAS40 in alcohol-induced myopathy" is stated. This goal appears to have been largely accomplished although in the absence of alcohol.

Weakness: 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. It appears this Pennsylvania Department of Health (PDOH) funding was used to supplement (funds for 1 Ph.D. student) a larger NIH study which had funding reduced. This study might have investigated these aims but such results are not included. Obviously, the aims laid out in the proposal could not be accomplished with 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.

Strength: Clearly the funding of a Ph.D. student is important and if that was the objective of this specific funding, then that objective was met. The findings are potentially important as they have increased the knowledge of the regulation of the AKT-mTor pathway.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The funded research accomplished the goal of better defining the role of PRAS40 in alcohol-induced myopathy. However, based on methods and results, it is not clear this hypothesis was tested.

The beneficial impact is not clear.

Reviewer 2:
While the development of alcoholic myopathy is likely multi-factorial, this project focused on mechanisms that effect skeletal muscle protein initiation, elongation, and translation. Results from this project may serve as solid, background information for future work; however, it does not appear that significant benefits to the human condition are immediate. For example, the role of PRAS40 in myogenesis was underwhelming and limited to proliferating myoblasts in culture. As such, future work should be dedicated to identifying the role of PRAS40 on myoblast size. Nevertheless, the work required to describe PRAS40 was considerable, the results are solid and properly evaluated, and the resultant publication should be of significant interest to the greater alcohol research community. As such, this reviewer believes the budgeted dollars were well-spent.

No major discoveries, new drugs, or approaches are attributable to this project.

The stated future of this research project "to better elucidate the potential role of PRAS40" will not continue as the protein does not alter muscle protein synthesis as anticipated. Nevertheless,

the training of the pre-doctoral student will benefit the future work in the Lang Lab. For example, these newly-acquired skills will be applied toward understanding the roles of other mTOR complex 1 proteins in regulating muscle protein synthesis.

Reviewer 3:
Strengths: This project funded a graduate student for 1 year and thus had beneficial impact on future research in this area. The findings included were used to bolster a NIH resubmission and thus likely had a large economic impact on the PI's university. It is difficult to ascertain how important the current findings were in obtaining funding from the NIH. Considering the relative low cost ~$45,000, the benefits (funded a Ph.D. student and bolstered NIH grant) appear to be of high impact. The project did not produce/report any major discoveries regarding the overall goal, but it did determine that PRAS40 is likely not a useful therapeutic target. This is important information as it can help limit further resources being spent on interventions targeted at PRAS40.

Weakness: This project only marginally progressed science towards answering the main question put forth by this project regarding alcohol-induced myopathy. If the overall aim (Experiment 1a-c) had been achieved the project could have substantially moved this field forward.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
This grant supported a graduate student to conduct studies that strengthened the resubmission of a competing renewal application to the NIH.

A new RO1 was funded and subsequently converted to a MERIT Award.

As stated by the PI, "The PRAS40 work in general will not continue because the results of research indicate that this protein does not alter muscle protein synthesis in the anticipated manner and therefore is unlikely to be a potential therapeutic target to combat muscle wasting."

Reviewer 2:
The graduate student's work was used, in part, to strengthen a renewal application to the NIH. This grant has subsequently been funded as an R37 MERIT Award. However, it appears the lab will no longer pursue the role of PRAS40 as a critical regulator of myopathy in alcohol models.

Reviewer 3:
Strength: The current project leveraged substantial funds (~1:8 ratio of PDOH to NIH funds) from the NIH. Furthermore, the findings from this project, at least in part, aided in the procurement of additional funding from the NIH.

Weakness: None.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
There is one published paper and one in preparation. Based on the funding amount, this productivity is appropriate.

Reviewer 2:
One publication was published in Molecular Medicine, titled "PRAS40 regulates protein synthesis and cell cycle in C2C12 myoblasts" (Mol Med 16: 359-371, 2010). The most recently published impact factor for Molecular Medicine is 4.469. This is a high-impact journal and visibility of this paper should be high.

In addition, a second paper was written and should be (or has been based on the timing of this review) submitted for review.

Reviewer 3:
Strength: 1 peer-review publication in a good journal (molecular Medicine); ISI impact factor 4.469. Additional papers are expected. One publication was expected in the initial proposal, and thus this meets the expectation but does not exceed it.

Weakness: A minor weakness is that the expected 2 conference presentations did not appear to materialize. It is possible/likely that this information was simply just omitted from the final report.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The project helped to fund a student when the budget in the NIH grant was cut. This helped the PI not cut the budget for research. This benefitted the PI, but not necessarily the institution, which is appropriate based on the amount of funding.

Reviewer 2:
No equipment was purchased with the allotted funds. The vast majority of these funds were used to support a pre-doctoral student, Abid Kazi.

Reviewer 3:
Strength: The grant allowed the PI to fund a PhD student and thus enhance the quality and capacity of research at the grantee's institution.

Weakness: None.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
No.

Reviewer 2:
No new collaborations with the greater research community have been established.

Reviewer 3:
Strength: None. No collaboration with partners outside of the institution appears to have occurred or to be planned as it relates to this project.

Weakness: Lack of community involvement, although that would be difficult to do given the research questions and strategy.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:
The central hypothesis was not tested.

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.

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 is intriguing.

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.

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.

ADDITIONAL COMMENTS

Reviewer 1:
The primary objective was to assess the effects of alcohol on protein synthesis and pathways associated with protein synthesis. What was presented was a characterization of the role of PRAS40 on protein synthesis and signaling events in C2C12 cells. The stated hypothesis was not tested.

Reviewer 3:
The objectives and hypotheses of this project were based on solid pieces of evidence in the literature and from pilot data by the Lang group. Unfortunately, the role of PRAS40 in the development of alcoholic myopathy was negligible and this line of research has been discontinued. Nevertheless, the key strength of this work was that a pre-doc student was sufficiently trained, an NIH grant was procured with help from this work, and re-focusing of the lab's overall goal (identification of mechanisms that drive decreased protein synthesis in alcoholic muscle) has occured.

Weakness: However, it is unfortunate that the leucine experiments have (apparently) not been performed. Further, the mechanisms that increase cell size in PRAS40 knockdown experiments do warrant further exploration, although the reviewer appreciates that these experiments are not the true focus of the lab.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The investigators did an excellent job in performing the proposed project during the short funding period. As outlined in the original proposal, mouse-specific induced pluripotent stem cells were produced during the first phase. The great majority of the funds received from CURE went towards the purchase of a state-of-the-art multi-color flow cytometer, leaving not enough resources to fully accomplish the in vivo studies proposed for the second phase. Even as is, the investigators made excellent progress and were highly productive. As expected from this experienced laboratory, there were no deficiencies regarding research design and methods.

Reviewer 2:
This hypothesis-driven project met the stated objectives within the framework of available funds. The objective of Aim 1 was straightforward, clearly presented, well-designed, and successful. Adult mouse fibroblasts were used to produce putative iPSC clones that exhibited both structural and functional characteristics of mouse embryonic stem cells (ESCs). The methods used for cell characterization were appropriate for the study and the data strongly supported the stated objectives and conclusions for this aim. The two-part hypothesis tested in Aim 2 involved: A) iPS cell differentiation to mesenchymal stem cells (MSCs) and; B) subsequent differentiation into osteoblasts and chondrocytes in vitro and bone and cartilage in vivo. The differentiation studies described in Aim 2A were successful in producing putative MSCs as judged by changes in cell morphology and the expression of several surface antigens that are characteristic of this cell type. These data strongly support the hypothesis stated in Aim 2A. Aim 2B was not fully completed due to limited funds (i.e., $30K direct costs). The completed in vitro studies suggested that MSCs are capable of calcium deposition and the expression of genes that are characteristic of the osteoblast lineage. These results supported the stated hypothesis to the extent that in vitro studies allow. However, the chondrocyte differentiation studies were unsuccessful. In vivo studies of bone and cartilage formation were not conducted. This is not surprising given the costs associated with animal studies and does not diminish the largely positive achievements of the overall project. In related project-supported studies, the PI's laboratory demonstrated that adult MSCs regulated cell migration and differentiation. In summary, these results met the majority of the stated project objectives.

Reviewer 3:
The central hypothesis of this project is that iPS cells can be directed to MSC differentiation and will give rise to osteoblasts, chondrocytes and adipocytes and will make bone in vivo. To test this hypothesis, the researchers have pursued the following aims: Aim 1, to generate and characterize mouse iPS from tail tip fibroblasts; Aim 2, to test the hypothesis that iPS cells can be differentiated into MSCs and the cells will form bone and cartilage in vivo.

The proposed experiments and outcomes from Aim 1 met the stated objectives; although, there was a shortage of originality or novelty in research designs and methods since the methodology how to generate iPS cells had been well established in Shinya Yamanaka's Nobel-winning paper published in Cell. Preliminary data demonstrated that the researchers are able to generate iPS cells from the tails of mice and cells from iPS that exhibit mesenchymal stem cell (MSCs) characteristics in terms of surface antigen expression.

The data and information provided were applicable to the portion of the project objectives listed in the strategic research plans in Aim 2. Some of research designs and methods in Aim 2 are novel. The data developed sufficiently to answer the research questions posed in Aim 2 regarding whether iPS cells can be differentiated into MSCs that show osteoblast differentiation in vitro. However, in Aim 2, the researchers planned to evaluate the iPS cells for chondrocyte differentiation in vitro and for bone and cartilage formation in vivo when the cells are incorporated in scaffolds and implanted into the mice from which the reprogrammed fibroblasts were harvested. The researchers showed that they had proposed to direct iPSC derived MSCs into cartilage cells, but were not successful in directing the cells to cartilage formation. No data or information in the progress reports or PI's publications address bone formation in vivo. Have researchers done any experiments ordo they have any ongoing experiments on those objectives? If both experiments of bone and cartilage formation in vivo are not successful, it may be doubtful that the MSCs generated in Aim 1 from iPS cells are real MSCs.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
This project falls under the high-risk high-reward category, and the data presented here show the potential that iPS holds for being used in musculo-skeletal repair and regeneration strategies. Thus, this project has significant clinical relevance and may ultimately change the way musculo-skeletal pathologies are treated.

Reviewer 2:
The reprogramming of adult cells to pluripotency with subsequent lineage-specific directed differentiation, holds tremendous potential for improving human health. These induced pluripotent stem (iPS) cells are promising candidates for regenerative medicine-related therapies and as platforms for drug discovery. Moreover, this approach bypasses the ethical concerns associated with the use of human embryonic stem cells for these therapeutic applications. In order to realize this therapeutic potential, fundamental studies regarding the conditions for

generating and differentiating iPS cells must be conducted. This project begins that process in mouse with a view toward future applications in human musculosketetal tissue repair and regeneration. This work is important because it defines the conditions by which iPS cells are produced and differentiated to MSCs and osteoblasts that express cell-type specific markers and genes. The PI proposes to capitalize on these discoveries by enriching a subpopulation of iPS cells that express a specific surface marker that is also found on human embryonic stem cells – cells that can be induced to differentiate into bone and cartilage cells.

Reviewer 3:
With aging, the yields of adult-derived stem cells decrease and studies have shown that the cells may exhibit reduced proliferation and differentiation potential. The goals of this project are to assess the potential of induced pluripotent stem cells (iPS) to differentiate toward mesenchymal stem cells (MSCs) and to make bone and cartilage in vivo, thus to understand the future application of the cells for the repair and regeneration of musculoskeletal tissues.

The research design in Aim 2 to reprogram fibroblasts harvested from the tail tips of mice, and then maintain the mice in which the iPS cells come from to later use the mice as cell recipients to assess bone and cartilage formation in vivo is novel. Unfortunately, they have no data on this novel designed experiment. If they have, it will offer an opportunity for the researchers and others to study stem cell function in vivo without using the expensive SCID mice.

The researchers have future plans for this research project in htat that they will submit a R01 application to the NIH based on the preliminary data from this project.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Dr. Niyibizi was successful in securing NIH funding and the promising data from CURE funding will be the basis for additional grants submitted to the NIH (RO1).

Reviewer 2:
The funds provided to the PI through this project led to the acquisition of NIH funds for the continuation of this work. Another NIH application to further this project is currently under review. Obtaining these additional highly-competitive funds underscores the importance of the work conducted with the support of this project.

Reviewer 3:
This project results in additional funding from the NIH (R21) and the PI of this project is planning to apply a R01 from the NIH.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The publication output from this grant with two manuscripts published by excellent journals is outstanding, particularly when considering the short funding period and that most funds were spent on an instrument.

Reviewer 2:
The project resulted in two peer-reviewed publications. One manuscript was published in 2009 in J. Cellular Biochem. This paper describes the bulk of the work from this project. A second manuscript was published in 2012 in BBRC and deals with related studies that were in part supported by project funds. No additional manuscripts nor commercial development opportunities are anticipated. The publication of two manuscripts in respectable, if not high-profile, journals is a sound return on this research investment.

Reviewer 3:
This project resulted in three abstracts in national meetings and two peer-reviewed publications in J. Cell. Biochem. and Biochemical and Biophysical Research Communications (BBRC). The impact factors of the journals are low. In addition, the paper published in BBRC is not directly related to the project objectives.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
A new multi-color flow cytometer with unique features was purchased and integrated with the Penn State research enterprise. That this new instrument has already been used for the analysis of about 20,000 samples from 20 laboratories demonstrates its high impact on the scientific community through infrastructure enhancement. The remaining funds were primarily used to support one undergraduate student and one post-doc, aiding the training mission at Penn State.

Reviewer 2:
A portion of the project funds was used to purchase a flow cytometer for the proposed and completed studies. This equipment has also been used by more than 20 Penn State investigators in 8 departments on campus to further their research efforts. Project funds were also used to train a post-doctoral fellow and to support a laboratory technician and a work study student in the PI's laboratory.

Reviewer 3:
The funds were used to pay for research performed by a post-doctoral student and supported one undergraduate student. The grant funding helped in acquisition of a new multicolor LSRII FACS

analysis instrument used for analyses in this work. Over 20 different basic and clinical research laboratories from across Penn State Hershey have been regular users of this machine, analyzing a total of close to 20,000 samples last year alone, demonstrating its broad applicability to multiple disciplines and diseases. There was no evidence in adding new researchers to the institution from this project.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
A collaborative effort with engineers at Penn State University Park is described involving the generation of cell culture scaffolds.

Reviewer 2:
The funded research led to collaborations with Penn State University Park engineers who are designing cell and tissue scaffolds.

Reviewer 3:
This project led to a collaboration with an engineer at Penn State University Park.

Section B. Recommendations

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.

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.

Project Number: 0864509
Project Title: Evaluation of mTOR as a Chemoprevention Target in Skin Cancer
Investigator: Shantz, Lisa

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The project met its stated objectives and resulted in a publication and the funding of an NIH R03 grant. The research design and methods were indeed adequate in light of the project objectives.

The PI's hypotheses on the potential role of mTorc1/2 as a mediator of the effects of UV on keratinocyte proliferation were well-tested and stood up to rigorous evaluation. Some additional experiments were added. The added experiments were entirely reasonable, well-justified, and explained and added to the strengths of the proposal.

The PI presented a series of panels in the final report that nicely documented the successful completion of the proposed experiments.

Overall, the PI of this proposal performed an interesting and informative series of experiments on the role of the mTorc1/2 complex on the response of keratinocytes, either in culture or in mice, to UV irradiation. The experiments were based on a solid and readily-testable hypothesis, high-quality data were generated by the various experiments presented. A strong case can be made for the importance of mTorc1/2 for the response of keratinocytes to UV irradiation.

Reviewer 2:
The project had two well-defined objectives as suggested by the reviewers of the funded NIH R03 grant. The project met the stated objectives partially. A study on the effect of mTOR inhibition by rapamycin on skin carcinogenesis in wild-type mice was proposed. The investigators showed that rapamycin sensitizes keratinocytes in vivo to UVB-induced apoptosis and inhibits UV-induced proliferation. The investigators generated the conditional mTOR-null mice lacking mTOR in the epidermis and showed that mTOR deletion blocks UVB-induced proliferation of the epidermis. However, no data on carcinogenesis has yet been obtained.

The research design and methods proposed are appropriate for the stated objectives.

The data were developed in line with two specific aims proposed. However, although the data obtained allow some preliminary conclusions on the role of mTOR in skin carcinogenesis, they are not yet sufficiently developed to fully answer the research questions.

No specific changes were made to the protocol, while a few additional experiments have been performed, for example, studies on Rictor-null fibroblasts.

Data provided indicate that the project made acceptable progress. The data and the information provided are directly applicable and very nicely aligned to the project objectives.

Reviewer 3:
This research proposal focused on the impact of mTOR as a potential target for chemoprevention of nonmelanoma skin cancer. The experiments in this one-year grant aimed to generate targeted deletion of mTOR in the skin of mice and to use this model to test the central hypothesis of the proposal that mTOR was important in skin cancer development and could thus serve as a therapeutic target. To this end, the research design did meet the expectations of the proposal objectives. The investigator set a clear path of genetically evaluating a complex pathway in skin carcinogenesis and the data generated answered the central question being posed. For a one-year grant, the data generated were of sufficient quantity and quality to show that indeed mTOR activity was required for tumorigenesis in the skin. The investigator also supplemented the mTOR knockout data with Rictor knockout cells. Their data clearly indicate that both mTORC1 and mTORC2 mediate distinct pathways in UVB-induced proliferation and survival.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
This project opens the door to a better understanding of the effects of UV light on non-melanoma skin cancer (NMSC) and also the potential utility of mTorc1/2 inhibitors as chemopreventive agents against NMSC.

As interesting as this research is, there is some question as to the likelihood that at-risk individuals would be encouraged to use mTorc1/2 inhibitors in the chemopreventive mode. Even if applied topically, such agents would have to be used over a long period of time as a chemopreventive and concern about on-target toxic side effects would have to be considered. NMSC is the most common form of cancer with over 3.5 million skin cancers in over two million people diagnosed annually. Most of these cancers are cured by surgical resection but a simple, safe and non-toxic chemopreventive strategy would reduce the burden of surgery required for these individuals.

The PI has expanded this project to investigate the role of Raptor and Rictor, which are essential components of mTOR complex 1 (mTORC1) and mTORC2, respectively, in skin carcinogenesis. This is the subject of an R01 application to the NIH/NCI.

Strengths are the high-quality research and a solid research publication (Inhibition of mTOR suppresses UVB-induced keratinocyte proliferation and survival. Carr TD, DiGiovanni J, Lynch CJ, Shantz LM. Cancer Prev Res 2012 5: 1394-404. PMID: 23129577).

Weaknesses include the low likelihood that mTorc1/2 inhibitors will gain traction/acceptance as chemoprevention agents in skin cancer.

Reviewer 2:
If confirmed, the objectives of this project could offer strategies to prevent UV-induced skin cancer, which is a significant health care burden.

The research completed implicates a role for mTOR signaling in the proliferative and apoptotic responses of mouse epidermal keratinocytes to UVB exposure. Although it is premature to extrapolate to these to actual skin cancer development in either mice or humans, the data have some limited value towards eventual skin cancer prevention.

The research being conducted may have impact on search for mTOR-based preventive strategies.

The completed research could contribute to new approaches for mTOR pathway-based prevention of non-melanoma skin cancers.

While it can be assumed that the future plans for this project will be to identify/develop methods to modify mTOR signaling by agents topically applied to the skin to prevent UV-induced skin cancer development, no such statement or any other statement on the future plans for this project was articulated.

Reviewer 3:
The significance of these findings is extremely high. While it was a fairly simple hypothesis, the data generated through the use of the genetic models are very convincing and point to a potential role of mTOR antagonists in a clinical setting of nonmelanoma skin cancer treatment.

Considering the small budget and single year of the project, the results are outstanding. mTOR antagonists already exist, so there was no need to investigate or to discover new inhibitors for clinical or pre-clinical use. This work will stimulate the use of mTOR inhibitors in the setting of skin cancer prevention and treatments. This project could be leveraged into a Phase I clinical trial using mTOR inhibitors or into an NIH R01 application aimed at understanding the downstream components of the pathway that are required for UVB-induced tumorigenesis in the skin.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
An R03 was awarded and an R01 is in development.

The award of an R03 is seen as a major strength.

Reviewer 2:
This project had the unique strength of leveraging the funds from an NIH R03 grant mechanism.

The researchers have used the preliminary data from these studies to apply for an R21, which was funded. An R01 application was submitted and reviewed. An amended application was planned for July 2013.

Overall, this effort to generate additional funding to continue and expand the research is highly commendable.

Reviewer 3:
No additional funds were leveraged during this project. The applicant has applied for long-term NIH funding under the R01 mechanism, but this project has not been funded according to the investigator and the NIH eReporter website. This is a major weakness.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
There was one publication: Inhibition of mTOR suppresses UVB-induced keratinocyte proliferation and survival. Carr TD, DiGiovanni J, Lynch CJ, Shantz LM. Cancer Prev Res 2012 5: 1394-404. PMID: 23129577.

The publication of the solid paper in Cancer Prevention Research is a strength.

Reviewer 2:
A peer-reviewed publication with the data from this project has been published in Cancer Prevention Research. This is a comprehensive publication in a well-respected journal in the field of cancer prevention research.

One additional publication is expected. No licenses or patents or any commercial development are planned.

Reviewer 3:
A single paper was published in June 2012 in the mid-tier specialized journal, Cancer Prevention Research. For a one-year project, this publication quantity is sufficient. It is a weakness that the quality of the journal was not higher. There were no patents or commercial developments from this project.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
A new cage washer for the vivarium was acquired and all researchers using that facility have benefitted from this system.

Two pre-docs were supported.

The provision of the new cage washer is a major benefit for the institution. The support of two graduate students is also a strength.

Reviewer 2:
Funding from this project contributed to the acquisition of an automatic stainless steel cage washer and dryer for mouse cages. This is a significant improvement to the infrastructure.

No new investigators were added.

Funds were used to pay for the research performed by two pre-doctoral students. One of the students listed is the primary first author of the published paper. This is clearly a strength in that the student's contribution to this project has directly resulted in a peer-reviewed publication.

Reviewer 3:
The application did result in the purchase of a LYNX model tunnel cage washer, dryer and bedding dispenser that will certainlybe an asset. Two pre-doctoral students were involved in the work.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
There were no research collaborations specified.

Reviewer 2:
No collaborations are planned. Although this may be appropriate, given that the research team has used the expertise of Dr. DiGiovanni for the published research, collaboration with other researchers may be beneficial.

Reviewer 3:
None listed.

Section B. Recommendations

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.

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.

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.

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.

ADDITIONAL COMMENTS

Reviewer 3:
Strengths: The project achieved its goal of identifying whether mTOR was involved in nonmelanoma skin cancer development. This led to a paper in Cancer Prevention research. The grant also included two pre-doctoral students and the purchase of a LYNX cage station for the institute.

Weaknesses: The paper was published in a specialized mid-tier journal with a lower impact than this work would seem to justify. Additionally, no long-term funding has been acquired.
Improvements: Attain long-term funding and continue more in depth studies of mTOR downstream activities involved in this tumor type.

Generic Recommendations for The Pennsylvania State Unviersity

Reviewer 2:
The research team, including the pre-doctoral students, is well positioned to generate and sustain research funding for the project. Any and all institutional support including encouraging collaborations could go a long way in bringing long term research rewards.

Project Number: 0864510
Project Title: IRES-mediated Synthesis of Proteins Integral to Adaptation to Hyperoxia
Investigator: Shenberger, Jeffrey

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The investigators aimed at determining if hyperoxia-specific effects on mode of protein translation could explain disparities of protein synthesis (identity, amount).

Strengths: The PI sought a different set of cell lines in order to address the difficulty with transfection and identified a construct that would respond with oxygen-specific alterations in translation that could later be used to adjust experimental conditions to further test the stated hypothesis.

Weaknesses: The investigator chose transformed cell lines and a degree of hyperoxia that would not mimic the redox stress likely faced by alveolar epithelium. For cells that do not "see" hyperoxia at air-liquid interface (e.g., HEK cells, HeLa cells), the degree of redox stress generated by the exposure conditions used is unrealistic, and could not adequately test the hypothesis. As an alternative approach, the investigator should evaluate changes in native translation. Instead, they confined their studies to changes in translation detected using reporter constructs. A proteomic approach, rather than a molecular approach, might have tested the concept more directly, providing the hyperoxia conditions more faithfully reproduced those likely to be experienced *in vivo*.

Reviewer 2:
The stated objectives of this project were to determine if exposure of human lung cells to high concentrations of oxygen alters the processes responsible for synthesis of specific proteins. The project's overall goal was to understand how high ambient oxygen concentrations increase the synthesis of some proteins while decreasing the synthesis of others.

The original specific aim of this grant was to characterize changes in the relative translational efficiency of cap-dependent and IRES-mediated translation of GADD45α and p53 mRNAs in human lung epithelial A549 cells. The working hypothesis was that hyperoxia would enhance the translation of GADD45α and p53 mRNA into protein through a relative increase in IRES activity. This clear and rational hypothesis was a strength of the proposal. Due to inefficiencies in transfection and stability of luciferase activities, the objectives and aims were modified to include HIF1α, VEGF, and ultimately BiP as targets, and HEK293 and HeLa cells were used in

place of the A549 cell line. Explanations for these modifications were largely appropriate, although no reason was given for the lack of investigation of GADD45α. The initial focus on these ultimately unproductive avenues reduced the time and resources available for more appropriate experiments.

Results of these studies indicated that cap-independent IRES activity of these constructs could be suppressed by hyperoxia, in contrast to the original specific hypothesis. Thus, the data were of sufficient depth to answer the research question as posed, and were appropriately modified to pursue the modified aims. By identifying appropriate cell lines, constructs and experimental timecourse and conditions for future work, these studies made acceptable progress that was applicable to the project objectives.

Reviewer 3:
Strength: This project is to determine whether hyperoxia results in changes of protein expression in human respiratory epithelial cells by altering cap-dependent and cap-independent mRNA translation mechanisms for genes GADD45A and p53, which are important in cell cycle and DNA repair. Either positive or negative data will help to understand the molecular mechanisms of hyperoxia-induced changes in gene expression and the related cellular effects.

The experimental design in the original proposal is clear and straightforward.

Weakness: Although reasonable progress was made toward achieving the original goal, the objectives were not completely met. In particular, experiments to study cap-independent translation of GADD45A in response to hyperoxia were not executed. In addition, the proposed BEAS-2B cells (airway epithelial cells) were not used even after the PI claimed "unsatisfied" data obtained from lung epithelial cells A549. On the other hand, cell lines (Hela and HEK293) that are not related to respiratory epithelia were alternatively used. Different responses to high concentration of oxygen between these cell lines are expected, and the hyperoxic data from Hela and HEK293 may not be applicable to respiratory epithelia.

The studies were not fully focused on the original hypothesis. There were lack of logical analyses and troubleshooting for the negative experimental data. Instead, the PI kept changing target genes and cell lines in order to fish out some "positive" result without providing convincible scientific justification. Therefore, the PI failed to obtain solid data to answer the research questions raised in original proposal.

There were inconsistencies for experimental conditions and data. For example, in their first progress report, A549 cell transfection efficiency was reported as "70%" while in the final report, it was mentioned as "20-30%." Moreover, the recovery time period between cell transfection and oxygen treatment was originally proposed as 16 hours in the experimental plan. However, in the executed experiments, the recovery time was much shorter (4 to 8 hours), and also varied between different genes and different cells, which make these data difficult to be evaluated and compared.

Studies on hyperoxia-induced changes of endogenous GADD45A and p53 gene transcription and translation were proposed in the original research plan by measuring their mRNAs and proteins,

which were supposed to be the foundation for further dicistronic reporter assay for their 5'-UTRs. However, these critical experiments were never performed.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strengths: Identified a reporting method (BiP/GRP78 expression) that may provide a tool that identifies the experimental conditions needed to generate oxygen-sensitive responses. This could be used to "titrate" cell culture conditions to mimic the conditions needed to generate responses that take place *in vivo*.

Weaknesses: The funds expended were quite limited, ~$48k. The project mainly aimed at identifying hyperoxia-dependent effects on alternative translation pathways for a number of proteins important to cell survival (*e.g*., GADD45a, p53). The number of potential targets tested was small, and there were no reported experimental results that actually showed effects on translation of proteins known to be important to epithelial cell survival or function.

Reviewer 2:
Insight into how specific protein levels are regulated in response to changes in oxygen conditions has important implications for understanding mechanisms of gene expression, with potential value for several disease and developmental conditions. If general principles for how differential translation is regulated during hyperoxia were identified, these could potentially be targeted therapeutically. The results of this particular study will have a modest beneficial impact, mostly in providing useful guidance regarding appropriate conditions for future studies. This impact is in line with the modest budget of the proposal.

Future studies will be directed toward understanding the regulation of the IRES elements in the BiP mRNA characterized here, as well as an FGF2 IRES regulated during oxygen-induced retinopathy.

Reviewer 3:
Strength: The research project in the original proposal has the potential to unveil new molecular mechanisms underlying hyperoxia-induced protein synthesis in respiratory epithelial cells, which may help to understand neonatal respiratory diseases such as bronchopulmonary dysplasia. The fundamental knowledge is also important in improving current oxygen therapy used in neonatal lung diseases.

Weakness: Since the research project was not carried out as originally proposed, the predicted conclusion could not be reached. Therefore, this funded research project could not result in any change in outcome and impact, and new discoveries. The future plan to study hyperoxia-induced protein synthesis of another gene BiP in Hela and HEK293 cells may not have any impact on respiratory diseases.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
According to the investigators' final report, the project did not leverage additional funds, but did provide some preliminary data for an NIH grant application. According to their final report, these funds have not yet yielded additional grant support.

Reviewer 2:
No leveraged funds were obtained. An R21 application is under preparation.

Reviewer 3:
Strength: The proposed project, if fulfilled as proposed, would be helpful to the PI's ongoing applications of NIH R01 grant and future R21 grant.

Weakness: It appeared that the PI was not successful in obtaining NIH R01 funding in the past 2 years. It is uncertain whether the data from BiP study will be useful for the PI's future R21 application that focuses on FGF2 IRES and its regulation during oxygen-induced retinopathy.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
There were no expectations that this project, given its projected scope, would generate any intellectual property, and no peer-reviewed publications were cited in the investigator's final report, although the report suggested some publications are planned.

Reviewer 2:
No publications were submitted. There is a plan to submit the findings in the near future. This may require additional funds to finish the BiP-related studies.

Reviewer 3:
Weakness: There was no publication or patent resulting from this funded project, although future manuscript submission was planned by the PI.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
According to the investigator's reports, there were no improvements in infrastructure, nor did the funds lead to the addition of new investigators or trainees, but the scope of this project was never intended to achieve this.

Reviewer 2:
One research associate (Zhang) received training through this project.

Reviewer 3:
Weakness: The funded project had no impact on the research capacity and quality of the PI's institute. There was no addition of new investigators or researchers to the PI's institute as a result of this funding. No pre- or post-doctoral students were involved in this project.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
According to the investigator's final report, there were no additional, outside collaborations that were developed, but this would not have been expected given the limited scope of this project.

Reviewer 2:
This project did not involve collaborating investigators. No planned collaborations are described.

Reviewer 3:
Weakness: No extramural collaboration were planned and developed from this funded project.

Section B. Recommendations

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.

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

Reviewer 2:

1. Failure to significantly pursue the described objectives: the study of GADD45α 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 GADD45α 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.

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

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

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.

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.

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.

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.

5. Data from the dicistronic reporter assay for p53 in A549 may just suggest no changes in IRES-mediated p53 translation upon O_2 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_2 exposure.

Generic Recommendations for Penn State University

Reviewer 2:
These proposals would be improved by requiring additional justification, rationale, and preliminary data for specific proposed experimental protocols.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The project met the stated objectives. While there were problems with the animal model which prevented the investigators from getting definitive answers to the questions they were asking, nevertheless experiments were conducted as proposed. The demonstration that the db/db model is not a good model is an important finding**.**

Reviewer 2:
The objectives of this project (prevention and treatment of stroke in type 2 diabetes) were scientifically important and clinically relevant. Unfortunately, the project did not meet its stated objectives. The first mouse model (ob/ob) exhibited striking variability in stroke damage. The next model chosen by the investigators (db/db mouse) was overly sensitive to cardiotoxic effects of the TZD drug. In addition, db/db mice were extremely insensitive to the glucose lowering effects of metformin and glyburide. Therefore, the investigators were unable to proceed to test their aims, namely to explore the effects of glucose reducing drugs on prevention and treatment of stroke damage. The data gathered in this series of studies was useful in excluding these animal models from their future studies of stroke, and the investigators will now use the NON mouse model of type 2 diabetes.

Reviewer 3:
The project did not completely meet the stated objectives. In the beginning of the project, the investigators encountered a significant problem with low response of the ob/ob mice to the hypoxia/ischemic insult. Then they decided to revert to the db/db mouse model to carry out the proposed experiments. However, this experiment turned out to be a failure because 40% of the animals receiving the darglitazone did not survive the hypoxic insults. In addition, no effects of metformin or glyburide on glycemia were observed in the animals. The investigators stated in the final report that "The outcome of these experiments was very disappointing and frustrating for all concerned." Currently, the investigators are in the process of characterizing a new mouse model for type II diabetes (the NONcNZO10/LtJ mice) to conduct the proposed experiments.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The investigators did an outstanding job in thoroughly looking at the negative and highly variable results they got. They will follow up with a different animal model to answer the questions they originally sought. However, these negative results should be published so that other investigators can benefit from their experience. This would have a great impact because it will prevent precious research dollars to be spent on this model in stroke studies.

Reviewer 2:
The current project, though unsuccessful in getting data on stroke, did provide useful information on the choice of animal models for future studies of stroke in type 2 diabetes. The future plans using the NON mouse model are credible and should yield very interesting results.

Reviewer 3:
Because the animal models that were proposed for the experiments did not work, there is little direct beneficial effects toward improving health outcomes. However, the study "has demonstrated the limitations of both the *ob/ob* and *db/db* as models for type II diabetes."

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Investigators already got an ADA grant.

Reviewer 2:
Leveraging of funds did occur, with the award of an ADA grant on diabetes and stroke. In addition, data from this study will help the planned renewal of an NIH R01 grant.

Reviewer 3:
The investigators have successfully obtained additional NIH funding for the project using NON mice.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Unfortunately, no publications; it is hard to publish negative data but variability in the db/db models needs to be reported one way or another.

Reviewer 2:
No papers have been published to date, which is understandable given the experimental difficulties with the ob and db models. However, the authors express confidence that the new experiments planned with NON mice with result in publishable data. I agree.

Reviewer 3:
None. The investigators plan to submit a paper for publication in the future.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Students were involved and provided a strong environment for research.

Reviewer 2:
Funds from the project also assisted in the purchase of a LYNX model tunnel cage washer with a stainless steel utility cabinet enclosure, LYNX model automatic cage dryer, LYNX model automatic bedding dispenser, and LYNX model cage and rack washer with a stainless steel utility cabinet enclosure. This will apparently benefit over 60 researchers at Penn State.

Reviewer 3:
It is likely that the project has helped to improve research infrastructure and train young researchers. The funds were used to pay for a graduate assistant.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The project did not result in any collaboration.

Reviewer 2:
No collaborations were established and there was no community involvement.

Reviewer 3:
The project appeared to enhance collaborations across multiple basic science departments.

Section B. Recommendations

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.

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.

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.

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.

Generic Recommendations for Penn State University

Reviewer 3:
The proposed project addresses an important and interesting research topic. Unfortunately, the proposed animal model did not work despite that the investigators made conscientious efforts to perform the experiments. Nonetheless, the results of the failed experiments demonstrated the limitations of both the *ob/ob* and *db/db* as models for type II diabetes. However, a pilot study with animal models would have been informative before the project is initiated. As the next step, the researchers will use a new mouse model for type II diabetes (the NONcNZO10/LtJ mice) to conduct the proposed experiments.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The project generally met its stated objectives. Results from the anesthetized animal recordings were well-described and have been published in a prominent journal. Results from acute and chronic unanesthetized recordings, the second major arc of this project, are described very briefly in this progress report and have not yet been published, making it hard to judge the completeness, quality, or success of this work. Figures 5 and 6 apparently represent some of the data from this project, but they are not clearly described and it is not clear how they fit into the overall research objectives. Some data are presented in the progress report that are not directly related to the stated objectives, but are nevertheless related to the broad research goals of the team.

My overall opinion is that although the PI did not adequately describe all of the key experimental results in this progress report, did not organize it in an optimal manner, and presented a little too much data on unrelated projects, it seems clear from the list of publications (and indications of manuscripts submitted or in preparation) that good, overall progress was made in reaching the stated objectives.

One weakness of the overall project is that the investigators used a single rodent model of Parkinson disease, the 6-OHDA-lesioned hemiparkinsonian rat model. While this model is potentially relevant to the study of PD, there are multiple other rodent models out there (esp genetic models in mice). No single model adequately recreates the human disease. For the overall research program to be more compelling and to strengthen the assertion of relevance to human disease, it would be important in my opinion to reproduce key findings in other animal models of PD.

Reviewer 2:
General Comments: The overall research objective of this multi-year study was to better understand how patterns of neuronal activity are altered by the transplantation of dopaminergic cell grafts in unanaesthetized hemiparkinsonian rats. Specifically, the investigators recorded electroencephalogram (EEG) activity from the cerebral cortex and local field potential (LFP) activity from the subthalamic nucleus (STN) after first inducing hemiparkinsonism in rats by

injections of 6-hydroxydopamine (6OHDA) into the striatum in 5 different treatment groups: Normal, Control, and Transplanted with one of three different strategies. The transplant strategies included: (1) Fetal ventral mesencephalon cells placed in the striatum, (2) Fetal ventral mesencephalon cells placed in the striatum and substantia nigra, and (3) retinal pigmented epithelium (RPE) cells placed in the striatum. The original plan was to record weekly from each rat in each of these groups following treatment, for a 3-month duration under both sleep and waking states, and compare the electrical activity patterns in each of the 5 groups, in addition to performing histological and behavioral analyses (to verify the lesion and recording sites and hemiparkinsonian state). The importance of the proposed work was established based on the need to understand the long-term safety and electrophysiological consequences of cell transplantation therapies for Parkinson's disease, including the potential basis for dyskinesias (uncontrolled abnormal movements). The contrast between the two original cell types proposed for use in the study (fetal ventral mesencephalon vs RPE) was claimed to hold direct translational potential for optimizing symptomatic improvement and electrophysiological normalization in Parkinson's subjects who might undergo transplantation therapy using such cell preparations.

The project met the stated objectives. In the first year, the investigators successfully implemented their model in a cohort of 7 rats, using surgically-lesioned rats obtained from Charles River Laboratories (CRL), which they subsequently placed into their 5 different treatment groups and implanted chronic electrodes for EEG and LFP recording. Verification of the accuracy of the lesion and recording electrode placements was demonstrated, along with behavioral validation of the hemi-parkinsonism itself throughout the recording time. Electrophysiological recordings were completed as planned on this initial cohort and preliminary data analysis performed. The progress report indicated successful generation of EEG and LFP data during both sleep and wakeful states, and also demonstrated clearly the need to separately analyze the spectral waveforms during these two epochs, which led to a split in their subsequent work into comparisons of the treatment effects in anesthetized and awake rats, and publication of a methods paper.

In the subsequent progress reports, the investigators continued to add more animals to their treatment groups, completing the analysis of electrophysiological, behavioral, and histological data from approximately 22-30 rats per year in the two behavioral states, and eventually produced a seminal study published in *Brain* (2011) on the work from the anesthetized groups. In the same time period, the investigators also developed and published a new analytical method termed multiscale autoregressive identification of neuro-electrophysiological systems, which they found to be highly useful on their accumulating data. Efforts to include data on the effects of L-Dopa administration in their hemi-parkinsonian rats were also initiated during this time period and included in the progress reports over the ensuing years.

Overall, several of the major findings from the work were among the first in the field, and specifically addressed the original and modified aims. In addition, with assistance from the work that was completed, two new lines of research were established that adapted their recording and analytical methodologies for studies in the non-human primate hemi-parkinsonian model, and also evaluated electrophysiological and other changes in the rat after treatment with a legume

extract from the seeds of the Mucuna pruriens plant, which has a long-established history of use for treating parkinsonism in Ayurvedic medicine.

The research design was novel, adequately powered, and appropriate to address the project objectives.

The sophisticated data analyses that were performed were clearly able to establish the changes in activity patterns that occur in the hemiparkinsonian rat during sleep and waking states in their 5 different treatment groups.

Only minor changes were made to the research protocol and they were adequately justified based on either the need for more rats or the need to separate rats in two different sleep/wake states. The data obtained were completely in accord with the original design of the research plan, with some modification as noted above.

Overall strengths: The completed research study had several major strengths, including the design, the analytical methods developed, and the painstakingly difficult validation of the model at each level, to ensure proper controls were in place. There are no notable overall weaknesses.

Reviewer 3:

The broad research objectives of this project are to better understand the normal electrical properties of the brain, how they are altered in Parkinson's disease and whether normalizing the aberrant electrical pattern is critical to restore normal behavior. Part of the overall objective is to understand the mechanisms by which cell transplants ameliorate Parkinson's disease symptoms. The specific research aim of this project was to answer the question: Do dopaminergic cell grafts modulate basal ganglia electrophysiology in the anesthetized and unanesthetized hemiparkinsonian rat? Planned sub-questions were to determine if the changes differed in asleep versus awake conditions; if fetal ventral mesencephalic cell grafts cause different changes than retinal pigment epithelium cell grafts, and if there was a difference between striatal-only grafts versus combined striatal-nigral grafts.

Excellent progress has been achieved over the tenure of this grant to address these aims. It is worthy of note that for a large part of the funding period of the TSF grant, work on non-human primates was in hand since the NIH-funded grant NS42402 titled Cell Transplantation in Parkinsonian Monkeys was funded in parallel. This was a strong asset in that it enabled the PI to assess if his interesting findings made in the rodent were conserved in the primate model of PD. The PI's studies in the rat model when combined with insights from the hemiparkinsonian monkeys provided some of the most interesting scientific data and conclusions from this research: i.e., that dyskinesias in PD is not a phenomenon that can be neatly isolated to nigrostriatal pathology but rather that network-wide changes in the cortex and its connections with the basal ganglia are cardinal to the genesis of the dyskinesias.

Thus, it is a shame that research using non-human primates has been discontinued at Penn State University Hershey Medical Center due to closing of the primate facility in the summer of 2011 due to lack of manpower to support its operation. This forced the PI to develop techniques in the 6-OHDA rat that simulate the same techniques used in the primate model.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
This preclinical research work explores some basic pathophysiological mechanisms of Parkinson disease, including the relationship between abnormal electrophysiological activity patterns and outward motor symptoms. The effects of dopaminergic cell grafts on basal ganglia electrophysiology and motor behavior are explored and represent a possible therapeutic strategy in humans. This preclinical work remains in a rather preliminary stage, in my opinion, and it is not clear whether this therapeutic strategy is going to be worth pursuing in humans; nevertheless, the work lays an important foundation for exploring this potential. Certainly, creative, preclinical work of this sort is of critical importance for a common neurodegenerative disease lacking any available disease-modifying treatment options. Therefore, even though the direct benefits of this research to humans is hard to estimate at this stage, it is worthwhile given the importance of the disease that it intends to treat.

Reviewer 2:
The project has potential significance for developing methods that help to characterize the changes in basal ganglia activity and combined cortical-basal ganglia activity that occurs in the parkinsonian state and following transplantation therapy of dopaminergic cells.

The specific data that were developed and acquired are not likely to be as valuable toward direct improvement of health outcomes as the methods that were developed. This is due, in part, to the relatively uncommon use of transplantation therapy at present compared to other invasive FDA-approved methods (i.e., deep brain stimulation) or the potential development of neural stem-cell based therapies. However, there was additional support obtained at several levels for the potential for the use of Mucuna pruriens extract in treating subjects with Parkinson's disease, which could have significant impact for improving health outcomes.

The refinement of the analytical approaches that were developed and validated in rats, and partially applied to the non-human primate model could hold much potential for helping to direct the choice of specific therapies that improve several of these outcome measures.

The potential for using Mucuna pruriens extract in treating subjects with Parkinson's disease could have significant impact for improving health outcomes, and although the idea has existed for millennia, it gained additional support through the work completed in this study. Other applications are more remote from direct applications.

The investigators have indicated their continued interest in exploring the use of Mucuna pruriens in their models due to its effectiveness, high tolerance and lack of any major side effects.

Major strengths: There are some direct, albeit preliminary, benefits of the work that was completed, including both methods development for electrophysiological monitoring in subjects with Parkinson's disease who may have had electrodes implanted for deep brain stimulation, as

well as potential long-term improvements that could be made from a pharmacological treatment perspective.

Minor weaknesses: In addition to the future directions indicated, the investigators may wish to consider how their methodologies could be used to study effects of iPSC-derived or fibroblast-derived pure neuronal populations at different differentiation states. The field has grown exponentially since the submission of the original grant proposal.

Reviewer 3:
The PI's seminal paper in *Brain* provided a unique suggestion for the notion that neuronal plasticity in the brain may be critical for the prevention and amelioration of drug-induced dyskinesias in humans. Clinical trials to test this notion are currently being contemplated.

Although not the primary goal of the research funded in this project, the techniques the PI and his group were able to develop have led to the further characterization and fractionation of *Mucuna pruriens* water extract. Their paper on evaluating the electrophysiological consequences of using Mucuna water extract in the parkinsonian rat has provided compelling data to test Mucuna in PD patients as an anti-dyskinetic agent. This is currently underway.

The PI plans to resubmit his NIH R01 NS42402 as an A1 resubmission after 3 additional publications that were funded in part by NS42402 and a CURE grant are published.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The funded work has resulted in a successful award of a small grant of $50,000 from the Grace Woodward Foundation. Multiple applications to the NIH for related work have not been successful, and one NIH application is still pending review, and other NIH grants are planned or have recently been submitted.

Reviewer 2:
The research completed in this study benefited considerably from the use of materials that were provided, in part, using funds from other sources, including both NIH (NINDS) and other. They clearly plan to continue to leverage such resources in the future to supplement any additional support they receive.

Reviewer 3:
The PI lists a number of submitted grants (4 NIH grants and one non-federal grant) since 2011. At the present time only the non-federal grant to the Grace Woodward Foundation has been funded. One NIH grant titled "Mechanistic Studies of an Ayurvedic Treatment for Parkinson's Disease" is pending review.

The PI plans to resubmit his NIH R01 NS42402 as an A1 resubmission after 3 additional publications that were funded in part by NS42402 and a CURE grant are published.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
This research team has been productive: At least 10 peer-reviewed publications have already been published as a result of the funded project, including a 2011 publication in *Brain*, a highly-cited, prestigious neurology journal; the funded PI was the senior author on this publication. This particular article was noteworthy because it was a proof-of-concept that implantation of dopaminergic cell grafts into the striatum successfully resulted in both improved electrophysiological firing patterns of substantia nigra and subthalamic nucleus, as well as improved performance on relevant behavioral tasks.

The remainder of the articles appear important if more incremental than the *Brain* paper, and are published in low-to-medium-tier journals. Nevertheless, it is notable that the research team is creative in exploring other aspects of Parkinson's disease in their animal models, ranging from the possible therapeutic effects of an Ayurvedic compound to a study of the electrophysiological effects of L-dopa treatment in monkeys. Monkey work had been funded by a complementary mechanism, but had to stop as their primate facility closed.

They mention 5 additional manuscripts that are in various stages of preparation. No licenses or patents have resulted from the funded work.

Reviewer 2:
The researchers produced an acceptable number of high-quality publications stemming from the funded project. The nature of the research methods (chronic long-term recording from rats over an extended period of time) dictates that the publications arising from these studies will not be large in number. Nevertheless, the report in *Brain* and other specialized journals are viewed as potentially high-impact. Additional publications related to the original work that examined the effects of the Mucuna pruriens extract were unfortunately not published in mainstream scientific journals, despite the rigorous nature of the design and methods and strong findings. This is the only weakness seen. No patents or commercial licensing were pursued.

Reviewer 3:
Outstanding track record in publication. This project has led to 10 publications in high-quality, peer-reviewed journals and five additional papers are in revision or preparation.

One of these papers published in *Brain*, a journal with an impact factor >10, showed that cell transplants have a unique and powerful effect on the basal ganglia circuit that had hitherto never been predicted. This study showed that continuous dopamine replacement via a striatal graft evokes neuronal plasticity that appears to affect the local striatum-GP-thalamic pathways more than the direct cortex to basal ganglia pathways.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The bulk of the funds paid for a very productive graduate student who led the main aspects of this research program; he is the lead author on several of the key publications from this program, including a prominent paper in *Brain*. Thus one interpretation is that these funds were instrumental in furthering the career of this promising young investigator who has now completed a doctoral dissertation.

The funds have partly supported another graduate student in the laboratory. The funding provided limited support for another faculty member and two members of the lab staff.
The research funds have supported lines of investigation that had not previously existed at the institution, and led to the establishment of new cross-institutional collaboration.

No specific infrastructure improvements are apparent as a result of this funding.

Reviewer 2:
Much of the funds awarded in this proposal were used to pay for personnel lines of graduate students or research associates whose contributions were essential to the successful completion of the study. With this in mind, after paying for the experiments themselves, the total funding was not viewed as adequate for substantial improvements in institutional infrastructure.

Reviewer 3:
The PI's lab funded through this grant brought optogenetics to Penn State University-Hershey Medical Center (PSUHMC). The PI's lab pioneered these techniques with Dr. Zhou from Penn State (PSU) main campus and for the first time PSU used laser activated *in vivo* experiments in awake behaving rodents. These techniques have been broadly adopted and many investigators have gained experience and advice by visiting the PI's lab and learning techniques from them.

This grant has been a major success in fostering collaboration across campuses and across institutions. Productive collaborations have been established between Electrical Engineering department (EE) and medicine across campus. Several papers have come from this collaboration with multiple members of the EE department including Drs. Lagoa, Monga, and Jenkins and with Dr. Zhiwen Luo leading to adoption of optogenetics in the PI's lab.

This research work also led to an inter-university collaboration with two separate labs at the University of Michigan; Dr. Joshua Burke, a leading expert in recording the brain from awake behaving rats, and Dr. Kennedy.

Funds from this project were used to support the research of 8 undergraduate and 3 pre-doctoral students.

The PI has run a highly successful summer internship program and the Central Pennsylvania

Brain Bee every year under the auspices of his laboratory. This internship program allows both local and nationwide high school, undergraduate and medical students to spend 10 weeks in the summer engaged in neuroscience research. The annual Brain Bee contest is a novel and effective way to gain community involvement in the PI's research.

The greatest success of this internship program came after the CURE funding began in 2009 and the largest class of student interns (9) enrolled in the summer of 2012. The PI's graduate student, Timothy Gilmour, Dr. Venkiteswaran, and Ms. Erin Handly, all supported in part by this grant, were actively involved in organizing the Brain Bee contest in 2010-2012; and in 2012 over 20 high school students participated.

This internship provided local high school students a taste of neuroscience research. In the summer of 2012, 3 high school students from central Pennsylvania who participated in this internship program presented their research at local science fair competitions, with 2 of these students winning first award and placement into state competition, clearly documenting the success of this program.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The project led to successful collaborations with investigators at Stanford and University of Michigan. A collaboration with Dr. Karl Deisseroth is especially exciting, and the team is the first, apparently, at their university to use optogenetics. This technique is very relevant to the goals of the project and the stated objectives.

Reviewer 2:
The researchers have established several important collaborations, in part, through the work funded in this project. These collaborations have allowed them to greatly expand the potential reach of their work into the clinical domains and is seen as a major strength.

Reviewer 3:
Yes, the PI is actively collaborating with Dr. Joshua Burke and Dr. Kennedy at the University of Michigan.

The PI has run a highly successful summer internship program. The greatest success of this program came after the CURE funding began in 2009 and has continued throughout the duration of this project.

Section B. Recommendations

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.

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).

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.

Reviewer 3:
None.

Generic Recommendations for Penn State University

Reviewer 2:
The work that was completed as part of the funded study has met its intended aims and achieved much more at no additional cost. The novel analytical methods that were developed and the new directions that have been identified by the investigator should be a high-impact area of research in the field of Parkinson's disease for at least the next decade. Because these are time-intensive studies requiring the contributions of numerous personnel, it would clearly be of benefit to ensure the investigator has adequate financial support to expand graduate student or postdoctoral involvement, as well as some involvement from institutional personnel who specialize in clinical/translational project development, which this work borders on.

Reviewer 3:
It is a shame that research using non-human primates has been discontinued at PSUHMC due to closing of the primate facility in the summer of 2011 due to lack of manpower to support its operation. This project, as well as many other projects at PSUHMC, would greatly benefit by providing support to enable reopening of the primate facility at PSUHMC.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The initial objective of this project (as indicated in the Strategic plan) was to investigate the sources within the CNS that account for the increased blood pressure variability in the absence of baroreflex control (sino-aortic denervation, SAD). The specific aim was to test the hypothesis that the RVLM contributes to the large blood pressure variability after SAD.

The final progress report describes work accomplished towards 2 major aims: the first was to investigate whether the source of blood pressure variability post-SAD lies within the CNS; the second was to examine the relative contribution of the aortic vs. carotid sinus baroreflexes to blood pressure variability. These aims made use of a rat model previously established in the PI's lab, namely the neuromuscular block rat (NMB) which allows to investigate cardiovascular dynamics while controlling for variables related to movement or respiration. The first aim used the experimental approach of blocking autonomic nervous system outflow with a ganglionic blocker (chlorisondamine) after complete sinaortic denervation, followed by infusion of vasopressor agents to restore average blood pressure. The second aim investigated the effect of bilateral aortic/ carotid sinus denervation followed by complete SAD and also unilateral aortic denervation in NMB rats. The results presented give support to the conclusions that the CNS is a major source of post- SAD increase in blood pressure variability and that the aortic baroreflexes play a more prominent role in attenuating blood pressure variability than carotid sinus baroreflexes.

Strengths: The research design and methods were adequate in light of the stated objectives.

The use of the special model, which is also very difficult to implement, is commendable.

The data obtained is developed enough to yield significant conclusions. This is confirmed by the recent publication of the PI, using the data presented in the final progress report (Tang X., Eur. J. Appl. Physiol., 2012, 112(6):2013-24)

Weaknesses: There is a perceived drift from the initial aims as indicated by the comparison between the project overview and the final progress report. It is unclear why the investigator

chose to give up studying the role of the specific CNS structure (RVLM). It is however understood that the data obtained following the changed specific aims is useful and significant, and well within the general area of the initial project.

Reviewer 2:
Strengths: This was a very interesting hypothesis and potentially important. The study was well-designed and carried out.

Weaknesses: The lack of publications is, overall, a little disappointing.

Reviewer 3:
The objective of the research was to determine the contribution of the arterial baroreceptors and the baroreflex to blood pressure variability and components of the blood pressure power spectrum. The hypothesis was that the central nervous system contributes to the increase in APV following sino-aortic denervation (SAD). The PI further hypothesized that the rostral ventrolateral medulla (RVLM) is the source of this variability. This latter aim was to be accomplished using RVLM lesioning in chronically-anesthetized and neuromuscularly-blocked rats. In addition, the PI was to investigate the role of the caudal ventrolateral medulla (CVLM).

Overall, the PI has made some progress toward his objectives. Given the small size of this award, I would not expect him to make a major contribution. On the other hand, some of the objectives were not met. As far as I can tell, the role of the RVLM or the CVLM in the generation of APV was not determined. As was indicated in an earlier progress report, there were some difficulties in getting rats to survive the ibotenic acid lesions of the RVLM. The PI went on to complete other aspects of the study and never returned to the role of the RVLM.

Predictably, the PI showed that SAD causes an increase in APV which can be reduced following ganglionic blockade. He further showed that the input from the aortic baroreceptors were more important than the carotid sinus input. The latter finding was also shown in humans by Sanders et al. (Circulation, 1988). SAD increased the very low frequency power (VLF) which is to be expected if this represents sympathetic tone. None of the data provided in the final progress report directly relate to the role of the CNS or specific areas of the CNS in generation of the APV.

In this reviewer's opinion, the data are predictable. More relevant information could have been obtained using a conscious model rather than the very difficult chronically-anesthetized and neuromuscular-blocked preparation which is very unphysiological.

As far as publication or grants are concerned, there have been none. One abstract related to this project has been presented at the Neuroscience meeting.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strengths: The research findings obtained contribute to progress in the understanding of a major mechanism involved in cardiovascular regulation. Although with limited direct applicability towards improving health outcomes, it provides important mechanistic insight.
Given the amount of funding, this progress is considered valuable.

Weaknesses: It is unclear what future research in the area will be developed following the completion of this project.

Reviewer 2:
Strengths: This data provides some increased insight into the regulation of arterial pressure variability, a measure that is gaining popularity as an index of cardiovascular disease in humans.

Weakness: The lack of significant publications limit access to the information.

Reviewer 3:
The impact of this project is modest at best. Given the budget, I would not expect much more; however, the likelihood that meaningful new information would come from this study was, in my opinion, low to begin with. The SAD studies were predictable and the lack of completion of the central studies make the outcome of very low impact. As I understand it, the study is complete so there are no future plans.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strengths: A NIH grant application stemmed from preliminary research funded by the present project. Although it has not been funded, this is commendable.

Reviewer 2:
There was an attempt to leverage for an R grant from the NIH. The leverage did not succeed.

Reviewer 3:
There were RO3 submissions that were not funded. There is no indication that any other grant submissions are planned.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strengths: There are 2 publications identified by this reviewer that have been published based on research funded by the project. This is considered commendable with regard to the funding provided for the specific length of time of the project.

Reviewer 2:
No publications yet, but potentially one in works.

Reviewer 3:
There were no publications that emanated from this work. One abstract was presented in 2011. One manuscript was submitted in 2012 to Autonomic Neuroscience: Basic and Clinical. The status of this manuscript was not apparent. No future papers were being prepared.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strengths: The project provided support for 3 young researchers.

Reviewer 2:
Strengths: Minor increase in the PI's ability to conduct research and collaboration with physicians.

Weakness: Little overall improvement of the institution's research abilities.

Reviewer 3:
It does not appear that any graduate students or post-doctoral fellows were paid from this award. There were collaborations with Drs. Dworkin and Norgren.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Weaknesses: Collaboration outside the institution, mostly directed towards the exploration of new areas and techniques has not apparently been pursued. This is encouraged and also necessary for the future development of the PI's scientific career.

Reviewer 2:
No evidence of much, except with research staff of the PI.

Reviewer 3:
None, that I can tell.

Section B. Recommendations

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.

Reviewer 2:
1. Lack of publications.

2. Lack of engagements of colleagues.

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

4. More aggressive attempts at funding.

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.

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.

ADDITIONAL COMMENTS

Reviewer 3:
The project resulted in low productivity. The data were not novel or innovative. The preparation was very invasive and unphysiolgical even if the state of the animal was continuously monitored and made as “physiological” as can be. Both APV and HRV can be modulated by cortical control. There was no way to assess this. No publications came out of this work.

Generic Recommendations for Penn State University

Reviewer 2:
There appears to be a need for better mentorship of junior faculty and integration into the research community.

Reviewer 3:
This was a poorly-designed study destined to have low impact from the beginning. I would not encourage further support; although, this is a moot point since the study has ended.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The project met the PI's stated objectives. The research design and methods were appropriate to achieve the project objectives. The results were developed sufficiently to answer the research questions posed and this was in line with the original research protocol. Sufficient data and information were provided to indicate that the project met its objectives and made acceptable progress. The data and information provided were applicable to the project objectives listed in the strategic research plan.

Strengths of the project include: the experimental approach, the controlled nature of the studies, the careful analysis of the results, and the valuable information generated as it relates to alcohol cardiomyopathy.

Reviewer 2:
This project fully met the designated objectives. The main objectives were: 1) to determine the effect of chronic ethanol administration on cardiac protein synthesis and expression, and 2) to identify which proteins were affected by the chronic ethanol treatment. The working hypothesis of the project was that massive ethanol consumption results in significant changes in the expression of cardiac proteins, resulting in the onset and development of alcoholic heart muscle damage (AHMD). By using the proposed iTRAQ approach, the applicant was able to identify a significant number of cardiac proteins, the expression of which was markedly altered following exposure of the donor animals (rats) to ethanol for 18 weeks. These proteins were located in different compartments of the rat hearts including cytoplasm and mitochondria. The process was rather specific in that several proteins increased in expression whereas others decreased in expression. Overall, the obtained results provide a new rationale to explain some of the long-term complications associated with the onset and development of AHMD in alcoholics.

Strengths: The data were properly developed to answer the research questions posed by the applicant. Two publications validate the significance and importance of the obtained data

Weaknesses: No major experimental weaknesses are noted. The applicant provides a valid rationale for the approach he has used and for the interpretation of the results. The next two challenges are: 1) to place the observed protein modification into a comprehensive frame to

explain the onset and progression of AHMD, and 2) to determine whether similar modifications can be validated in human patients.

Reviewer 3:
Strengths: The proposal met the stated objective, especially in successfully applying the mass spectrometry approaches with the time and funding limits of the support mechanism. The best evidence for this is the two publications resulting from the study and demonstrating the use of iTraq mass spectrometry was successful and yielding interesting, and in some cases, novel data. The data are applicable to the original objectives and advanced understanding by the inclusion of sex-related differences in the alteration of protein profiles.

Weaknesses: The weaknesses are relatively minor considering the challenges of successfully applying iTraq technology to the questions asked. The findings are extensive and no attempt has been made to do some sort of cluster or network analysis to guide the next steps in the application. Much was made of the possible involvement of oxidative stress, but no attempt was made to determine potentially interesting post-translational mechanisms contributing to the cardiac disorder.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The significance of this project is the advancement in understanding of the mechanisms responsible for cardiac failure in chronic alcohol-consuming individuals. This is a health care burden that affects a significant proportion of the adult population in the United States. The findings provide insight into the mechanisms responsible for cardiac failure, and in the future can lead to druggable targets to improve health.

Reviewer 2:
This project and the results it has provided are of great significance for the general population. A significant percentage of the U.S. population consumes large amounts of alcohol (>80-90 g ethanol/day for more than 5 years). This consumption results in the development of AHMD in about one-third of these chronic alcoholics. As indicated by the PI, AHMD is multi-factorial in nature but alterations in protein synthesis are at the bases of the pathology.

Strengths: The information provided by this research presents the essential background to understand the onset and development of AHMD and its cardiac manifestations, and to possibly find a beneficial treatment to their development and progression to limit and possibly reverse the progression of the alcohol-induced modifications on cardiac proteins.

Weaknesses: No major weaknesses are noted. The obtained results are important for future studies and would certainly benefit the human population affected by the disease, although not in the immediate future, as it is not clear how these modifications can be reversed aside from preventing massive alcohol consumption. It would have been useful if in the final report the

grantee would have commented about the possibility that similar modifications may also be at the basis of the wasting in skeletal muscles observed in chronic alcoholics.

Reviewer 3:
Strengths: Although it is too early to tell the beneficial effects of the findings, published results advance the field and are of relatively high impact in the context of the budget appropriated. The results set the stage for more focused investigations that may lead to diagnostics or therapies. Findings on sex-related differences may provide a basis for personalized medicine approaches. An NIH grant has been submitted with a budget that should allow pursuit of some of the important yet understudied aspects, especially redox-related modification and involvement of the proteosomal control mechanism for protein degradation.

Weaknesses: It seems possible that more could have been accomplished with regard to some focused mechanisms No mention is made of a role for autophagy or mitophagy, although this would appear to be an important future direction.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The project led to the generation of preliminary data that allowed NIH funding to be secured by the PI.

Reviewer 2:
The project succeeded in securing additional funding. Indication of an NIH-AAA grant is provided in the final report. This financial support is key to continue the project initiated through this “intramural” support, and further expand the research topic of the grantee.

No weaknesses noted.

Reviewer 3:
Strengths: In view of the longstanding interest of this group in effects of chronic alcohol consumption on cardiac dysfunction, it was clearly a primary goal to take the information from the published studies in leverage for a larger and more comprehensive NIH grant. This has been accomplished much to the credit of these investigators.

Weaknesses: No significant weaknesses.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The project resulted in peer-reviewed publications and in presentations at national meetings where findings were disseminated. The number is appropriate for the funding received.

Reviewer 2:
The project resulted in several publications related to the research topic described in this project. All the publications directly and indirectly related to the research topic described in this project are of high-quality and published in well-respected peer-review journals.

No weaknesses noted.

Reviewer 3:
Strengths: Two publications are listed and provided with the progress report. One is published in a speciality journal, which is highly regarded in the field of alcohol-related research, and the other in the more general and highly-regarded journal, *Physiologial Genomics.* This is excellent progress in view of the extent of information derived from the studies.

Weaknesses: No significant weaknesses.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
There were no direct improvements to the infrastructure. Funds were used to support trainee research.

Reviewer 2:
There is no clear indication of improvements made to the existing infrastructure at the grantee's institution. However, the noted financial improvement (grant for the NIH-AAA for 5 years) and the technical improvement to the proteomic approach (establishing the iTRAQ methods in the grantee's laboratory) can be considered valid and significant improvements generated by this proposal.

One (female) master student was added to the project and supported through the money provided by this grant. In addition, the grantee acknowledges that part of the grant's money was used to support a mass spectrometrist involved in the project for 45% of his/her time. Both these aspects should be considered as significant achievements considering the amount of money granted and the costly type of research carried out by the PI.

No major weaknesses noted.

Reviewer 3:
Strengths: Although not clearly stated, it is generally held in proteomic facilities that successful application of techniques such as ITraq will be of benefit to other investigators. The success in publishing also provides evidence that the infrastructure associated with mass spec and proteomics is working and should be sustained to benefit other investigators. Support did benefit a master's student.

Weaknesses: The response to the question related to the impact of the funding on infrastructure was surprisingly vague with a repetitive statement regarding progress.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Not evident by the report.

Reviewer 2:
The project did not develop collaborations with partner(s) outside the institution, and no specific new involvement with the community was indicated in the final report.

Reviewer 3:
No clear evidence of seeking outside collaborations is presented.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:
None.

Reviewer 2:
1. Overall, this is a very strong project that met the major objectives it was designated to investigate. The identification of alcohol-induced modification in numerous cardiac proteins, however, raises the challenge of incorporating many of these modifications into a comprehensive patho-physiological scheme that can help to better understand how AHMD develops and progresses. Because of the nature of the modifications observed, another challenge is to define an approach that can help limit the progression of these modifications and possibly reverse it. In no form and shape, these challenges subtract from the results achieved by the PI.

2. It would be interesting to determine whether these protein modifications are restricted to the heart or also take place in skeletal muscles.

Reviewer 3:
A recommendation for future improvement is the identification of post-translational mechanism including especially redox-related modifications as well as phosphorylations and acetylations.

Generic Recommendations for Penn State University

Reviewer 1:
This is a commendable effort to support research by scientists at the institution. Clearly this type of support is conducive to future extramural funding as was the case for Dr. Vary's project.

Reviewer 3:
The progress in the project suggests that there should be continued sustaining infrastructure support for proteomics.

Project Number: 0864515
Project Title: Development of Nanoliposomal Therapeutics for Leukemia
Investigator: Claxton, David

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
This project had three stated objectives to reach the overall goal of using nanoliposomes to develop a new therapy for leukemia. These specific objectives include: 1) Optimization of nanoliposomal ceramide for therapy of leukemia mice; 2) Development of ceramide-cationic nanoliposomes containing anti bcr-abl siRNA for therapeutic depletion of bcr-abl; and 3) Evaluation of the anti-leukemic effect of anti bcr-abl with and without ceramide in an in vivo mouse leukemia model. The investigator did an excellent job of pursuing all of these objectives. There are some weaknesses to the results, however, that are due to the inability of siRNAs to target bcr-abl in the model cell line (described in detail below).

The PI designed the study to utilize bcl-abl induced leukemia as a model for development of improved therapies. Although there are small molecule therapies for this form of leukemia, there are two reasons why selecting this oncogene as a model is important. First, most patients develop kinase inhibitor resistant mutations in bcr-abl, highlighting the need for new avenues of therapy. Second, bcl-abl provides an excellent, tractable model system for development of siRNA cancer therapies for other leukemia subtypes. The methods of the proposal were well-formulated and squarely aimed at addressing each of the specific aims.

The data obtained partially addressed the specific aims and were sufficiently developed to answer the research questions. With respect to Aim 1, the PI demonstrated that nanoliposomes containing ceramide were modestly effective at preventing growth of tumor cells in vitro, reducing tumor burden in vivo, and extending lifespan. However, the addition of vincristine enhanced the anti-leukemia activity of ceramide nanoliposomes against leukemia cell lines. These results mirror the expected results in the original proposal and support testing this combination in additional leukemia models and potentially the future development of this combination as leukemia therapy. With respect to Aim 2, the PI showed that anti-bcr-abl siRNA could inhibit the growth of K562 cells (which express the BCR-ABL fusion). However, these compounds could not block the growth of the 32D bcr-abl containing cell line. This is a curious and unfortunate observation. Based on this failure of siRNA to target 32D cells in vitro, aim 3, which was designed to test the siRNAs in vivo in the 32D transplantation model, had to be modified. As such, the PI changed Aim 3 to study the effect of the cytotoxic agent vinblastine on the activity of ceramide. He found that vinblastine indeed showed in vitro synergy with ceramide

nanoliposomes (Lip-C6) both in vitro (KG1 cells) and in vivo (xenograft of one AML sample). These results support the development of combinational therapies that include ceramide nanoliposomes and vinblastine.

There were no significant changes to Aim 1. The one additional experiment was the development of xenograft models of primary leukemias, a reasonable and positive extension of the work that was utilized in Aim 3. There were significant changes made to aim 3, because Aim 2 did not achieve its goal. It isn't clear why K562 cells were not used in vivo as an alternative way to test the anti bcr-abl/ceramide combination. If K562 cells could not be used, the PI could have turned to a bcr-abl transduction/transplantation model for the in vivo study. Nevertheless, Aim 3 was revised to investigate whether vinblastine would enhance the efficacy of ceramide in other models of AML.

The annual progress reports and the final summary provided excellent data to support the project's success in achieving its modified goals.

In summary, the results of Aim 1 and modified Aim 3 are strong. They provide rationale to pursue the use of liposomal ceramide in combination with other agents such as vinblastine as anti-leukemia therapy. The weakness of the project is the inability to test the ability of anti-bcr-abl siRNAs to target bcr-abl positive leukemias in vivo. Overall, the project met many of its stated objectives and was adequately modified to achieve acceptable progress.

<u>Reviewer 2:</u>
Strengths: The PI made substantial progress in establishing primary xenografts from patients with AML; the cationic lipid experiments show some progress; the data from this funding was used as a part of a Program Project grant application.

Weaknesses: No publications have emerged; the delivery of siRNA is a good first step but not particularly novel; and the effects of ceramide are not that strong.

<u>Reviewer 3:</u>
This project met the stated objectives in three specific aims. The PI optimized the nanoliposomal ceramide for therapy of leukemic mice, and showed that C_6-ceramide liposomes (Lip-C_6, 36 mg/kg) significantly reduced bcr-abl leukemia cells and human AML samples in mice and prolonged the survival of leukemic mice. A ceramide-cationic nanoliposomes containing anti-bcr-abl siRNA was generated and tested in K562 leukemia cells. Additionally, this project showed that vinblastine enhanced the efficacy of Lip-C_6 in human AML cells and in mice with hAML.

The strengths of the project performance are that they tested the effects of Lip-C_6 (ceramide) in AML cells that were derived from patients, and Lip-C_6 has significantly synergistic effects with low concentration of vinblastine.

The weakness is that it is not clear whether the nanoparticles can enhance C_6-ceramide delivery and therapeutic efficacy in vivo.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
There has been little progress in developing new therapies for a number of subtypes of leukemia. This project investigated the activity of nanoliposomal ceramide against human leukemia cells in vitro and in vivo and demonstrated there is anti-leukemia activity, especially in combination with cytotoxic agents. This may lead to new strategies for treating leukemia in patients and represent a strength of the project. The future plans were not discussed, so the means of furthering the research into patients is unclear.

Reviewer 2:
The impact is moderate. On one hand, useful models were generated and the work will be pursued. On the other other, the scientific progress was only moderate. However, given the relatively modest amount of funding, this was more successful than many such proposals.

Reviewer 3:
It indicates that Lip-C_6 has the potential to be developed as an effective agent for AML treatment. Based on the achievements of this project, the PI submitted several grant applications to NIH and the "Targeted sphingolipid metabolisms for treatment of AML" was considered for funding.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
A strength of the project is that it contributed to four NIH grant applications. One of these grants, “Targeted Sphingolipid Metabolism for the Treatment of AML” received an outstanding priority score and may be funded.

Reviewer 2:
The PI has not received additional funding but is likely to receive funding as part of a Program Project grant (NIH).

Reviewer 3:
The PI submitted four grant applications to the NIH, to expand the research.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The project did not lead to a publication or patent application.

Reviewer 2:
No publications or IP filings.

Reviewer 3:
Two manuscripts are prepared: "Engraftment of human acute myeloid leukemia defined by integrated genetic profiling in NOD/SCID/IL2ry ^{null} mice for preclinical therapeutics evaluation"; "Nanoliposomal ceramide and inhibitors of autophagy for therapy of myeloid leukemia".

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The project allowed the recruitment of Dr. Brian Barth, an expert in sphingolipids. This addition is expected to aid in the development of new therapeutics in AML. Funds were used to support one post-doctoral fellow (at 5% effort). The remaining personnel were research technologists or wage staff. Several pieces of equipment were purchased.

Reviewer 2:
The models generated will continue to be an important resource. Moreover, the methods established will facilitate other research. A number of undergraduate, graduate and post-doctoral scientists were supported.

Reviewer 3:
This project enhanced the quality and capacity for research at the Penn State University. It improved the equipment for cell study, and lipid analysis. It allowed recruitment of a new investigator to this research, and paid for one undergraduate, one pre- and one post-doctoral student.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
New collaborations were established with leukemia experts Dr. Martin Tallman and Dr. Ross Levine as well as Dr. Schultz who has expertise in AML xenograft models.

Reviewer 2:
The PI had several collaborators both in and outside of Pennsylvania.

Reviewer 3:
It established a new collaboration with three investigators, including Drs Martin Tallman and Ross Levin at Memorial Sloan-Kettering Cancer Institute, and Dr. Lenny Schultz at Jackson Laboratories.

Section B. Recommendations

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.

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.

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.

Reviewer 2:

1. PI should try to publish their data.

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

Reviewer 3:
None.

Generic Recommendations for Penn State University

Reviewer 1:
Grant recipients should be encouraged to support graduate students and post-doctoral fellows.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The investigators hypothesized that the presence of alterations of both (simultaneous) the p16 gene and the BRAF gene in papillary thyroid cancer would give rise to a particularly aggressive form of this disease.This project had 2 specific aims: the first aim was to analyze surgically resected tumors for p16 alteration and BRAF mutations. The second aim was to correlate the presence of these changes with patient outcomes.

The technical aspect of the work was well carried out. Assays for both p16 and BRAF were developed and applied successfully.

The analysis, however, suffered considerably from a small sample size and a rudimentary approach. The sample size was inadequate for testing the hypothesis.

Reviewer 2:
The PI assessed BRAF mutation and p16INK4A expression in 32 papillary thyroid cancer (PTC) specimens. Research design and methods were adequate. The PI proposed analysis of 32 archival PTC cases and the PI conducted such analysis on the proposed numbers of specimens. The PI nicely documented the results of the experiments.

Overall, a relatively limited analysis of BRAF mutation and p16(INK4A) expression in archival PTC specimens. Analysis was conducted thoroughly, but it is hard to know if there are any real conclusions to this study given the relatively small number of specimens analyzed.

Reviewer 3:
The project had two specific aims: a) to analyze surgical specimens of papillary thyroid cancer (PTC) for the BRAF mutations and p16 alterations, and b) to correlate the co-occurrence of these molecular changes to patient outcomes. Although Aim 1 is seems to be completed, Aim 2 has not been accomplished.

Only 32 patients and 32 controls were analyzed with no statistical justification and power requirements to analyze the clinical outcomes. This is a weakness of the design. Another

weakness is p16 is evaluated by non-quantitative IHC and BRAF mutation was assessed by ASPCR.

No changes were made to the protocol; target goal of 30 patients was completed.

The data and information provided indicate that the project met one of its objective (Aim 1) but not the second aim.

The data and information provided are in line with the project objectives.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The potential benefit could be significant if a subclass of cancer was discovered to be particularly aggressive. These tumors could be targeted for additional treatment. However, given the minimal data presented, it is impossible to really assess the data. It seems unlikely that further funding will be forthcoming for this work.

Reviewer 2:
No relationship of BRAF mutation and/or p16(INK4A) expression to clinical outcome was presented.

Reviewer 3:
Molecular markers that accurately predict the behavior of PTC are needed to guide the treatment of PTC. This project addresses this need by analyzing BRAF and p16. However, it is not articulated how the correlation of alterations in p16 and BRAF mutation to aggressive PTC can be translated to better management of PTC patients.

The research completed to date is merely correlative and unlikely to improve health outcomes in the near term.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
No leveraging was anticipated and none occurred. Additional funding seems unlikely.

Reviewer 2:
No leveraging of funds was expected or occurred; project seems to be completed with no follow-up planned.

Reviewer 3:
This project did not leverage any additional funds. There are no plans to apply for additional funding in the future to continue or expand the research.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
No manuscripts were published, although they indicate that one is planned. The data, as presented, do not lend themselves to publication.

Reviewer 2:
The PI claims that a paper is being prepared for submission. Hopefully, the PI will follow through and publish the results of this study.

Reviewer 3:
To date no peer-reviewed publications, licenses, patents or commercial development opportunities resulted from the project. The researchers are preparing a manuscript for submission.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES
Reviewer 1:
None

Reviewer 2:
Threre were no obvious enhancements to the quality and capacity for research at the grantee's institution.

Reviewer 3:
It is stated that the project supported collaborative studies among investigators from diverse departments. However, since there are no published, peer-reviewed publications, it is not possible to evaluate the impact of this collaboration. No new investigators were brought into the institution. No pre- or post-doctoral students were supported by the funds. One medical student was supported for 1% effort.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
None.

Reviewer 2:
No collaborations specified.

Reviewer 3:
No collaborations with research partners outside of the PI's institution are listed.

Section B. Recommendations

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.

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.

Reviewer 2:
Even given the limited funding provided, analysis of a larger patient cohort should have been possible under the aegis of this proposal.

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.

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.

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.

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?).

ADDITIONAL COMMENTS

Reviewer 3:
This project did not accomplish anything novel or significant. The design of the study is weak. No statistical reasoning for selecting 32 patients was provided. No quantitative IHC analysis for p16 was considered. Curiously, both BRAF mutation and p16 levels were present in similar (78%) and a much larger proportion of cases than reported in the literature. No explanation has been offered for this unusual occurrence. No detailed analysis of data in Table 3 was provided to help the reviewer to understand their significance. For example, nearly the same proportion of patients had tumor recurrence irrespective of whether they had only BRAF mutation or p16 alteration or both. It is not clear what can be concluded from these data.

This project did not provide opportunity for any pre- or post-doctoral students to learn the methods in clinical/patient-oriented research. It would be useful to have pre- or post-doctoral students participate in this type of research.

Generic Recommendations for Penn State University

Reviewer 2:
This type of hypothesis-free, highly-descriptive research is, in my humble opinion, largely a waste of money. Insufficient numbers of patients were analyzed and so any analysis of outcome is intrinsically unlikely to be of statistical significance. Moreover, this proposal supported 9 individuals over 2 years but it was hard to see what the justification for even a very limited amount of effort might be. Finally, the data presented in the final summary could likely have been generated and analyzed in two months rather than two years.

Reviewer 3:
The institution might consider providing the investigators with additional (subsidized?) institutional resources to accomplish the larger goal of identifying markers that predict the behavior of PTC.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Two specific aims have been proposed for this project. In Aim 1, Dr. Mailman planned to test the central hypothesis further in the "Atherosclerosis Risk in the Communities" (ARIC) prospective cohort. His group for the first time, provided evidence that low cholesterol was associated with a faster progression of Parkinson's disease, although initial case control studies found a negative association between statin usage and PD occurrences, which possibly caused by possible neuroprotective properties of the statin. Their follow-up study suggested that ApoE4, a risk factor in Alzheimer's disease, was actually protective in PD by showing in 786 patients and 1537 controls, as compared with participants with ApoE3/3, ApoE4 carriers had significantly lower odds for having PD, whereas ApoE2 carriers did not. In Aim 2, Dr. Mailman proposed to test the hypothesis that peripheral cholesterol-APOE status affects the metabolism and or central entry of toxicants that can cause Parkinson's like damage. In text, they claimed that in Figure, 1, the only significant difference was that depletion of dopamine was actually greater in the high cholesterol group challenged with MPTP. However, the data showed in Figure 1 did not agree with their statement. Additionally, no animal numbers per group were demonstrated and no statistical analysis was performed in Figure 1, which could not tell if their original proposed experiments were correct or not. In their follow-up study, they performed a modified larger scale experiment. However, their data in Figure 2 are quite questionable. From data shown in Figure 2, as compared to controls, both MPTP and Rotenone treatments did not affect striatal dopamine levels, indicating their conclusion was not right as it looks like both MPTP and rotenone induce dopaminergic neuronal damage. Without showing their experimental designs and dopaminergic neuronal quantification, one cannot provide further comments on their data.

Strength: Aim 1 was successfully completed. Data were well-justified and significant.

Weakness: Aim 2 study was incomplete and animal models need to be validated and successfully developed.

Reviewer 2:
Strengths: These studies were based on findings that support that individuals with low cholesterol may be at greater risk for developing PD. The present studies extend these findings

with an excellent analysis of the both the DATATOP and PRECEPT data, which involved pre-statin versus post-statin era cohorts of well-studied patients with PD. The initial case control studies found a negative association between statin usage and PD occurrences, which has been reported by two groups and refuted by two others. However, this is the first group to show that low cholesterol is also associated with faster progression of PD. In addition, the ApoE4 allele (associated with high cholesterol) may be associated with a lower risk of PD. These studies resulted in preliminary animal studies to address the effects of peripheral cholesterol-APOE status on metabolism and/or central entry of neurotoxins/effects of neurotoxins. These preliminary studies did not support the central hypothesis, that peripheral cholesterol levels affect metabolism of catecholamine neuronal systems or the effects of the neurotoxins, rotenone and MPTP.

Weaknesses: As admitted by the PI of this project, brain cholesterol levels are regulated independently of the CNS levels; therefore, the rodent studies do show that peripheral cholesterol changes do not affect DA metabolism or the effects of neurotoxins, but these studies do not address if changes in CNS levels of cholesterol will alter metabolism of DA systems or the effects of DA neurotoxins.

Reviewer 3:
This project is based on the PI's earlier findings (supported by data from other groups) that associated increased prevalence of Parkinson's disease with lower cholesterol and presence of the apolipoprotein E ε2 allele (both of the latter generally considered "good" characteristics.) The first aim was to test *the central hypothesis further in the "Atherosclerosis Risk in the Communities" (ARIC) prospective cohort.* When the PI became aware of a second cohort that would allow prospective testing the hypothesis that lower plasma cholesterol may be associated with faster progression (in addition to prevalence of PD), the studies were extended to cohorts from the Parkinson's Study Group DATATOP and PRECEPT. The second aim of this project was directed at *testing the hypothesis that a peripheral cholesterol-APOE status affect the metabolism and or central entry of toxicants that can cause Parkinson's-like damage.*

The studies conducted under Aim 1 provided additional evidence in a variety of different types of clinical studies (above and beyond the ARIC cohort that was originally noted) showing the association of high cholesterol with a lower incidence of PD. Moreover, the secondary analysis of the DATATOP trial provides the first evidence that higher total serum cholesterol concentrations is also associated with a slower clinical progression of PD. Findings in animal models addressing specific Aim 2 were negative indicating that altered cholesterol status at least in the mouse did not affect the central availability of dopamine neurotoxicants. Although the PI's data refuted his working hypothesis the information is important for proceeding on to other mechanisms that might actually be related to PD. The mechanism that relates high cholesterol to lower incidence or neuroprotection in PD remains an open question and worthy of further investigation.

The results also suggested the need to now focus on brain mechanisms, specifically those related to genetic factors that influence whether an individual has high LDL-cholesterol and the associated lower risk of PD, and contributed to the co-investigator, Dr. Huang receiving funding

as the PI of a new NINDS-funded biomarker project that seeks to determine structural or biochemical markers of Parkinson's disease.

By means of the funding of Dr. Huang's U01 grant, the PI will now have the unique opportunity to access extensive numbers of patient samples and controls gathered under stringent conditions that will allow his group to examine those genes that are linked to LDL-cholesterol (e.g., biosynthetic and degrading enzymes, processing enzymes using cholesterol as precursor, carrier proteins like apolipoproteins, etc.). With NextGen sequencing capacities purchased to advance this and other projects at the College of Medicine, it will now be feasible for the PI to investigate which of these genes are related to both high-LDL cholesterol and lower risk of PD, a study not previously possible. This provides a new direction and a strong foundation for future funding of this project.

The PI has done an outstanding job of addressing the original and extended aims of this project.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strength: The hypothesis was further confirmed in clinical research studies. Thus, the results of this proposal have a big impact on the Parkinson disease research. In this study, they provide the first evidence that the high serum cholesterol concentration is associated with a slower clinical progression of PD. A future study was well-proposed. If the hypotheses are correct, it would argue against use of cholesterol-lowering drugs in Parkinson disease patients and otherwise healthy individuals. This could result in decreased morbidity, as well as cost savings from unnecessary or harmful use of such drugs in otherwise healthy people.

Weakness: The impact of this proposal may be somewhat diminished by their incomplete/unsuccessful animal studies.

Reviewer 2:
Strengths: It can be argued that this work has potential major beneficial impact in that it attempts to determine whether the use of statins, to lower cholesterol, is truly beneficial and/or decreases the incidence of neurodegenerative diseases, such as PD. In addition, it begs the question if there are genetic mechanisms that cause higher cholesterol, which may relate to neuroprotective mechanisms that can be determined and possibly used for novel drug target development. As such, the principal investigator has worked with a co-investigator to secure NIH funds for MRI markers of pathology in PD and foundation for a possible NIH program project grant that is being pursued.

Weaknesses: No major issues, even though the studies to date have been meta-analyses and animal studies that do not support the central hypotheses.

Reviewer 3:
It is noteworthy that during the course of this project, an article appeared entitled ”Cholesterol Drugs For The Healthy Still Debatable” in Drug Discovery & Development, June 29, 2010. This article summarized a current controversy about whether healthy people with low cholesterol should take a pill to lower their cholesterol even more in the hopes of preventing heart problems. The PI’s data supported by their DATATOP study suggest that statins may nether be neuroprotective nor innocuous in otherwise healthy people. It is premature at this time to make a clear judgement concerning this issue without further studies. However, if the PI’s hypotheses are correct it would argue against the indiscriminate use of cholesterol-lowering drugs in otherwise healthy people.

This information, if validated, could result in decreased morbidity, as well as cost savings from unnecessary or harmful use of statins in otherwise healthy people.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The questions have been adequately answered by PI and his collaborators. They have successfully brought in and planned to apply additional funding.

Reviewer 2:
Strengths: This project was excellent in leveraging funds from two grants: one for $25K (non-federal) and one for $650K (NIH) that focused on the major issues. In addition, the PI is working on a NIH Program Project Grant, which will likely be submitted that will go after multi-year funding.

Weaknesses: None noted.

Reviewer 3:
The PI was very successful in leveraging additional funds for this project. He received non-federal funding from the Parkinson’s Study Group and Federal funding from the NIH for a project titled, “Multimodal MRI Markers of Nigrostriatal Pathology in Parkinson’s Disease.”

This project has also provided the foundation for one project on a NIH Program Project grant that is being worked on for submission in 2013.

In addition, through funding of Dr. Huang’s U01 grant, the PI will now have the unique opportunity to access extensive numbers of patient samples and controls gathered under stringent conditions that will allow his group to examine those genes that are linked to LDL-cholesterol (e.g., biosynthetic and degrading enzymes, processing enzymes using cholesterol as precursor, carrier proteins like apolipoproteins, etc.). With NextGen sequencing capacities purchased to advance this and other projects at the College of Medicine, it will now be feasible for the PI to investigate which of these genes are related to both high-LDL cholesterol and lower risk of PD, a

study not previously possible. This provides a new direction and a strong foundation for future funding of this project.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strength: Three publications and more manuscripts in the future.

Weakness: None.

Reviewer 2:
Strengths: Three quality peer-reviewed articles resulted from this work. More are planned and should appear from this work.

Weakness: No patents or intellectual property at this time.

Reviewer 3:
The project resulted in three peer-reviewed publication in quality journals. The PI plans to submit articles for publication from the ARIC cohort as well as a short communication on the animal results in 2013.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strength: This project has helped the grantee's institution develop a critical basis for a new program grant application on Parkinson's that is currently under development and will further expand its research base in the neuroscience. The NextGen sequencing instrumentation capacities added through this project will have an ever-expanding role as a crucial part of their new Institute of Personalized Medicine and Genome Sciences facility.

Weakness: No graduate students and post-doctors/new investigators were involved in this project.

Reviewer 2:
Strengths: The project helped the institution develop a critical mass of active research related to PD. This provides the basis for a new NIH Program Project Grant, which is under development. In addition, the NextGen sequencing instrumentation, which has been added through the expanded projects of this proposal, have an ever-expanding role in a new Institute of personalized Medicine and Genome Sciences facility.

Weakness: None noted.

Reviewer 3:
This project has helped Pennsylvania State University (PSU) develop a critical mass of active research related to Parkinson's Disease. This provided the basis for a new Program Project Grant application on Parkinson's that is currently under development and will further expand PSU research base in the neurosciences.

The NextGen sequencing instrumentation capacities added through this project will have an ever-expanding role as a crucial part of the new Institute of Personalized Medicine and Genome Sciences facility. The sequencing instrumentation benefits Penn State Hershey researchers across the research enterprise and facilitates both basic and clinical research. Over the past five years, a large number (sixty-one) of the research laboratories at Hershey have used the Genome Sciences Facility, but nationally "traditional" methods of genotyping of known SNPs such as microarrays are being augmented by whole genome and exome sequencing methods which can reveal new mutations associated with a disease or condition. The additional capacities provided by the HiSeq and MiSeq instruments have already been used for example to obtain results for extensive population studies of autism (complete genome sequencing of 30 twins with autism and their families using the HiSeq - Dr. Daniel Notterman); RNA-Seq studies of Diabetic Retinopathy (HiSeq - National Eye Institute study funding to Dr. Willard Freeman), and for DNA methylation studies of the epigenetic basis of drug craving (MiSeq –National Institute on Drug Abuse funding – Dr. Willard Freeman, Dr. Sue Grigson). With the variety of molecular biological approaches possible with this instrumentation, investigators across a number of clinical and basic science departments and divisions will be aided by the addition of its capability. This project has led to a dramatic improvement of the infrastructure of PSU.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strength: Collaborations have been successfully established.

Reviewer 2:
Strengths: Collaborations have now been started with investigators at Harvard Medical School (Drs. Alberto Arturuo and Michael Schwartzchild), NIEHS (Dr. Honglei Chen), University of Mississippi, Jackson (Dr. Tom Mosely) and the University of Minnesota (Dr. Alvaro Alonson).

Weaknesses: None noted.

Reviewer 3:
This project has led to new collaborations with experts in the field, including Drs. Alberto Arturuo and Michael Schwartzchild of Harvard Medical School, Dr. Honglei Chen of the NIEHS, Dr. Tom Mosley (ARIC PI at the University of Mississippi, Jackson) and Dr. Alvaro Alonson (University of Minnesota).

Section B. Recommendations

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.

Reviewer 2:
None.

Reviewer 3:
None.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The project meets the stated objectives.

Reviewer 2:
Strengths: The main objective of this project, "preparing ISC-4 for clinical evaluation," was achieved by completing all the preclinical testing and licensing the compound to Melanovus Oncology for further development through a Phase 1 clinical trial.

The research design was very well-developed and appropriate to achieve the goals of this study. It included logical steps to achieve the objectives: 1. Preparation of a scaled-up batch of ISC-4 and evaluation of the compound's physiochemical and biological characteristics in preclinical models compared to current small-scale lab generated batches; 2. Development of suitable formulations; and 3. Pharmacokinetic/ADMET studies of the compound in a second animal model.

Weaknesses: None.

Reviewer 3:
The project was designed to develop an agent to inhibit the development of melanoma. Melanoma is a cutaneous tumor, which develops from transformed melanocytes whose function in normal skin is the generation of melanin pigment important in the protection against UV radiation from the sun. Individuals who are sensitive to sun, can develop the disease. It begins as localized lesions which spread and invade more superficial regions of the skin. Following the initial development of tumor, tumor cells can escape the original lesion, and via lymphatics or vasculature can seed other anatomic sites. Tumor cells can be detected in peripheral blood, circulating tumor cells (CTCs), and these cells will take up residence preferentially in certain organs and tissues which can support their further growth as metastases. Preferential sites of metastasis are colon, lung, liver and eventually bone and brain. The disease has limited conventional therapies available, which show significant promise over the years. The key factor in promoting survival is early detection resulting in surgery, site targeted radiotherapy and in the case of metastatic disease, chemotherapy using agents such as Dacarbazine. Unfortunately, as the

PI states, additional effective chemotherapeutic agents do not exist which show significant promise.

The purpose of the study was to develop a novel agent which could inhibit a pathway important in regulating apoptosis, a natural means whereby cells die. Apoptosis is a pre-programmed cellular mechanism which occurs throughout development and is the result of the interaction between immune effector cells, such as cytolytic T cells, and natural killer cells and tumor. The agent developed showed promise in inhibiting AKT signaling, thus promoting apoptosis. Interestingly, a novel therapy for melanoma has been developed at the National Cancer Institute and other labs and clinics around the world. This therapy is immunotherapy and it targets tumor cells by exploiting natural immune effector mechanisms. Immunotherapy has evolved from using nonspecific innate mechanisms to the recent development of vaccines designed for therapeutic interventions. It would be interesting, as a future application, to pursue the combination therapy of the agent described, ISC-4 and immunotherapy. However, that was not the purpose or design of the current study. This suggestion is for the future.

The current study was designed to do a number of things, all leading to the generation of adequate data to file for IND status with the FDA. The project did not set as a goal human Phase I or II trials. Those human trials were designed to be developed concurrent with and following IND application and eventual success thus allowing the use of the drug in humans. There were a number of goals established by the PI and his team, to the best of this reviewer's expertise; it appears that most of the goals were attained in a timely manner. After significant investment in state of the art equipment and supplies, a research team headed by the PI successfully completed the goals stated in the original application. In general, those goals included the generation of a large batch of the agent, which behaved similarly to smaller research-size batches in prior evaluation. Formulation of the drug was to be explored, tablet vs. capsule, but eventual use as a cream was discussed and seems to be the current focus. It is not clear what the status of the tablet vs. capsule investigation was beyond including the agent in chow given to mice. However, the development of a cream or lotion is probably a more relevant application of the agent. Finally, Pharmacokinetic studies were done in mice and supposed to be done in a second animal model prior to IND submission. It is not clear what the status of the second animal model is (either rats or dogs, beagles). This reviewer feels that the stated goals were achieved for the most part and it appears that other than the second animal model, convincing data exists to apply for an IND and eventually begin human studies.

In regards to design and methods, those described seem adequate and clearly answered the questions posed. The initial observations focused on an agent, naturally occurring in leafy vegetables, which had an inhibitory effect on melanoma growth. Utilizing siRNA in in vitro studies, the PI showed the agent was targeted to the AKT pathway, a pathway which inhibits apoptosis through the caspase 3 pathway. The PI utilized expertise available to the group in the design of a suitable drug which decreased the doses needed for effective action as shown in earlier studies using natural products. Realizing that those quantities of natural products were not feasible for use in humans, the PI designed a synthetic drug and then modified the drug to include selenium, that in addition to an increase in chain length led to a drug design which performed at reasonable physiologic levels in vitro and in in vivo murine models. The PI was able to obtain a large batch of drug, kg quantities, which by all studies presented appeared to

show similar activity to smaller research size batches. This is an important point considering the technology used to make small vs. large batches are different and pose different problems in terms of purification and the levels of contaminants which could be present. The PI presented supportive data showing the large Kg batch performed well in comparison to the research batch when effects such as proliferation and induction of apoptosis were examined. Where appropriate, in vitro and or in vivo studies were performed. Data was well presented with appropriate statistical measures applied. Finally, pharmacokinetic studies were done and showed that the product was safe in regards to other normal tissue and organ functions. Thus the product designed to inhibit the development of melanoma, or as shown in murine models to reduce the size of tumor lesions was successful.

This reviewer has two suggestions in terms of the present study. It is not to suggest that the results obtained are not valid or convincing. Rather, these are suggestions. It is important in cancer therapy to be clear and distinguish between the events that occur during development of disease, small lesions, vs. treatment of established disease and metastatic lesions. The PI simply needs to be clear in the presentation as to what is being addressed. As one reads the experimental design, it is clear what is being studied, the issue is in the original application where studies are proposed. Secondly, if additional changes were made to the research protocol, additional melanoma lines would have been investigated. Most in vitro studies utilized a limited number of lines (1-3) to show efficacy. Additional melanoma cell lines which are readily available should have been examined in addition to more control samples. It is clear that the reagent tested has some effect on normal fibroblasts. The question of course is whether the fibroblasts that were studied were indeed normal or had characteristics of transformed cells. It would have been helpful to see normal, biopsy-provided fibroblasts, normal peripheral blood monoculear cells and other normal cell targets which can be purchased or obtained from other investigators. Again, there is 5-fold more activity against tumor; however, why is there an effect on normal cells and should it have been addressed further. In terms of other tumor lines, inclusion of other lines addresses the issue of specificity. Finally, as mentioned earlier, there is an important role that the immune system plays in the eradication of melanoma. It might be important to now evaluate effects on the immune system in terms of activation of antigen presenting cells, presentation of antigens, T cell reactivity and the potential influx and/or generation of inhibitory immune cells including Tregs and MDSCs.

Finally, as the studies progressed, it appears that the PI made appropriate adjustments to the study in order to maximize the generation of relevant results. Appropriate changes were evident in experimental procedures in order to efficiently investigate the biologic activity of the compound. Overall, it is this reviewer's opinion that the PI reasonably met all stated goals and achieved all the objectives stated in the strategic plan. In addition, progress reports reflected progress made in each calendar year. The PI was realistic in the goals stated and achieved the bulk of the research results following successful implementation of resources and staff.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The significance of this project is helping to develop better anti-cancer therapy and to improve the cancer survival rate.

There is value of the research completed towards eventual improvement in health outcomes. May reduce the risk of death from cancers.

No major discoveries, but, the project may lead to develop new drugs and new approaches for treatment of cancers that are attributable to the completed research project.

The future plans are reasonable.

Reviewer 2:
Strengths: There is potentially high impact on improving treatment options and therapeutic outcomes for melanoma patients, if clinical trials confirm the data obtained in this proposal in preclinical models that ISC-4 is a potent agent that inhibits melanoma development.

Weaknesses: None.

Reviewer 3:
Melanoma is a major killer of men and women in the United States. As stated by the PI, few therapeutic options exist. Surgery is possible if detected early. However, the disease is aggressive, often leading to metastatic sites. When this occurs, radiation therapy directed against localized areas of disease are feasible, and a limited number of chemotherapeutic agents can be used for metastatic disease. The current project proposes a novel drug, one targeted to a pathway which inhibits apoptosis. It is a novel approach and as shown by the PI, can be utilized against cutaneous disease. This is actually advantageous in the long run. Granted, this approach will not work against metastatic disease unless the PI figures out an oral dose. However, the approach goes hand in hand with early detection. One could envision utilizing the cream on the margins of a surgical site as well as use it to treat small newly-formed lesions.

To this reviewer, there are no additional new drugs that have arisen from the development of this reagent. However, information regarding a recently-funded RO1 were not provided. It is possible that this work has led to the development of new reagents that were not discussed. Future plans for this project include obtaining an IND and through local collaborations initiate human trials. Human trials will include toxicity studies and dose escalation studies leading eventually to studies designed to evaluate efficacy.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The researchers plan is to apply for additional funding in the future to continue or expand the research.

Reviewer 2:
Strengths: The project leveraged additional NIH funding of $232,566.80. In addition the investigators also plan to submit R01 or other R-type applications.

Weaknesses: None.

Reviewer 3:
Additional funds directly related to this agent were not obtained, at least to the best knowledge of this reviewer. An RO1 was obtained during the funding period, it is believed that the experience of the group was helpful in obtaining this funding. The equipment which was procured from funds made available from the tobacco settlement should have been instrumental in obtaining these extramural funds from the NIH. The PI does mention that an SBIR was not funded, both an original and a revised submission. It is interesting because the amount of data obtained might have predicted approval.

From reading the final progress report, it seems that additional avenues for funding are not being explored. But, it may be that this point was not clarified.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
There was one high quality publication.

Reviewer 2:
Strengths: The project resulted in a very high-quality publication in *Cancer Prevention Research*.

Weaknesses: None.

Reviewer 3:
The work described resulted in one significant publication in Cancer Research. This is a high-quality journal which reflects the quality of the work. It is surprising that more publications were not attempted. It is not clear why this is so.

In terms of commercial development, an interaction has been initiated with a venture called Melavirus Oncology. This interaction should lead to eventual clinical efficacy studies as proposed.

A patent has been filed as cited in the final progress report.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Part of funds were used to pay for research performed by post-doctoral researchers.

Reviewer 2:
Strengths: This project has enabled a better translational research potential, and the purchase of Becton Dickinson FACSAria III SORP cell sorter benefited multiple investigators within the PI's institution.

Weaknesses: None.

Reviewer 3:
In this reviewer's opinion, the infrastructure was improved dramatically by the funds made available. The PI obtained significant resources in terms of capital equipment, the most significant being the flow cytometer which has cell sorting capability. The PI cites the use of the instrument to be vigorous, clearly improving the quality of research on campus. In addition, other equipment such as PCR-related equipment should be helpful if shared by other members of the University.

It does not appear that researchers were brought in from out of state as the result of the funds obtained.

Two post-doctoral fellows obtained support from the funds obtained. It is unfortunate that the work done by the post-docs has not resulted in additional publications.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
No evidence of collaboration with other researchers.

Reviewer 2:
Strengths: This project resulted in collaboration with the commercialization partner - Melanovus Oncology.

Weaknesses: None.

Reviewer 3:
It was not evident that other researchers became involved in the work described. The association with Melavirus Oncology will result in utilization of the reagent developed in melanoma clinics located locally and across the state.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:
Published papers in high-impact journals are limited.

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.

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.

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.

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).

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Although the project did not achieve completely the initially-stated goals, the PI and research team has changed questions and made reasonable progress to generate new information related to understanding retinopathy of prematurity.

The original proposal was designed to investigate the specific role of protein synthesis in retinal neovascularization in ROP model. Investigators had access to 4E-BP1/2 knock out mice as well as the parent species Balb C and showed preliminary evidence that KO mice had better response to oxygen insult and that they express more of the major angiogenic growth factor VEGF.

In year 1 progress report, investigators pointed out difficulty in breeding of KO mice and in year 2, they stated that was no difference between control and KO in terms of retinal neovascularization; so, they dropped the original plan and focused on studying the WT animals to examine alteration in mTOR signaling pathway and investigate the protective effect of mTOR inhibitor Rapamycin on retinal neovascularization in ROP model. These experiments are still in progress. There is promising but not yet complete results.

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.

Reviewer 2:
The progress report provided sufficient data and information to indicate that the research team was able to determine that 4E-BP1/2 did not alter retinal neovascularization in an animal model of oxygen-induced retinopathy (OIR). As a result, they were not able to define the effect of 4E-BP1/2 on VEGF expression in a meaningful manner. This was not predicted at the time of grant application. The research team then decided to show whether a mouse strain Balb/c, which was not commonly used in the field, could also be used for (OIR). This is a useful piece of information that may be beneficial for studies that require the use of Balb/c mice. In addition,

they were also able to demonstrate that mTOR activation in Phase II of OIR has identified a potential therapeutic target for retinopathy of prematurity (ROP). Although the outcome was not originally anticipated (beyond control of the research team), with the added experiments, the research team made acceptable progress.

Reviewer 3:
Strengths: Some of the stated objectives were met and rationale for modifying the objectives and experimental design were provided for the majority of the changes.

New data was provided for the response of the Balb/c mouse to the OIR model.

Weaknesses: More quantitative analysis of the data would have better supported the conclusions i.e., quantitative analysis in a blinded fashion vs. conclusions based on gross observations. 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), but it would not hurt to state this explicitly.

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.

Several inconsistencies were apparent when reviewing the annual progress reports vs. the final progress report. The planned duration of the project was adjusted several times. The initial estimate of time had to be increased based on needed data to support the use of Balb/c mice in this study. However, the apparent quick cessation of activity was not explained. The progress report for July 2010 - June 2011 stated an expected end date of Dec 2011 and future analyses were planned; yet the progress report for July 2011- June 2012 indicated that the project ended in the prior fiscal year and no further data is provided in this or the subsequent annual progress report. In the final progress report, however, there is additional data provided to show that work did continue, albeit, presumably via funding from the Children's Miracle Network. The new work showed the change in research focus. However, there were some inconsistencies with conclusions reported in the final progress report and those reported in the annual progress reports. For example, in the 2009-2010 annual report, data is presented and the conclusion is drawn that “loss of 4E-BP1/2 ameliorates OIR-induced neovascularization coincident with an increase in VEGF and FGF2.” Plans were made to investigate whether the changes in growth factors represented a direct effect of 4E-BP1/2 knockout or were reflective of an accelerated restoration of appropriate vascular development. These studies were apparently not done as the 2010-2011 progress report indicated efforts to continue experiments to correlate changes in vascular density with alterations in levels of VEGF and FGF2. No more experiments were carried out past June 2011 [despite an anticipated ending date of Dec 2011]. In the final progress report (page 7), it is stated that “we could identify no difference in neovascularization due to the loss of E4-BP1/2” and this was used as justification for the change in research focus from VEGF and FGF to the mTOR pathway in OIR. No new data is provided to back up the change in conclusion about this reported lack of differences in neovascularization between the WT and DKO retinas, just a simple picture of retina flat mounts and the statement that gross examination

showed no differences. 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 a better explanation of the abandonment of the original hypothesis should have been provided. There was no attempt at explaining the paradoxical increase in VEGF, for example, in the face of ameliorated neovascularization, if that was supported by the work completed at the time. New data provided in the final progress report and carried out after the end of the funded project (with alternate funding), does indeed support a new potential means of intervening in retinopathy of prematurity.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Due to detailed problems of breeding above and lacking a complete set of results, the likely beneficial impact is small**.** Results are not ready for publication to share with the scientific community or to be extended to improve health outcome. However, investigators are hoping to continue working on the project to publish the results as manuscript.

Reviewer 2:
The study with mTOR activation may be beneficial to the establishment of improved treatment for ROP. The research team will continue to work on one aspect in this area. In addition, this study provided additional information regarding a mouse strain, Balb/c, in OIR model, which may be potentially useful for OIR studies that have to be conducted in this strain.

Reviewer 3:
Strengths: The project had potential to add to our understanding of the pathogenesis of retinopathy or prematurity, a condition associated with long-term visual impairment and blindness, and thus provide insight for therapeutic intervention (i.e., drive improvement in health outcomes). Recent preliminary data *collected after completion of this study [using funding from the Children's Miracle Network]* suggest a potential path for intervention that could alter neovascularization in the developing retina.

Weaknesses: No major discoveries resulted from this work. 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.

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).

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
An R21 application was submitted to the NIH back in Feb 2010 and it was not funded.

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

Reviewer 2:
The PI applied for an NIH grant but was not materialized. They expressed a possibility of future applications for funding.

Reviewer 3:
Strengths: R21 proposal (and presumably a resubmission) sent to the NIH in 2010.

Weaknesses: No major discoveries/outcomes from this research. The PI 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.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
As stated by investigators, having no difference between WT and KO hindered the progress of the project. The new direction on the role of mTOR pathway seems promising, and hopefully a manuscript can result after completing the proposed studies.

Reviewer 2:
Except for a published abstract, there was no peer-reviewed publication at this time; although, the progress report indicated the preparation of manuscript for publication in one of the leading vision research journals. The focus should be on the publication of data in the future.

Reviewer 3:
Weaknesses: 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.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strength: A pre-doc fellow was trained through support of the project.

Weakness: No addition to infrastructure was added.

Reviewer 2:
The research is beneficial to the grantee's institution by providing support for students.

Reviewer 3:
Strengths: Research experience gained by pre-doctoral student; project contributed to the science base for research - a priority for Penn State's Children's Hospital.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
No new investigators were recruited or new collaboration was established.

Reviewer 2:
None noted.

Reviewer 3:
Weaknesses: No new collaborations or extension of work outside of the institution.

Section B. Recommendations

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.

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

Reviewer 2:

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

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.

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

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

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.

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).

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.
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.

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.

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.

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.

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.
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.

Generic Recommendations for Penn State University

Reviewer 1:
CURE grants represent a unique way to nurture investigators at Penn State University and help to establish strong research foundation within the institution as well as initiate collaboration with another institutions.

Reviewer 3:
Encourage collaborations with individuals outside of the institute/institution to broaden research capabilities within the institute/institution.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
No, objectives were not met. Initial observation that uninfected red blood cells were more readily phagocytosed from infected mouse were not confirmed. Further studies did not yield informative data.

Data were presented to show that the model did not permit the analysis of red cell ligands and macrophage receptors in their in vitro phagocytosis assay. These data were informative.

Strength: The funded investigator did conduct studies to test the hypothesis that uninfected red blood cells (RBCs) from infected mice were more susceptible to phagocytosis than uninfected RBCs from uninfected mice. The data showed that in their severe malarial anemian (SMA) model (infection with Plasmodium chabaudi followed by P. berghei) that the levels of phagocytosis were the same in infected and uninfected mice.

Weakness: The data showed that their SMA model did not confirm their initial hypothesis. As a result the Specific Aim 1 studies could not be pursued. Specific Aim 2 apparently showed no effect of malaria pigment or GPI on phagocytosis; therefore, this line of research was also not pursued.

Reviewer 2:
Unfortunately, the specific aim objectives of the proposal were not achieved.

SA1) The red cell ligands and macrophage receptors involved in phagocytosis of unfected red cells were not identified.

SA2) The very interesting and intricate sequential infection model developed in the PI's laboratory when functionally tested did not demonstrate a reproducible enhanced erythrophagocytosis of uninfected red cells in their rodent severe malaria anemia model.

The investigators tested multiple mouse models including a sequential infection scheme, multiple sources of monocytes/macrophages, and applied pharmacological agents to attempt to enhance

activity, without technical success.

Reviewer 3:
The aim of this project was to elucidate the mechanism of severe malaria anemia (SMA) using a mouse model. It had 2 aims: (1) To identify the ligands and receptors involved in erythrophagocytosis, and (2) determine if and how hemozoin and glycosylphosphatidylinositol (GPI) affect this process.

The investigators first developed fluorescent in vitro assays for erythrophagocytosis involving labeling RBCs with a dye whose fluorescence would increase when the red cell was phagocytosed. This was an important accomplishment in that the method will be useful for future research.

Next, the investigators measured the susceptibility to phagocytosis of infected and uninfected RBC from different murine malaria models. For one of the models, *P. chabaudi,* more phagocytosis was seen in vitro for uninfected RBCs from *P.chabaudi*- infected mice than uninfected RBCs from uninfected mice. However, this difference was not seen with their two other models: *P.berghei*-infected mice the "SMA" model (*P.chabaudi* followed by *P.berghei*). These results were contrary to what they expected and do not support the investigator's hypothesis that SMA is largely due to phagocytosis of uninfected RBCs during a malaria infection.
The investigators also attempted to determine if hemozoin, TNF, or other cytokines affected in vitro phagocytosis, but no effect was seen.

One weakness is that none of the figures contain error bars, nor are any p-values given. No information is given as to the number of measurements that were used to construct each bar/graph point. Error bars and p-values will be needed if the investigators wish to publish these data or use them as preliminary data for future grant proposals.

The investigators were not able to prove their initial hypotheses, but this is not necessarily a weakness. However, it is a weakness that alternative hypotheses are not entertained. For example, their data might indicate that dyserythropoiesis plays a bigger role in their anemia model than erythrophagocytosis.

A final weakness is that murine malarias are different from the human malarias in many ways. It would have been a good idea to also look at the phagocytosis of infected and uninfected RBCs from cultures of *P. falciparum,* the most important human malaria.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The topic is very important - severe malarial anemia (SMA) has a major impact on young patients with malaria and is the leading cause of deaths from malaria in infants. Dr. Stoute has

been developing a mouse model to mimic SMA in humans. Identifying the mechanisms responsible for SMA and finding ways to reduce SMA is a very important and justifiable goal. However, the findings from this study are too preliminary to have a meaningful impact on health outcomes in patients with malaria.

The investigator has developed a useful model of SMA in rodents and needs to find additional ways to leverage this model to identify useful intervention strategies. How he will pursue this was not apparent. He did see increased phagocytosis of uninfected RBCs in the P. chabaudi infection model and states that it will be important to validate and explore this further. A detailed plan for such studies, however, was not provided.

Weaknesses: Found that this model was not suitable to study phagocytosis of uninfected RBCs and, therefore, was not able to investigate red cell ligands or macrophage molecules or to screen for any compounds that would block phagocytosis and reduce anemia.

Reviewer 2:
The significance of these results at this point cannot be translated to improving health in human malaria infections.

The investigators state plans for a NIH grant submission, but the results do not support use of this mouse model if similar specific aims are proposed.

Reviewer 3:
The investigators are taking an innovative approach to an important problem, and this is a strength. Malaria kills about a million children each year; malaria-associated anemia contributes to many of these deaths. A better understanding of the process could save many lives.

The investigators did not specify future plans (such as writing new grants) and this is a weakness. They state that they are planning to continue this research (item 12), but don't go into details other than that they will next use hepatic Kupfer cells to measure erythrophagocytosis.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
No other funds were obtained at the time of the report. The investigator states that further funding is being sought. The article from Dr. Stoute's lab that describes this model (Harris et al. 2012, Infect. Immun.) states that other funds had been available including from the NIH.

Strengths: This is a priority area of research and Dr. Stoute is a well-recognized expert in this field. He has published a good number of articles in this area.

Weakness: This work to date has only resulted in one research publication (cited above). That reduces its competitiveness for NIH funding.

Reviewer 2:
No additional funds were leveraged. The investigators state plans for a NIH grant submission but the results do not support use of this mouse model if similar specific aims are proposed.

Reviewer 3:
The investigators do not discuss future plans for grant proposals.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strengths: One article from this subject area was published in a well-respected journal. The data appear to have been generated from experiments not covered in this current project proposal.

Weakness: The current study does not seem likely to lead to publication, at least not without significantly more research.

Reviewer 2:
The authors have published an excellent paper in Infection and Immunity that describe their sequential P chabaudi and P berghei infection that leads to severe malaria anemia and demonstrates erythrophagocytosis in the liver. This is the model that did not provide the anticipated experimentally tractable evaluation of uninfected red cells isolated from mice infected with these murine malaria parasites, however.

Data presented from this application could be published, but currently represent negative data, which is a challenge to present.

Reviewer 3:
There is one peer-reviewed publication, and it is a strong paper in a good journal. Overall, funding was modest so this is good productivity.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strengths: Funding provided support for Dr. Stoute to pursue an important line of research at his institution.

Weakness: While Dr. Stoute indicated a local collaborator (Dr. Channe Gowda), there was no clear evidence of a vigorous collaboration.

Reviewer 2:
Improvements to infrastructure was not requested. Support for post-doc Mbuga were provided by this award, supporting the potential development of another expert in this understudied field at the intersection of hematology and immunology.

Reviewer 3:
A new post-doctoral fellow (Amos Mbugua) was brought in to work on this grant and developed new methods for these types of studies. No pre-doctoral students were supported. Again, given the modest size of the budget, this was appropriate.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strength: There is major interest in a rodent malaria model of severe anemia and it is likely that exciting data from this model would lead to many groups becoming interested in working closely with Dr. Stoute.

Weakness: The model is still quite preliminary.

Reviewer 2:
No collaborations developed, although if the mouse model is developed further , it is likely that opportunities would exist.

Reviewer 3:
This project involves collaboration with Dr. Gowda, but it is not clear if this is a new collaboration or not.

Section B. Recommendations

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.

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.

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?

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?

3. Investigators published erythrophagocytosis in the sequential P chabaudi and P berghei infection model, and anemia in C3 -/- 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.

Reviewer 3:

1. Improve statistical analysis and presentation of data.

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

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

ADDITIONAL COMMENTS

Reviewer 1:

The studies performed during the study were adequately designed to test whether this model was suitable to pursue the specific aims. The results showed quite clearly that the model was not suitable to pursue the stated aims in any particular detail. The funding was also very limited and in reality did not permit studies beyond those pursued herein. Specific Aim 1 was totally untouched. Specific Aim 2 was apparently pursued at an initital stage, but the data did not support any modulatory effect on phagocytosis. Unfortunately, those data were not shown.

Reviewer 2:

The investigators embarked on a very ambitious goal to illuminate the basis for the loss of uninfected red cells in an acute malarial infection. Well-controlled experiments based on excellent hypotheses were performed, but unfortunately negative results were developed. Is it possible that the percol gradients that were used to separate infected red cells from uninfected red cells also separated out co-factors necessary for phagocytosis by macrophages ?

Generic Recommendations for Penn State University

Reviewer 2:

This project was an ambitious, high risk, high-potential reward proposal. Well-controlled

experiments based on excellent hypotheses were performed, but unfortunately, negative results were developed. Further investigations that integrate information and approaches gleaned from hematological studies of sickle cell anemia "bystander hemolysis," human malaria vaccination studies (resulting in bystander hemolysis), and pharmacological testing of clinically-useful drugs like eculizumab , or novel-imaging approaches may help the investigators with this very difficult project.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
On this project, the applicant has proposed to examine the anti-tumor effect of nanoliposomal ceramide using a mouse heptocellular carcinoma model in vivo. This project is based on their previous results showing anti-proliferative and anti-survival effect of liposome encapsulated ceramide on a breast adenocarcinoma cell model in vitro. The applicant wanted to extend the in vitro observation to an HCC model in mice in vivo.

This is a highly-focused project with limited novelty and significance. However, the applicant has completed the originally-proposed aims and got some interesting results, with one paper published in the journal "Gut," for which the applicant is the first author and this specific funding source is acknowledged.

Reviewer 2:
This project met the stated objectives in three specific aims: 1) determine the efficiency of ceramide liposome (lip-C_6) on inhibiting solid tumor growth in murine model; 2) determine the efficiency of ceramide liposome to inhibit proliferation and promote apoptosis in tumor vasculature; and 3) mechanisms of action of ceramide liposome on pro-mitogenic, pro-survival signaling cascades. The PI successfully determined that nanoliposomal C_6-ceramide prevented the growth of human HCC xenografts by reduced proliferation and promoted apoptosis of cancer cells and decreased tumor vascularization. The PI also characterized that the anti-tumor effect of Lip-C6 is associated with decreased phosphorylation of AKT pathway.

The strengths of the project performance are that they tested the effects of Lip-C_6 (ceramide) in HCC tumor xenograft by systemic administration (36 mg/kg, every another day, iv) for 44 days. The weakness is there is no comparison of the C_6-ceramide to Lip-C_6 to show the advantage of nanoparticle delivery.

Reviewer 3:
The objectives of this project were threefold: first, to determine the efficacy of ceramide liposome in inhibiting tumor growth in a mouse model; second, to determine the ability of ceramide liposome to inhibit cellular proliferation and promote apoptosis in tumor vasculature;

and third, to determine the mechanism of action of ceramide liposome effects on cellular signaling cascades.

Broadly speaking, the project met its stated objectives, and this is viewed as a strength. The research design and methods were adequate to meet the original objectives, and the data generally answered the proposed questions. Furthermore, the results support the conclusions that are drawn.

It appears that a different mouse cancer model was used other than the one that was originally proposed. The rationale for this change is not stated, but this is not a significant weakness, as the model that was used is appropriate. Nevertheless, the reason for this deviation should have been provided.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
This is a very small and highly-focused project. To the applicant's credit, she has performed and completed the proposed experiments. However, the reported experimental results are very much expected, with limited impact on cancer research and therapy.

The project, as presented, lacks novelty and in-depth mechanistic analysis, but is considered reasonable based on the small amount of funds allocated to the project.

Reviewer 2:
The project provides a fundament to develop an effective approach targeting hepatocellular carcinoma based on C_6-ceramide. It is very interesting that the systemic administration of Lip-C_6 significantly inhibited the tumor growth in vivo.

Reviewer 3:
The significance of this research lies in the fact that the results support the further development of this particular approach in the generation of novel cancer therapies. This may eventually lead to improvements in cancer outcomes. The likelihood of this is difficult to judge at this stage, but this is true of any preclinical research. Overall, the likely beneficial impact is therefore a moderate strength.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
No funds were leveraged.

Reviewer 2:
There is no grant application submitted.

Reviewer 3:
No grant applications were submitted, and none are planned. This is a weakness.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The applicant listed one publication in which she is the first author. The paper acknowledged funding from the Pennsylvania Department of Health.

The data reported in the paper did not suggest a robust effect of liposomal ceramide; it is unclear how much translational value this method may implicate in liver cancer treatment.

Reviewer 2:
It resulted in one peer-reviewed publication: HR Tagaram et al. Nanoliposomal ceramide prevents in vivo growth of hepatocellular carcinoma. *Gut* 2011 60: 695-701. Another manuscript was under preparation.

Reviewer 3:
A peer-reviewed manuscript was published in Gut, which is a very high quality journal. A second manuscript is planned. This is an excellent level of productivity in relation to the budgeted funds, so this is viewed as a strength.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The applicant used part of the funds to purchase a new piece of equipment, which would clearly benefit the institute. No students were involved in the research.

Reviewer 2:
This project enhanced the quality and capacity for research at the Pen State University. It improved equipment for 3D image analysis. It supported the independent research of new investigator, Dr. HR Tagaram.

Reviewer 3:
A computer and image analysis software were purchased that will enhance the quality and capacity for research at the institution. Therefore, this could be considered an improvement to the research infrastructure. However, there is no justification given as to why this was needed at the

institution, why such a large portion of the budget (>50%) was spent on this single item, and why this item was necessary for this specific project. The lack of these details is a weakness.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The publication resulting from the project was accomplished in collaboration with colleagues in several departments at Penn State University School of Medicine. Dr. Tagaram, a research assistant professor, has contributed and will continue to contribute to the development of cancer research.

Reviewer 2:
None.

Reviewer 3:
There were no collaborations with research partners outside of the institution or new involvement with the community. This is a minor weakness.

Section B. Recommendations

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.

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.

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.

Generic Recommendations for Penn State University

Reviewer 1:
It is recommended to give small amount of funding to researchers who do have innovative and also risky ideas, rather than small and safe projects without novelty**.**

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The project called for 40 patients. It is not clear if a power analysis was done to determine that 40 patients would be needed. It appears the 34 patients were enrolled in the first year, and 20 of them were in the final analysis. It is not clear why enrollment didn't continue until 40 patients were enrolled and completed the full study.

Data that was collected was not used to calculate sensitivy and sensitivity to determine poor neurologic outcome. The statistical methods used could detect significance between two groups, but not the clinical utility of such a test.

Reviewer 2:
Single biomarkers have not provided predictive value to the physician in determining which aneurysmal subarachnoid hemorrhage patients go on to develop delayed cerebral ischemia. The applicant tested the hypothesis that multiple inflammatory serum biomarkers has predictive value in determining which individuals progress to delayed cerebral ischemia. The study design was straightforward with the research design adequate to test the proposed hypothesis. The approach appeared to be carefully designed and implemented. There were no changes in the original research protocol. There were no target numbers for patients to be enrolled in the study. This is reasonable since the inclusion criteria for enrollment constitutes only a small portion of patients seen in the facility. Nevertheless, an estimate, based on previous hospital admission, would have beneficial for the initial proposal. The project was able to generate sufficient data and made acceptable progress toward the original objectives. While the data did not support the hypothesis being tested, it did add important information to our understanding of delayed cerebral ischemia. The study did uncover several interesting biomarker candidates for further studies and pointed to the idea that inflammation may not be the underlying cause of delayed cerebral ischemia.

Reviewer 3:
Project objectives were partially met in terms of analysis of serum for mediators of inflammation with respect to clinical parameters and time after aneurysmal subarachnoid hemmorrhage (SAH). Missed goals on patient numbers and analysis of cerebrospinal fluid (CSF).

Appears to have had lack of sufficient awareness of the statistical power required to meet objectives. This could have placed more emphasis on patient numbers from the beginning. Table 4 was very difficult to understand and given the number of comparisons made, I suspect the P-value should be much less than 0.01.

Their initial participant goal was 40, 34 were admitted into the study and 20 patients analyzed. Based on the number of tests and confounding parameters age, sex, smoking, it would appear that even the initial goal in terms of the number of patients would not be sufficient, given the number of factors analyzed.

On the other hand, one could consider this number of patients and tests consistent with the money expended and could form the preliminary data for a larger grant application.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The study appears underpowered with only 20 patients in the final analysis. Thus, it can only suggest that further studies be done.

Reviewer 2:
This study represents a "spoke" in a much larger wheel. Small bits of information ("spokes") collectively come together for scientists to understand the underlying disease process. While this study did uncover some potential unstudied biomarkers, the data may point to the idea that inflammation is not predictive of delayed cerebral ischemia following subarachnoid hemorrhage. Scientists need to look for other markers and possible other mechanisms for delayed cerebral ischemia. While the hypothesis was reasonable and worthy, it did not seem to hold up to testing. Future plans include assessing the rate of biomarker change as it relates to delayed cerebral ischemia. The investigators state that a manuscript is in preparation to be submitted to a peer-reviewed journal. Furthermore, "The results from these studies will likely be combined with other work to generate an application for further grant funding for the study of delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage."

Reviewer 3:
The strength of this project rests on the initial goals. They provided sufficient background evidence that indicated that mediators of inflammation might play a role in DCI. Their approach was unique in using cutting-edge techniques to study changes in a panel of 27 cytokines and growth factors with time after SAH. The hypothesis was that changes between day 1-3 and later times would provide biomarkers for DCI.

A weakness was the relatively low number of patients which appears to preclude any strong validation of their negative results. Stringent negative results would also be of importance if it could be shown that inflammatory factors were not involved in DCI. However, as the investigators state "a major weakness would be the lack of statistical power and high probability

of false negatives." Positive results could have led to development of biomarkers, which could narrow the focus for the underlying causes of DCI.

This study did not investigate these potential markers in CSF, which may have been a better source of more concentrated material, lowering the number of patients required for stringent statistics. Negative results could also be important in that they would shift the field away from inflammation factors as a primary cause.

As noted above, with more patients, this work could have significant value and the researchers indicate that they will apply for a grant to continue this work.

Excellent goal and research plan, but execution below expectation.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
No, but may do in the future. Several of the cytokines appeared promising. And no linear combination analysis was done to determine if a combination of the factors would have better predictive value.

Reviewer 2:
The investigators did not identify any additional research partners, new involvement with the community, or additional funding to continue the project (present or pending). However, given that additional studies may be needed for some of the biomarkers, there is an opportunity to develop additional collaborations with other institutions. However, such collaborations were not mentioned by the investigators. The authors did state, "The results from these studies will likely be combined with other work to generate an application for further grant funding for the study of delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage."

Reviewer 3:
No grant applications were submitted.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
No peer-reviewed publications came of this project.

Reviewer 2:
No publications, licenses, patents, or commercial opportunities were derived from the studies. The investigators did not make claims to any of the above in the original proposal. Nevertheless,

suitable progress was made with the project and the investigators are on a reasonable pace.

Reviewer 3:
No publications.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
No.

Reviewer 2:
Two MD/PhD studentsparticipated in the reseaerch.

Reviewer 3:
There was M.D./Ph.D. student participation in the project.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
No.

Reviewer 2:
The investigators did not identify any additional research partners or new involvement with the community. However, given that additional studies may be needed for some of the biomarkers, there is an opportunity to develop additional collaborations with other institutions. However, such collaborations were not mentioned by the investigators.

Reviewer 3:
A broader base of hospitals and health care professionals may have helped in generating patient numbers.

Section B. Recommendations

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.

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.

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.

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.

ADDITIONAL COMMENTS

Reviewer 2:
Delayed cerebral ischemia is a common occurrence following aneurysmal subarachnoid hemorrhage. It also is associated with a high rate of mortality and morbidity. The ability to predict which patients develop delayed cerebral ischemia following aneurysmal subarachnoid hemorrhage would be beneficial in determining treatment modalities. To date, single biomarkers have not provided predictive value to the physician. The applicants tested the hypothesis that multiple inflammatory serum biomarkers have predictive value in determining which individuals progress to delayed cerebral ischemia. The study design was straightforward with the research design adequate to test the proposed hypothesis. The approach appeared to be carefully designed and implemented. The project was able to generate sufficient data and made acceptable progress toward the original objectives. While the data did not support the hypothesis being tested, it did add to our understanding of delayed cerebral ischemia. The study did uncover several interesting biomarker candidates for further studies or possibly points to the idea that inflammation may not be the underlying cause of delayed cerebral ischemia.

The applicant did state that there may be several biomarker candidates for further study or that the rate of change of the biomarkers aneurysmal subarachnoid hemorrhage may be significant in predicting which patients are at risk for delayed cerebral ischemia. The studies did meet the outlined objectives and may be worthy of continued funding in order to help understand and predict delayed cerebral ischemia following aneurysmal subarachnoid hemorrhage.

Generic Recommendations for Penn State University

Reviewer 1:
Provide bridge funding for the addition of more patients and CSF data to enable a high-quality publication and application for NIH funding to continue this project.

Project Number: 0864523
Project Title: Tim2 Expression on Oligodendrocytes: A new Immune System Target
Investigator: Connor, James

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The hypothesis to be tested by this study was that the existence of the protein, Tim2, on oligodendrocytes renders these myelinating cells of the central nervous system susceptible to be targeted by immune cells in individuals with multiple sclerosis.

This project had two stated objectives: 1. To examine whether Sema4A protein, a ligand for the Tim2 receptor, was expressed in higher abundance in myelin lesions of human neurospecimens from individuals that had had multiple sclerosis, and to associate any changes in Sema4A expression with markers of inflammatory cell types; and, 2. To determine whether the cerebrospinal fluid of individuals with multiple sclerosis had measurably higher amounts of Sema4A protein compared with non-diseased individuals.

Strengths: The work supported by this funding mechanism sought to provide important and relevant new information on the targeting of oligodendrocytes by immune cells in the neurodegenerative disease multiple sclerosis. Based on the data provided, the investigators met their stated objectives and these data provided were developed in line with their original research protocol.

Using human tissues and clinical samples, the investigators sought to identify, based on their previous studies, whether expression of the Tim2 ligand, Sema4A occurs in association with myelin lesions in the human multiple sclerosis brain.

The investigators made reasonable and sufficient progress toward their stated objectives.

Weaknesses: 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.
An attempt at this type of analysis was provided in Figure 4 of the progress report (which was mislabled 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 allude 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.

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.

Reviewer 2:
The project somewhat met the stated objectives and generated a small amount of data. The research design and methods were not adequate in light of the project objectives. The data collected by the investigators were sparse, and were not developed sufficiently to answer the research questions posed and were not developed sufficiently in line with the original research protocol. Their immediate plan is to resubmit the manuscript with the data from the autopsy samples. They will also continue to seek funding through the NIH and private foundations.

Reviewer 3:
Aim 1: Strengths are the novel concept that shared expression of Tim-2 between oligodendrocytes and the immune system mediates OL death and inhibition of OPC-induced remyelination through Sema4A.

While the impetus of this work stemmed in part from work in EAE mouse, the importance of this project was to discover the relevancy of this mechanism in human MS.

The purpose of Aim 1 was to determine whether Sema4A was upregulated in MS lesions. This was performed by staining paraffin sections with an antibody that the PI showed was specific for Sema4A. Staining of MS patients was higher than normal tissue in the white matter. Staining appears in immune cells predominantly.

Weakness***:*** Quantitation of immunoblots appears to show that Sema4A protein levels were lower than normal patient controls (less than 1), and specifically that there was no difference between NAWM and chronic lesions, suggesting that the levels of expression are not physiologically related to facilitating the MS phenotype as originally hypothesized. In a similar fashion, this data from Figure 4 also showed that the uncleaved Sema4A was lower in active lesions of MS. Also there appears to be a significant reduction in the cleaved form compared to NAWM. Therefore, the interpretation of these results are not consistent with the data shown.

Aim 2: Strengths of Aim 2 was a novel concept of discovering the levels of Sema4A in CSF as a potential biomarker.

True efforts were made in attempt to find Sema4A in CSF. Although it could not be detected with the antibody used, the completion of this aim was not accomplished.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
There were two strengths of this project. The first strength was the hypothesis being tested. Based on a prior publication by the Principal Investigator, the work undertaken in this project provided a logical and potentially insightful corrolary of this previous study. The second strength of this work was that it provided support for two trainees; a post-doc and a graduate student. The scientific outcomes of this work did not validate the utility of screening Sema4A as a clinical biomarker for multiple sclerosis. Thus, the significance and value for improving health are minimal. However, the knowledge that Sema4A would not be a practical biomarker has value to the scientific community.

The investigators state that they have identified a potential interaction between Tim2 and Sema4A in association with a neurological infection, cerebral malaria. This is intended to be a future direction for this line of investigation by the investigators.

The likely benefit is therefore small given the short time frame and limited budget of this work.

Reviewer 2:
The goal is to advance the understanding of disease pathogenesis and treatment of multiple sclerosis by finding a way to promote remyelination by endogenous oligodendrocyte progenitor cells, allowing them to repair myelin and, therefore, prevent secondary axonal loss and neurodegeneration. The investigators have shown that Tim-2 protein is selectively expressed on cells of oligodendrocyte lineage. Tim-2 is present on mature oligodendrocytes and oligodendrocyte progenitors and functions as receptor for extracellular H-ferritin and Sema4A proteins. Considering that both Sema4A and Tim-2 have an important function in immune regulation, the investigators propose that existence of Tim-2 receptors on oligodendrocytes may predispose these cells to immune attack. The results of this research remotely support the possibility that the Sema4A and Tim-2 receptor interaction may underlie the demyelination. Further investigation in more detail and depth is needed to draw meaningful conclusions. The beneficial impact of this project is unclear.

Reviewer 3:
Strengths: Looking at the role of Sema4A and TIM-2 is highly significant for understanding the pathology of the M.S. Others have just recently shown that elevation of Sema4A may drive T helper cell skewing in human MS patients (Nakatsuji J Immunol 2012) lending support to the PI's hypothesis that Sema4A along with TIM-2 plays a pivotal role in this process. The original goal was to determine if the expression of Sema4A was contributed by either immune cells or oligodendrocytes.

Weakness: There was no discussion of future plans of this information moving forward to drug or diagnosis development, as it is too preliminary at this point.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
According to the materials provided, this work did not leverage funding from federal or private funding sources. Two grant applications were listed intended for the National MS Society and the Department of Defense.

A strength of this work was that they determined a lack of Sema4A in CSF and potentially identified its expression in myelin lesions in MS. The hypothesis tested is original and interesting.

A weakness of this work was the limited interpretation of the findings. However, given the limited funds and time frame for this project, the productivity was not inappropriate.

Reviewer 2:
None.

Reviewer 3:
Based on responses from applicant: Two NIH grants were initiated in 2010 and 2011 but were not funded. There was a plan to submit a EUREKA grant in 2013.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The findings of the funded work have not as yet been published in a peer reviewed journal. It is indicated by the investigators that it is their intent to extend some in vitro studies on the effect of Sema4A on oligodendrocyte survival to complement their pathology data that was funded by a different source. A strength of this work, therefore, would be that a publication expected from these studies, if these additional experiments are successful, will make a meaningful contribution to the field. A weakness of this work would be that the funded work did not result in any peer-reviewed publications.

There were no commerical developments, patents or therapeutics developed as a result of this study.

Reviewer 2:
None.

Reviewer 3:
Based on responses from applicant: None submitted, but there is a plan to submit OL cell culture

work to Journal of Neurochemistry.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
A strength of this project was to provide support for a post-doctoral research fellow and a graduate student. An additional strength of this funding may be the future investigation into the association of Sema4A and Tim2 in cerebral malaria. In light of the alloted budget and time frame of this work, there would be minimal weaknesses in terms of enhancing the quality for research at the grantee's institution.

No new investigators were added in order to carry out the objectives of this research project.

Reviewer 2:
The project has some moderate impact.

Reviewer 3:
This project supported the salary for 16.7% post-doc and 49.25% graduate student.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The investigators indicate that a future direction for their studies on Tim2 and Sema4A could include cerebral malaria. It might be expected that this could include a collaboration, although this was not explicitly stated by the principal investigator in their final report. If future collaborations are developed toward this new area of study, this could be viewed as a strength for the institution and as an outcome for this project.

For the funded research, no research collaborations developed. Given the scope of this project, the minimal impact on the community would not be viewed necessarily as a weakness.

Reviewer 2:
None.

Reviewer 3:
Based on responses from applicant: None.

Section B. Recommendations

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.

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 refering 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.

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.

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.

Reviewer 2:

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

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.

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.

ADDITIONAL COMMENTS

Reviewer 2:
The beneficial impact of this project is small. Although the investigators have shown some evidence indicating that Sema4A can be detected in lymphocytes in demyelinating plaques, but not in normal white matter from human tissue collected at autopsy, and that there are higher concentrations of Sema4A protein in MS patients compared to controls. The data are sparse.

Reviewer 3:
Strengths: The premise of the work was shown by others last year to play a pivotal role in human MS. Specifically they showed that Sema4A is elevated in immune cells in MS patients and that elevated protein levels are found in the sera of patients. Therefore, it will be important to determine how Sema4A is expressed and affects OL survival.

Weakness: Although, the work in this project did not completely address the issue of whether Sema4A is expressed by OLs (this is not clear in Figure 2, background too high compared to controls) or has a role with OL cell survival in MS, the PI of the grant is in a good position to study this issue.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Major strength: The investigators made good progress on Specific Aim 2. Highly-significant results were observed and this data led to a publication. The data are well-presented in the final progress report and demonstrate that significant progress was made on this aim.

Strength: There were difficulties with the techniques in Aim 1, but the investigators were able to generate at least some useful data by attempting alternative strategies.

Weakness: Unanticipated problems arose in the completion of Aim 1. The investigators identified problems with the lentiviral vector delivery system, which hindered their ability to perform the objectives of Aim 1. While these data were useful for the laboratory and others in the field, they did not allow the investigators to address the main objectives of Aim 1. This is a considerable weakness of the project.

Reviewer 2:
The broad objective of this project was to elucidate the mechanisms of microsatellite emergence, mutation, and degeneration in individual human genomes, using an interdisciplinary, computational and experimental approach. The overall goal of this project was to provide direct experimental evidence in support of a new collaborative model, in order to improve the competitiveness of an NIH-R01 application.

The experiments in this project were specifically designed to test whether in vitro results accurately reflect microsatellite mutagenesis occurring in human genomes, a concern of the NIH grant reviewers in the first NIH submission. The goals of this project have been accomplished: an interdisciplinary, multiple principal investigator (MPI) NIH grant has been obtained, and a co-authored, interdisciplinary manuscript describing the results of this research has been accepted for publication.

The design and method were met and adequate to achieve these goals. The findings are applicable to the project objectives

Reviewer 3:
A strength of this project is a series of new findings that have distinct health-related implications. Dr. Eckhart and associates found that dinucleotide repetitive sequences are expanded in mismatch defective cells; previous studies of this show that this expansion was dependent on DNA polymerase κ expression. In this project, they try to repeat the κ experiments using greater amounts of κ and were not really successful. However, their experiments on repeat length are quite elegant and they show repeat length expansion and deletions as separate functions. This provides information for a detailed molecular analysis, which is going to be carried out in a collaborative project that has been submitted and funded by the National Institute of Health. The health-related implications are the function of repeat length for sensitivity studies on mutagenesis by microsatellites, which will have implications for mutation detection−particularly with respect to smoking.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strengths: New discoveries were made during this project that add to knowledge in the field of microsatellites. These findings are relevant in the basic science realm, and perhaps eventually in the clinical realm as well.

The problems with Aim 1, while a major setback to accomplishing the goals of Aim 1, allowed for a number of technical difficulties to be remedied. These will benefit the lab and others in the field using similar methods.

The beneficial impacts of this project are reasonable, considering the amount and time period of the funding. The investigators were quite productive in such a short time period and with the limited budget.

Reviewer 2:
The project provided new mechanistic insights into microsatellite biology. However there is no path forward to translate this into a clinically-useful diagnostic. Given that this was a modest-size project, the progress is reasonable and adequate. The project resulted in a publication, albeit in a non-high impact journal.

Reviewer 3:
The strength of the project is the new discoveries and the very careful experiments that have been carried out.

The weaknesses of the project are they did not use controls in the experiments with pol κ. It would have been nice to have a xeroderma pigmentosum deletion control to show the role of pol η, 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 the funding was clearly only a tokenism to get them started for an NIH grant and I hope a lot of studies will be

considered.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Major strength: Two NIH R01 grant applications were directly related to this project. One of these was unfunded and the other funded.

Strength: Two additional grant applications were planned, relating to this project. One of these is the renewal of the above-mentioned R01, and the other to the Crohn's and Colitis Foundation of America.

Reviewer 2:
The project very successfully leveraged progress to obtain an NIGMS R01 with this project as additional data to respond to the first round of critiques. However, overall the PI is not planning to take this research forward further.

Reviewer 3:
A grant application has been funded as a result of this project.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strength: One manuscript was published in "Genes, Genomes, Genetics." Since one of the specific aims was not accomplished, it is impressive that the investigators were able to publish the findings based only on the second specific aim.

Reviewer 2:
The project resulted in one peer-reviewed publication.

Reviewer 3:
One important article been published that contains most of the information found by these investigators.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strength: Funds were used to pay for one student for a year.

Reviewer 2:
The project supported a graduate student, which helped improve the overall learning environment of Penn State University. There were no new researchers recruited to PSU.

Reviewer 3:
It surely increased the quality and capacity for research at the grantee's institution. An interdisciplinary grant has been submitted and approved.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strength: Collaboration was extended to include Drs. Makova and Krasilnikova at Penn State, University Park.

Reviewer 2:
The PI is hoping to collaborate with other groups to extend this work.

Reviewer 3:
This projet led to an interdisciplinary NIH grant that has been approved. It brings together computational analysis into the length of polynucleotide expansion.

Section B. Recommendations

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.

Reviewer 2:
1. Would have been nice to have publications in more impactful journals.

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.

3. No new techniques developed.

Reviewer 3:
The weaknesses of the project are they did not use controls in the experiments with pol κ. It would have been nice to have a xeroderma pigmentosum deletion control to show the role of pol η, 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.

Generic Recommendations for Penn State University

Reviewer 2:
Matching funds for these grants would be very helpful to leverage them.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
This proposal performed correlative studies on samples from ongoing Phase I/II clinical trial at Oregon Health and Science and Pennsylvania State University evaluating combination epigenetic therapy SAHA and cladribine along with anti CD20 blockade with rituximab or SCR trial in 40-50 patients with relapsed B cell malignancies. The authors proposed to study: 1) global methylation and histone modifications with response to treatment, 2) perform global expression profiles in pre-and post-treatment samples and identify candidate genes and/or miRNA and then, 3) validate mechanism of action of candidate targets using overexpression and loss of function studies.

Strengths: Overall, the investigators have promising clinical data which was presented at ASH in 2012 with overall response rates of 100% and 74% achieving complete remission in 27 patients with mantle cell lymphoma (MCL). None of the patients who had a complete response have relapsed with median f/u of 18.2 months. Patients with blastic mantle cell lymphoma did not respond. Global methylation data shows hypomethylation in 6/6 patients as well as loss of histone methylation. Several upregulated genes were identified but they are not shared across patients. One of the candidate genes DUSP2 has some correlation in primary samples as being reexpressed by RTPCR and some increase in protein.

The mechanism of action of cladribine acting on histone methyaltion appears to be robust with Western blots showing loss of h3k9 and h3k27 trimethylation both in cell lines and primary samples and loss of even mono and di methyaltion at these residues in Granta 519 cell lines.
In understanding the mechanism of action, some studies on a polymorphism for cyclin D1 are performed and the GG genotype appears to correlate with response rates potentially with cyclin D1 functioning as a transcription factor and interacting with cyclin D3 when it's overexpressed as with the AA genotype.

Weaknesses: Overall, although SCR therapy is efficacious, the mechanism of action studies are not clearly delineated and this would be helpful to understand what has been accomplished. Summarization of the global studies on expression and methyaltion that were performed including number of patients where these studies were done would be useful. The global methylation assay data (HELP assay) results are shown on subsets on MCL and CLL in Figures

3 and 4 in the final report, but again unclear how many patients was this performed on. Several genes became hypomethylated but the numbers or % are not clear...is it the 7 circles or 7 genes out of 14,000 genes in Figure 3 or 8 genes in Figure 4? A summarization of this data would be helpful to understand if hypomethylation is indeed the mechanism of action or only a handful of genes are hypomethylated.

Similarly, expression profiling was done on a subset of patient samples. How many samples, what platform was used? MiRNA studies had been proposed, were those ever done?
The Western data on DUSP2 is promising but then CHIP studies are performed to understand the mechanism of action of its reexpression but only H3lysine 27 tri methyl mark is studied and not the activating mark H3K4.

The dosage of cladribine changes between the experiments ranging from 20uM for Western assays and then some of the CHIP studies are done with 100nM of cladribine. What was the rationale for changing doses? What are the equivalent levels in clinical trials? This is the dose that should be used in the in vitro assays.

It would be useful to have summarization of all the patients on the clinical trials to understand if the efficacy is more of a global phenomena and if correlative studies have been done on those patients and show similar results.

Reviewer 2:
The major strength that has driven this project is the clinical trial being carried out parallel to these studies and not funded by this research project. The use of cladribine with vorinostat and rituximab for the treatment of B cell malignancies is being evaluated in a Phase1/2 setting at Penn State in collaboration with the Oregon Health Sciences University.

A strength of this project is the proposal that the cladribine used in this study is acting via an epigenetic mechanism, causing a loss of methylation at critical sites in the promoter regions of growth-promoting genes, and also decreases in the level of methylation at histones regional to these positions. The purpose of this project is to gather information on whether these effects are happening and, to the extent possible, whether epigenetic phenomena are involved in the therapeutic benefit being seen in the combination trial.

A weakness of the project is that the data is rather unconvincing and inconclusive. The effects of CDA on DNA methylation are actually being carried out at Albert Einstein Cancer Center as a collaboration, and the histone methylation studies and chromatin immunoprecipitations (ChIPs) and RT-qPCR experiments are being pursued at Penn State in Dr. Epner's laboratory.

The DNA methylation changes in cell lines are being studied using a ligation mediated amplification of HpaII/MspI-treated DNA followed by a CHIP analysis. Multiple hypomethylation events are detected at numerous genes in cell line studies, with some being followed at Penn State by RT-qPCR on both cell lines and patient peripheral blood cells. There is no consistent pattern in the data being collected, and the data listed on the most promising gene, DUSP2 is most consistent with random variations in the data. The investigators instead concluded that there were differences in some patients but not all, with no control data

showing consistency in the techniques used.

The data on DNA demethylation leading to re-expression of individual genes is unconvincing.

A strength of the performed research is a convincing demonstration of a much lower level of methylation of histones globally at H3K27me3 and me1 as well as H3K9me1,2, and 3 in a B cell line. Previously, related experiments had been shown only in a few papers from a Polish group.

A weakness of the project is that the analysis did not extend to other histone modifications, specifically H3K4me3, as well as H3AC and H4Ac. Should the data show a specific depletion of the histone methylation marks associated with inactive loci but no effect on the methylation marks associated with active promoters, they would have an interesting case. As it is, they seem to only have examined the histone modifications that would agree with their hypothesis. Likewise, histone methylation depletion was shown in a patient sample identified as being treated with cladribine, but it is most likely that the patient was involved with the ongoing Phase1/2 trial of the triple combination of cladribine, vorinostat, and rituximab, so there is no way to tell which drug was responsible for the effect, and to imply it was cladribine is viewed as a weakness.

Another major weakness is that the work conducted under this support has not resulted in any publications, in spite of the listing of 7 abstracts presented under this support over four years. The only paper listed as in preparation is aimed at PLOSOne and involves other research supported, to this reviewer's eye, by another funding source.

Reviewer 3:
The investigators have made advances in the therapy of B cell malignancies such as CLL and MCL. More specifically, they have identified a nontoxic combinatorial therapy which is highly signficant. The combination of cladribine and rituximab was found to be not only nontoxic, but, most importantly, a potent approach to CLL and MCL therapy.

Therefore, the objectives were met and the research design and methods were appropriate for achieving the objectives. The data and information provided were applicable to the project objectives.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strengths: The clinical trial (SCR trial) on which the correlative science was performed in this grant appears to be highly promising against certain B cell malignancies including mantle cell lymphoma, chronic lymphocytic leukemia and other indolent B cell lymphomas. The investigators state that they have initiated clinical trials based on these results. The response rates for subsets of these patients have been described such as 15 patients with mantle cell lymphoma with response rates of 100% and 12 patients having complete remission.

Weaknesses: The results of the entire trial in synopsis would be useful to understand how efficacious the combination therapy will be. In addition, what trials have been initiated based on the clinical and laboratory data would also be useful.

Reviewer 2:
The clinical trial that is being carried out in parallel to this correlative work may have an important effect on the treatment of cell malignancies, but the studies carried out specifically under the support of this funding would not appear to have any long-term effect. The research carried out under this support has been inconclusive on the involvement of epigenetic phenomena on the clinical activity of cladribine. The activity of cladribine as a single agent in B cell malignancies was known prior to this project.

Reviewer 3:
This project is signficant in improving health because B cell malignancies take many lives each year and advances in therapy for this disease are highly important. This project will improve health outcomes through the development of combinatorial approaches that are nontoxic and potent in treating B cell malignancies.

Future plans are to extend investigations to the mechanism and activity of action of this therapeutic approach in order to explore further approaches to therapy.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strengths: The project team has been able to make excellent use of this pilot data to leverage for multiple other grant mechanisms. In addition, the data in this proposal were correlative studies based on an ongoing clinical trial which was based on other funding mechanisms. The investigators obtained $125,000 from the Lymphoma Research Foundation which funded subset of these studies. In addition, they have applied for funding from Lymphoma Research Foundation for multiple (3) projects in Feb 2013 based on initial data from this grant with funding amounts requested ranging from $250,000 each for "Combined epigenetic therapy and monoclonal therapy for treatment of chronic lymphocytic leukemia" and "Combined epigenetic therapy for treatment of mantle cell lymphoma" as well as $600,000 requested for "Combined epigenetic therapy for treatment of follicular lymphoma". All these awards are pending.
In addition, future grants are planned investigating role of cyclin D1 for NIH funding and future trials funding is also being sought.

Weakness: None.

Reviewer 2:
The investigators have shown diligence in seeking external funding of correlative clinical studies related to this project. However, no external funding has yet to be awarded that stems from this work.

Reviewer 3:
There are three pending grant applications as a result of this funding opportunity. All of the pending applications have been submitted to the Lymphoma Research Foundation. The total amount requested of these three pending applications is $1,100,000. This represents an outstanding funding effort.

In addition, there are plans to submit a grant proposal on the transcriptional role of Cyclin D1 in mantle cell lymphoma. Importantly, this application will be submitted to the NIH according to the information provided in the final progress report.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strengths: The research team has suitable plans for publication. One publication was submitted to PLOS one in Jan 2012 but it is unclear if this has been published. Other publications are planned on the clinical trial along with its accompanying correlative data. Two other papers are planned including one on CD20 regulation and its role in combination epigenetic therapy and CD20 therapy resistance and another on the role of cyclin D1 as a transcription factor.

Weakness: The investigators could have updated on the status of their PLOS One paper if it was submitted in 2012.

Reviewer 2:
The investigators expect to submit several papers on this work, but it is not clear from the data presented whether they have a data set that would support any publications. The one PLOS One submission they attached as a potential publication from this work does not appear to stem from this funding and represents a collection of data from another previously-funded project.

Reviewer 3:
A manuscript for publication has been submitted to PLoS One entitled "Oncogene addiction/substitution in Cyclin D1 deregulated B cell malignancies." PLoS One is a leading journal and the PI is listed as the senior author on the submitted paper.

There were seven abstracts/meeting posters presented during the project period. The awardees are planning at least three further publications based on the data derived from the work on this grant (one clinical paper on the SCR trial results, one on CD20 regulation and a third on Cyclin D1 activity as a transcription factor).

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The proposal was successful in improving the research infrastructure of the host institution. Funding was used to support two pre-doctoral students. No out-of-state researchers were recruited for this proposal, although collaborations were performed with out-of-state investigators.

Strengths: The project expanded the scope of clinical trials at Penn State University and performance of cutting-edge research and allowed collaborations with other institutions such as Albert Einstein.

Weaknesses: None.

Reviewer 2:
There were no enhancements in research infrastructure as a result of this funding, nor were there new investigators added to PSU faculty or staff.

Two pre-doctoral students were supported by this project during the period of funding. The role of these students in the described work was not elucidated in the text, nor whether degrees were awarded.

Reviewer 3:
Funds were used for two pre-docs who were conducting studies related to the project and were involved in the presentation of the data derived from this funding.

This project also expanded the scope of clinical studies at Penn State(Hershey) and facilitated patient access to experimental therapies.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strengths: The project was successful in having collaborations with outside institutions including Dr. Samir Parekh at Albert Einstein College of Medicine for high throughput screening of methylation patterns and gene expression profiles of pre- and post-treatment lymphoma samples. In addition, a collaboration was formed with Dr. Mirit Aladjem at the NCI for investigating possible resistance mechanism to combination epigenetic and anti CD20 monoclonal therapy by downregulation of CD20 protein.

Weakness: Although the collaboration with Dr. Parekh is described, it is unclear what the results

are from the collaboration at NCI.

Reviewer 2:
This project supported a collaboration with an investigator at Albert Einstein School of Medicine.

The project was one that sought to fund correlative studies on an ongoing clinical trial but did not support the trial itself.

Reviewer 3:
There are two major collaborative efforts.
1. Collaborations are ongoing with Dr. Amir Parekh at the Albert Einstein College of Medicine.
2. Collaborations have been developed with Dr. Mirit Aladjem at the NCI.

These are significant collaborative efforts and will likely be important to further success of the efforts aimed toward continuing progress on the research developed from this funding opportunity.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:
1. The investigators have been successful in leveraging a small clinical trial which has shown encouraging results to understand the mechanism of action. It would be helpful to summarize the results of the entire trial and the exact number of samples that were interrogated with the global methylation and expression assays, to understand the mechanism of action of the triplet therapy.

2. Although the investigators propose that cladribine acts as a hypomethylating agent, the only data that is shown is the HELP assay on ? number of samples. It is unclear how many genes get demethylated and if the investigators validated demethylation by another strategy such as bisulfite sequencing.

3. The investigators mention activation of apoptosis pathway with caspase activation briefly in one of their reports but little detail is provided again in understanding the mechanism of action.

Reviewer 2:
1. The DNA methylation studies had identified specific gene loci at which DNA methylation was modified in a cell line and in patient samples after treatment with CDA. The later RT-qPCR studies on the patient samples were not consistent with any single molecular change and were most consistent with random changes in the level of expression of the genes identified for study by the DNA methylation data. The cell line data could be amplified to include repeated analyses, histone methylation and acetylation studies of marks associated

with positive effects on transcription, as well as the limited survey currently conducted on only H3K9 and K27.

2. With the level of change in global histone methylation shown in the cell line figure, changes at individual genes should be detectable.

Reviewer 3:
None.

Generic Recommendations for Penn State University

Reviewer 2:
More careful reiview of projects should occur prior to project selection for a grant. The level of funding granted to this project ($ 434,000) is substantial and is not in balance with the results shown.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The experiments reported by Hajnal et al, in this formula grant, met the stated objectives. The designs were appropriate given the stated methods. The data were explored and in line with the stated objectives of the proposal. Overall, this was a highly successful project, that lead to 2 conference abstracts and 1 published paper, with another in preparation. No weaknesses were noted.

Reviewer 2:
The project "Novel Multielectrode Recording Techniques for Assessment of Taste Function in the Brain" had the goal of testing two hypotheses concerning the role palatability plays in guiding excessive food intake in obesity. First, it was hypothesized that, compared to normal body weight animals, fat-induced obese rats would show an increased neural activity in response to high sucrose concentrations and a lower response to low sucrose concentrations. Second, it was hypothesized satiation to sweets would have a blunted effect on neural activity for obese rats. The results showed neural taste responses paralleled behavioral responses in that appetite was increased in the obese animals and the "feeling" of pleasantness to sweets was blunted. Experiment 1 tested the effect of various diets on their efficiency in developing obesity and altering taste perception of sweet. During an 8-week testing period, a very high-fat diet significantly increased body weight (a difference not related to energy intake) compared to the effects seen with a high-energy diet or regular rat chow. Experiment 2 tested the effects of satiation on neural coding in genetically-obese rats. As hypothesized, compared to normal body weight animals, the obese rats showed a reduced neural response to lower concentrations of sucrose and an exaggerated response to higher sucrose concentrations.

Reviewer 3:
The stated objective of this project was " . . . to develop novel multielectrode recording techniques necessary to obtain critical pilot data for a future NIH R01 grant application." This, it was proposed, would be accomplished in two experiments. Experiment 1 employed purely behavioral procedures to examine the influence of diet composition on body weight, body composition, daily energy intake and weekly caloric efficiency. Experiment 2 examined the

effects of satiation on neural coding in the parabrachial nucleus (PBN) for gustatory stimuli (citric acid, sodium chloride and sucrose) in free-moving rats.

Weaknesses: The objective suggested to me that the enterprise was about the development of some new recording technique. Disappointingly, I see no text in the progress reports that details how this was achieved. Rather, the technique seems to have been fully realized at the outset of the project and employed in one of the two proposed experiments. Is this a weakness? Clearly, this research was less about the development of a new recording technique and more, perhaps, about gaining experience with that approach.

Strengths: The objective of obtaining NIH funding was successfully accomplished.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The likely benefits of basic science are normally unknown. However, there is some promise that understanding the effects of obesity and energy balance on taste reward may lead to novel strategies for therapy. The only weakness noted is that the basic science work is very far removed from potential benefits to patients. For example, these data may not eventually translate to human anatomy or beneficial therapies.

Reviewer 2:
Obesity is a global problem. A further understanding of the behavioral and neural responses in animal models to obesity may eventually lead to more effective and lasting treatments of weight problems in humans. This project provides another piece in the understanding the complex problem of the etiology of obesity in humans.

Reviewer 3:
Although the work is framed in terms of obesity, it was not, as far as I understand, intended to have any direct influence on improving health. Rather, it’s a basic science feasibility study of taste function in the rat.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The experiments reported here led to an important publication and also facilitated a new NIH grant to study the effects of obesity and energy balance on taste function. This is an important strength for the institution and the local economy.

Reviewer 2:
The senior investigator was awarded a five-year NIH grant (R01 DC000240-29; Neural Systems of Ingesting Behavior).

Reviewer 3:
A competing renewal of the NIH R01 grant entitled "Neural Systems of Ingestive Behavior" was obtained (amount awarded: $1,647,940).

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Thus far, the funded project has produced 1 publication in a good-quality journal. The PI states that an additional publication should be forthcoming. Given the amount funded for this project, the publication record is a solid strength.

Reviewer 2:
So far, one peer-reviewed publication ("Gastric Bypass Surgery Alters Behavior and Neural Taste Function for Sweet Taste in Obese Rats" American Journal of Physiology, Gastrointestinal and Liver Physiology 299:967-979, 20120) and two conference presentations (Acute surges of blood glucose affect pontine taste processing differentially in lean and obese rate, presented to the Society of the Study of Ingestive Behavior, 2009 and Parabrachial taste responses to sucrose, fructose and Polycose in the rat, presented to the Association for Chemoreception Sciences, 2010.)

Reviewer 3:
One article was published:
Hajnal, A., Kovacs, P., Ahmed, T.A., Meirelles, K., Lynch, C.J., and Cooney, R.N. (2010). Gastric bypass surgery alters behavioral and neural taste functions for sweet taste in obese rats *American Journal of Physiology, 299*, G967-79.

An additional manuscript is mentioned but no details were provided. This untitled work will acknowledge partial funding from Pennsylvania Department of Health.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
An undergraduate participated in the research.

Reviewer 2:
One undergraduate student was funded, no recruitment of an out-of-state researcher was reported

and an NIH R01 was funded for 5 years in part because of the preliminary data collected using the multielectrode recordings.

Reviewer 3:
The work aided, in unspecified ways, the improvement in chronic multi-electrode recording in the PI's lab. No new investigators were added/brought to the Penn State Medical School. However, an undergraduate student participated in some unspecified way in this project.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
None.

Reviewer 2:
None identified in the progress report.

Reviewer 3:
No, the final progress report details no new collaborations.

Section B. Recommendations

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.

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.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Overall, the project achieved the majority of the initially stated objectives. The original proposal had 4 specific aims that were eventually consolidated to 2 specific aims and resulted in 2 publications. Research team has performed all stated experiments and generated several pieces of new information for changes in retina in type 1 diabetes.

Research design: The original first and second aims to investigate the impact of glycosphingolipids on insulin signaling and cell death in ex-vivo retinas and examine the protective effects of inhibiting glucosylceramide synthase on the metabolic milieu. Studies related to the first aim are completed and resulted in a publication. Studies related to Aim 2 were performed, however there is inconclusive results in diabetic retinas. Alternative strategies using knocking down expression of glucosylceramide synthase were performed, however it did not produce effective protection as expected.

The original third and fourth aims were consolidated to one aim that focused mainly on analyzing lipid metabolites in particular circulating sphingolipid metabolites by LC-MS/MS from diabetic mice. Results showed increases in sphingosine-1-phosphate and decreases in neuroprotectiveomega-9 nervonic acid. Authors concluded that these changes can serve as biomarkers for type 1 diabetes. The results were compiled into a manuscript that was published in J. Lipid Research. Clearly this part of the project was completely executed and generated new information.

Reviewer 2:
The PI addressed most of the objectives initially proposed. The research design and methods used to address Aim 1 were excellent. The data obtained supported previously published data as well as data obtained by other investigators in other tissues. The PI expanded published data by examining mechanisms involved in the regulation of the retinal insulin receptor signaling pathway and showing that cholesterol-enriched membrane microdomains are necessary to mediate insulin-stimulated Akt signaling and downstream signaling. His work demonstrated that PKC-inhibition of insulin signaling results from impaired translocation of Akt to the lipid microdomains. To further expand the work proposed in the present project, the PI has assessed the possible role of glycosphingolipids on inflammation by performing studies in a microglia cell

line (BV-2). In this cell line, LPS-stimulated activation of NFkB in presence of glucosylceramide synthase (GCS) inhibitors was evaluated and shown to be reduced. However, since an siRNA approach to diminish GCS expression was unable to block/reduce NFkB activity, the results may imply that some of the chemical inhibitors used to block GCS may inhibit NFkB activity by mechanisms other than GCS inhibition. This point, as the PI recognizes, needs to be further addressed, since it will considerably impact the interpretation and significance of the results obtained in the second aim of this project.

The PI also expanded his quest to uncover other lipids within lipid microdomains that could impair insulin signaling and lead to decreased survival or decreased protection against apoptosis of retinal cells. The data obtained is interesting but quite limited. A considerable amount of lipid-derived second messengers are released during oxidative stress, which is markedly increased in diabetes, and therefore, there are multiple candidates with a possible role in retinal cell apoptosis and survival. The PI focused his interest in the decrease of phosphatidic acid but since PA may interact with multiple proteins it is unlikely that it may represent an ideal target for therapy. Data obtained in Aim 2 of the proposal requires validation. The results in the two animal models tested (STZ-treated diabetic rat model and Ins2 Akita model) to assess the efficacy of GCS inhibition on diabetic retinopathy, are promising, however essential validation is missing and without it, proper interpretation of the results cannot be made. Data showing that GCS in the diabetic retina of the two animal models was decreased after administration of the GCS inhibitors and that the inhibitor reached the retina are essential to validate the studies performed. Studies performed in Aim 1 using cultured cells did not exclude the possibility that the anti-inflammatory effect of the chemical GCS inhibitors may not be mediated by GCS. Therefore, the effect of GCS inhibitors in reducing the development of retinopathy in the animal models studied could be secondary to the anti-inflammatory action of the GCS inhibitors and not to a decreased concentration of GCS in the retina.

The PI mentions several possible projects that could be an important next step in the future development of his research. However, until the validation of the results obtained in the animal models and a clear explanation why GCS siRNA inhibition did not lead to reduced inflammation are provided, research in this area cannot be pursued with any assurance that the results will be meaningful or have any therapeutic implications.

The work performed examining the circulating sphingolipid levels in models of type 1 diabetes is quite interesting and very relevant. Increased focus in ceramide-induced development of retinopathy would certainly be of great interest and it has great potential to lead to therapeutic strategies.

<u>Reviewer 3:</u>
Aim 1 was successfully completed with 3 papers (2 official and 1 related).

Aim 2 was not completed. What the PI did accomplish produced negative data.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The results of the completed studies provided new and exciting information about how type 1 diabetes causes specific changes in sphingosine lipid metabolite in plasma and retinas in 2 different animal models. Also, the results elucidated how alteration in lipid rafts can inhibit survival signal of insulin resulting in neurodegeneration in the diabetic retina.

The research team, led by the PI, was keen on executing the planned experiments, analyzing data and communicating the new information to the scientific community through publications (3) and obtaining extramural funding, too. As such, the project has great beneficial impact.

Two major discoveries are documented by the results of completed studies: First, that changes in lipid metabolites can be detected systemically and can serve as biomarker for diabetic retinopathy, and secondly, that results identified omega-9 fatty acids (nervonic acid), nutritional supplementation as a putative preventative treatment of diabetic retinopathy.

Weakness: Experiments using inhibitors of the glucosylceramide synthase were promising but were not conclusive. Investigators should continue to invest in optimizing the use of inhibitors, as they could be druggable targets for treatment of diabetic retinopathy.

Reviewer 2:
Sphingolipids are very important molecules since they are important mediators of cell behavior and cell signaling. Therefore, studies examining the role of sphingolipids in disease pathways are extremely important and very relevant. Sphingolipids as mediators of cell signaling constitute a rather novel and quite important field of research.

This project has potentially high impact in influencing health outcomes, if the studies performed uncover possible targets of treatment to reduce or prevent the development of complications, specifically retinopathy, in diabetes.

Some of the data derived from work performed in this project may help to uncover important diabetes-induced changes in sphingolipids and be eventually used to recommend the use of nutritional supplements to prevent the development of complications, specifically retinopathy, in diabetes.

Some future studies delineated in the progress report do not show great promise because they are based on data not yet validated and that will need to be validated prior to pursuing any further studies. However, some of the proposed studies aimed at broadening the spectrum of sphingolipids measured in tissues of diabetic animals have the potential of uncovering important information concerning the role of sphingolipids in the development of diabetic complications. Therefore, the data obtained in the previous funding cycle can open novel and important areas of research.

Reviewer 3:
The work derived from Aim 1 was valuable. The work in Aim 2 was not completed and this would have had a larger impact on human health. No changes in risk factors or information on disease was generated.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Yes: Studies to Verify Dysfunctional Toll-Like Receptor Signaling and Diabetic Retinopathy, PA Check-OFF program, DOH $178,619.

Reviewer 2:
The PI has applied for and obtained funds from JDRF to verify dysfunctional toll-like receptor signaling and retinopathy, based on preliminary data obtained during this funding period.
The PI states that he will use some of the data obtained through PA-CURE funding to submit a competing renewal of his present NIH Grant.

A number of possible future projects derived from data obtained in the present funding period are mentioned by the PI in his progress report and it looks interesting and clinically relevant. Some of the future projects being considered are quite promising and likely to obtain funding.

Reviewer 3:
One grant from JDRF was generated from the data from this grant. The PI states that some of the data may be used for a competing renewal for March 2013

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Excellent productivity and publication profile related to the project:

Fox TE, Young MM, Pedersen MM, Giambuzzi-Tussey S, Kester M, Gardner TW. Insulin signaling in retinal neurons is regulated within cholesterol-enriched membrane microdomains. Am J Physiol Endocrinol Metab. 2011 Mar;300(3):E600-9.
Fox TE, Bewley MC, Unrath KA, Pedersen MM, Anderson RE, Jung DY, Jefferson LS, Kim JK, Bronson SK, Flanagan JM, Kester M. Circulating sphingolipid biomarkers in models of type 1 diabetes. J Lipid Res. 2011 Mar;52(3):509-17.

Fox TE, Young MM, Pedersen MM, Han X, Gardner TW, Kester M. Diabetes Diminishes Phosphatidic Acid in the Retina: A Putative Mediator for Reduced mTOR Signaling and

Increased Neuronal Cell Death. Investigative Ophthalmol & Visual Sci.;53(11):7257-67, March 2011. PMCID: PMC3478036

Patency: provisional patent filed before start of PA CURE grant. Research team plans to continue discussion with pharmaceutical companies.

Reviewer 2:
Three publications were listed in the progress report which were associated with the aims of this project. The number of publications was adequate and they appeared in prestigious, high-ranking peer-reviewed journals. The 3 papers were of excellent quality.

According to the PI, an invention disclosure entitled " Method and system for altering dysfunctional lipid flux in diabetic complications" was submitted by Drs. Kester and Fox and describes several modalities to restore lipid homeostasis and minimize diabetic complications. A patent was filed for this invention, however, in 2005, prior to obtaining funds to the PA-Cure project.

Reviewer 3:
Three papers (2 official and 1 related) were generated from this work. They were published in strong journals (IOVS, Am. J. Physiology, and J. Lipid Research.

One patent was filed in 6/05 with the provisional patent filed before this grant activated. There is no license of the patent and no success in commercial partners.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The funded project did enhance the quality and capacity for research at the grantee's institution at multiple levels.

Funds were used to partially purchase ABSciex 5600 Triple TOF to perform proteomic, metabolic, lipidomic analyses.
Funds were used to support research conducted by undergraduate student as well as by a post-doctoral fellow.

New researcher was brought to the institution to earn a degree. Xianming Wang, M.S., obtained his master’s degree from Penn State University, Department of Pharmacology.

Weakness: None.

Reviewer 2:
The project did definitively enhance the quality and capacity of the research at the Penn State University. The development of methodology to measure circulating sphingolipids is extremely

important since few institutions are equipped to perform these measurements. Interest in sphingolipid measurements is likely to grow and these measurements are a real asset in research. They may also eventually become quite important for clinical purposes.

Improvements were made in the infrastructure since a new piece of equipment (BASciex 5600 Triple TOF) was purchased with partial funds from the PA-Cure project. This instrument allows to perform proteomic, metabolomic and lipidomic analyses. According to the PI, the instrument has been used by multiple PIs and in multiple research projects across a wide range of disciplines.

New investigators were brought into the institution to perform the work proposed in the project and both one undergraduate student and a post-doctoral fellow participated in the project.

Reviewer 3:
A major piece of equipment--ABSciex 5600 Triple TOF was purchased. The PI states this provided data for over 20 separate PIs within PSU.

Two new investigators were brought in through the project,1 undergraduate and 1 post-doctoral fellow.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The project initiated new collaboration with University of Oklahoma (Dr. R.E. Anderson) as well as continued collaboration with University of Michigan (Dr. Thomas Gardner).

Successful collaboration is documented by co-authored publication in J. Lipid Research 2011.

Reviewer 2:
Collaboration was previously established and it will continue with the University of Michigan (Dr. Thomas Gardner) and new collaboration was established with the University of Oklahoma (Drs Pederson and Anderson). The collaboration has been well-demonstrated by the publication of manuscripts involving the investigators listed above.

Reviewer 3:
One outside recruitment of Wang. Established collaborations with University of Oklahoma. No new benefits to the community.

Section B. Recommendations

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 glucosylacermide 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.

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.

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.

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.

Reviewer 3:
1. 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.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The Pennsylvania State University provided research support to William B. Reeves, M.D., to investigate the cross-talk between renal epithelial cells and dendritic cells in acute kidney injury. Three specific aims were proposed to perform these studies. Specific Aim 1 was proposed to confirm the presence of defective TLR4 signaling in proximal tubule epithelial cells. Renal epithelial cells of mouse and human origin will be cultured *in vitro* and exposed to TLR4 agonists. Specific Aim 2 was proposed to demonstrate specific TNF-alpha gene recombination in the proximal tubules of GGT-cre/TNF flox/flox mice. Proximal tubules will be microdissected from GGT-cre/TNFflox/flox mice and DNA recombination and mRNA expression of TNF-alpha will be determined. Specific Aim 3 was proposed to generate a dendritic cell specific TNF-alpha knockout mouse. TNF flox/flox mice will be bred with a dendritic cell specific cre-transgenic mouse to create a dendritic cell-specific TNF knockout. The proposed studies are of vital importance in acute kidney injury (AKI) since drug toxicity and ischemia-reperfusion is the leading cause of AKI and 5-7% of all hospitalized patients suffer from renal disease. The cellular and pathophysiological mechanisms involved in the development of AKI are not well understood. The studies proposed by the PI are toward examining these mechanisms.

The strengths and weaknesses of the studies are as follows:

Strengths: The PI has successfully completed most of the proposed studies and provided novel information that dendritic cells play a renoprotective role in cisplatin nephrotoxicity.
The PI has demonstrated for the first time that endogenous IL-10 is involved in providing protection from cisplatin nephrotoxicity. In these studies, the PI clearly showed that IL-10 produced by dendritic cells contribute significantly in providing a protective effect in cisplatin nephrotoxicity and associated inflammation.

The studies proposed in Specific Aim 1 to confirm the presence of defective TLR4 signaling have been successfully completed. The PI has elucidated the mechanism of TLR4-mediated lower production of TNF-alpha and showed a defect in mRNA translation but not in TNF-alpha mRNA transcription. However, on overexpression of TLR4, LPS-induced TNF-alpha production was demonstrated to be significantly increased.

The studies proposed in Specific Aim 2 to demonstrate specific TNF-alpha gene recombination in the proximal tubules of GGT-cre/TNF flox/flox mice were apparently accomplished. The PI has successfully used the Cre recombinase approach to generate proximal tubule-specific TNF-alpha-deficient mice. It was shown that expression of GGT-Cre mice was delayed until 7-14 days postpartum; thus, recombination occurs late in renal development.

The PI has successfully accomplished the experiments proposed in Specific Aim 3. These studies accomplished the generation of dendritic cell-specific TNF-alpha knockout mice. TNF-alpha floxed mice were bred with dendritic cell-specific cre- transgenic mice to generate dendritic cell-specific TNF-alpha knockout mice.

Weaknesses: None.

Reviewer 2:
Strengths: Overall, each specific aim was addressed systematically and methodically. In Aim 1 both mouse and human renal tubular epithelial cell lines showed similar results. Aims 2 and 3 generated state-of-the-art mouse models which will be used to further our understanding of inflammatory processes occuring during AKI. This information will be useful in designing specific therapies to be used in patients.

Weaknesses: It is not clear how the results of Aim 1 will be followed up with additional experiments, and the physiological/pathological significance of the phenomenon they have uncovered is not discussed at all. Since only immortalized cell lines have been used so far, testing this in primary cultures would be useful.

Reviewer 3:
All the proposed goals to make a proximal tubule and dendritic cell specific TNF KO mice were completed. They were then used in two specific papers and were essential to getting a NIH grant.

All the goals were met; data and information were applicable to the project objectives. Given the money involved, which only could partially fund the objectives, this project was very successful.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
A large segment of the population suffers from AKI resulting in significant morbidity and mortality. The risk of mortality is greater than 60% among patients who develop AKI and subsequently require hemodialysis. The financial burden related to AKI is estimated at about $12 billion per year. This proposal was designed to understand the cellular and molecular mechanisms responsible for toxic and ischemic AKI and associated inflammation. The studies undertaken have provided evidence that dendritic cells play an important role in providing protection from cisplatin nephrotoxicity. The proposal has clinical significance in that it provides

evidence that dendritic cells and IL-10 rescue from cisplatin nephrotoxicity. Thus the proposal is important toward eventual improvement in health.

Strengths: The PI has provided evidence that administration of renal dendritic cells improved renal function and histology in a mouse model of cisplatin-induced AKI. The PI further demonstrated that renal dendritic cells showed high expression of IL-10 in response to cisplatin treatment and that cisplatin worsened renal function and caused more severe tubular damage in IL-10 deficient mice compared to wild-type mice. These studies demonstrated that IL-10 produced by renal dendritic cells play a protective role in cisplatin nephrotoxicity and associated inflammation.

The PI has also successfully accomplished studies proposed for Specific Aims 2 and 3. The PI has successfully generated proximal tubule-specific and dendritic cell-specific TNF-alpha knockout mice. In future studies, these mice will be used by the PI to (a) determine the role of proximal tubule production of TNF in the pathogenesis of ischemic and toxic acute kidney injury using cell specific knockouts developed in our laboratory and (b) determine the role of dendritic cell production of TNF in kidney disease using a cell-specific knockout mouse developed in the laboratory.

Weaknesses: None.

Reviewer 2:
The current results themselves will have little impact on improving health; however, these studies and newly-generated mouse models can be used as a foundation for future higher impact studies. The beneficial impact is very reasonable in terms of the dollars budgeted.

Reviewer 3:
The project has helped define the source and role of TNF in renal injury.

One might consider the use of a drug like enteracept for the treatment of acute renal failure if one could overcome the delivery issues.

Continue to use these new animal models to explore the role of this pathway in the pathogenesis of different models of renal disease. Likely will help the PI get his NIH grant renewed and may someday (5-10 year time line) lead to the development of new treatments for renal disease.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
After completing the project, the PI was able to secure funds from the National Institute of Health (NIH). For this project, the PI did not receive funding from any other source during the project period. The PI worked on this project from 7/8/2009 through 6/30/2010 at the Pennsylvania State University.

Weaknesses: None.

Reviewer 2:
Strengths: The PI successfully used the data generated during this grant period to successfully compete for a large NIH grant on epthelial cell/dendritic cell crosstalk in AKI. Future related grant applications are planned.

Reviewer 3:
This project led directly to the funding of a related NIH grant totaling 1.66 million dollars.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The results obtained from this research led to the following publications:

Tadagavadi RK, Reeves WB. Renal dendritic cells ameliorate nephrotoxic acute kidney injury. *J Am Soc Nephrol* 21(1):53-63, 2010.

Gao G, Zhang B, Ramesh G, Betterly D, Tadagavadi RK, Wang W, Reeves WB. TNF-α mediates increased susceptibility to ischemic AKI in diabetes. *Am J Physiol Renal Physiol* 304(5):F515-21, 2013.

These publications are in highly-respected journals.

Reviewer 2:
Strengths: Two nice publications have been published using data generated from this project: one in Journal of Immunology and one in American Journal of Physiology Renal Physiology. Several more publications are planned using the mouse models generated during this project.

Reviewer 3:
Two papers were submitted. Both are in excellent journals and are high-quality work. Neither paper, however, used the animal models produced by this application but the supported student worked on these projects. More papers using the animal models will likely be forthcoming.

No patents or commercial developments.

Given the modest level of funding, the productivity is adequate.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strengths: The funds provided training for research staff and helped generate sufficient preliminary data for the NIH grant application for new funding.

The project has also helped in generating reagents and tools that will help other investigators in the institute to use these reagents and knockout mice for their studies.

Weaknesses: None.

Reviewer 2:
Strengths: This project supported the research of a predoctoral student. In addition, this grant supported the purchase of an Agilent Bioanalyzer for DNA and RNA analysis which is used by numerous (almost 70) research labs in both clinical and basic science departments at Penn State College of Medicine.

Reviewer 3:
One new useful piece of equipment was purchased. No additional researchers. A post-doctoral fellow was supported.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
No.

Reviewer 2:
External collaborations have not resulted from the current project. Generation of these mouse models may provide opportunities for outside collaboration in the future.

Reviewer 3:
No, and not really applicable to the limited scope of the proposal.

Section B. Recommendations

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.

Reviewer 3:
None.

ADDITIONAL COMMENTS

Reviewer 3:
The goal was to make two specific animal models and the project was successful, facilitated funding of a large RO1 grant, and supported the training of a post-doctoral fellow. The animals will likely be used in new animal models in the future.

The only criticism is, as yet, no paper specifically using the generated animal models has been published.

Generic Recommendations for Penn State University

Reviewer 3:
Very nice project from a strong investigator that generated a 20 fold return in new grant money to the institution. This is a successful program and an outstanding outcome.

Project Number: 0864529
Project Title: Synergistically Acting Targeted Therapeutics for Melanoma
Investigator: Robertson, G.P.

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The project met its stated objectives. The research design and methods were adequate. The data developed sufficiently answered the posed questions and were in line with the original proposal. The data and information provided were sufficient and applicable to the project objectives listed in the strategic research plan. There is no significant weakness that is identifiable.

Reviewer 2:
The project met its objectives, and was accompanied by a very sound, well-controlled research design. The strengths are numerous. First, the authors were able to show that targeting mutated BRAF and Akt3 simultaneously had anti-melanoma effects which are clinically relevant. Second, the authors show that siRNA targeting various oncogenic proteins can be formulated and delivered both topically and systemically in vivo to elicit on target effects against melanoma. Third, this work resulted in a funded NIH R01 grant. The weaknesses are minor. Namely, there could be a greater discussion on what kinases besides Wee1 were discovered through their siRNA screen since it is likely that more than a single kinase could be playing a role in the biology of this disease.

Reviewer 3:
The PI did an excellent job in assessing the interaction(s) between siRNAs targeting BRAF(V600E), AKT3 or WEE1. The PI applied relevant statistical tests (Chou & Talalay Isobologram analysis generating a combination index) to test for synergistic, additive or antagonistic effects. The PI further tested and validated new nanoliposome formulations to deliver siRNAs in the pre-clinical setting and with potential future utility in the clinical setting. Overall, the PI did an outstanding job in delivering on the goals and aims of the proposal.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The beneficial impact of this is high because new kinase target and novel siRNA therapeutics

have been discovered and developed.

Reviewer 2:
This project is likely to benefit the field of melanoma therapy, as well as cancer therapy in a significant manner. In addition to validating a novel treatment combination for a deadly disease, it also advances upon optimizing nanoliposomal technology for delivery of specific targeted siRNA. Given the fact that the investigators took their initial investment and generated additional extramural funding that can lead to further advances, this represents a very significant accomplishment with high potential for beneficial impact.

Reviewer 3:
This research is very high in value with potentially strong importance to eventual improvement in health outcome: potential new synergistic drug combinations for the treatment of melanoma, new drug delivery vehicles for siRNAs that target key signaling nodes within the cell, and clear demonstration of efficacy in the pre-clinical setting. The PI proposes to continue this area of research, which is both timely and important. Moreover, the PI's track record of success indicates a high likelihood that he will be successful in bringing this research forward, closer to the clinical arena.

Strengths: Synergistic pathway targeting of melanoma will be required to elicit durable remissions.
New approaches to delivering siRNAs to tumors are required.

Weaknesses: Not clear of siRNAs have any future in the clinic.
Small number of melanoma cell lines tested means that it is unclear how general the observations are in BRAF mutated melanoma.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Additional grant application has been submitted. The researchers are planning to apply for additional funding.

Reviewer 2:
There were additional funds obtained via an NIH R01 grant, which is quite impressive in the current funding climate. This work will likely set the stage for additional future funding, as commented on by the investigators.

Reviewer 3:
The PI was awarded a highly-competitive R01 from the NIH/NCI to support his research program. In the current funding climate, the award of a new R01 for this project represents a major achievement that reflects very well on both the project and the PI.

If PI wishes to renew the R01, he will have to show evidence of scientific productivity in terms of published manuscripts.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The project has resulted in publications and patent applications. A patent has been filed, although the patent application seems to be on a different therapeutic than the one investigated in this project.

Reviewer 2:
The project has resulted in an invention disclosure form, which could result in a future patent. In addition, this work has resulted in a manuscript currently under development that will be submitted in the future. This level of accomplishment is quite reasonable in the time period and given the scope of the studies.

Reviewer 3:
A patent has been filed (but not issued) on the concept of a combination of cancer drugs, a proof of hypothesis has been tested, and a model of a therapeutic efficacy has been developed (a nanoliposomal formulation has been created) and tested in working models demonstrating preclinical efficacy (tumor development in animals is inhibited).

The PI says that a manuscript is in the planning stage. The absence of a publication represents the one major deficiency in this project. However, I assume that it is simply a matter of timing before a manuscript is submitted for consideration.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The funding enhanced the investigator's research program. There were no new investigators brought in, but the funds were used to pay for research performed by pre- or post-doctoral students.

Reviewer 2:
This project had a positive impact on the grantee’s institution as it brings in indirect costs to help support the research infrastructure and bring national recognition to the melanoma program. It appears that funds were used to support the research of a young assistant professor, Dr. Sharma. This research project did not directly add to the infrastructure of the institution, and did not bring additional researchers to the institution.

Reviewer 3:
This project increased the sponsored project research base at Penn State and at the Hershey Cancer Institute.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
There were no new collaboration identified.

Reviewer 2:
This project did not appear to bring additional collaborative partners into the research from within or outside the institution. Scientifically, it was relatively isolated. However, due to the clinical relevance of the project, and the potential for translating the findings into the clinic, it could lead to future advances in cooperation with other institutions.

Reviewer 3:
No collaborations listed.

Section B. Recommendations

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.

Reviewer 3:
1. The PI is strongly encouraged to publish the results of the described studies.

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

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The stated objectives were not met.

The authors have not shown a relationship between contractile protein levels and contractile responses in arterial vs venous grafts in diabetic patients.

Sufficient data were not supplied to answer the objectives of the project.

Insufficient amounts of LIMA grafts do not allow for contractile analysis for this conduit.

The investigators have not demonstrated important differences in the contractile function of these grafts in diabetic vs non-diabetic conduits.

Reviewer 2:
The strength of the project is the concept to be evaluated in human subjects, and the use of human tissue to study diabetic-induced changes in smooth muscle cell contractile protein content and vascular contractile physiology in saphenous vein bypass grafts and in internal mammary artery bypass grafts. The investigator has continued to pursue this topic, and in fact this project led to National Institutes of Health funding for the project. Other strengths are the adaptation and verification of methods for the analyses proposed; the technical methods were indeed developed and employed. The weaknesses, which are substantive, were the recruitment of only one diabetic patient; thus, no publications were developed and no meaningful data were shown. It is also unclear why all analyses (PCR, vascular rings, immunoblotting, immunohistochemistry) were not available for all samples. From a knowledge of the likely procurement issues, one would suspect that adequate tissue should have been available.

Reviewer 3:
The project did not meet the original goals of the study. Especially, the number of patients that were recruited was much smaller than planned and only some of the analyses proposed were performed. As such, very limited data was obtained and the original hypothesis could not be tested.

The original design and methods of analysis were appropriate for the proposed study. However, due to very low recruitment and limited number of tests that were performed, it is concluded that the design was not adequate for the proposed study and the location of the research. Very limited data was generated and thus, it could not be used to rigorously test the hypothesis. The data obtained was a portion of the planned study.

The data presented was relevant to the proposed study; however, it failed to achieve the amount of data as originally proposed and expected.

Strengths: Development of methods to analyze contractile properties of vascular grafts.

Weaknesses: Limited number of patients recruited, limited number of tests performed.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
There is no impact of the results, so far, on improving patency of saphenous vein grafts in diabetic patients undergoing CABG surgery.

There are no discoveries from this grant that will improve health outcomes.

The investigators hope to obtain more specimens to continue the study; however, the impact of these results will be minimal since surgeons are moving to an all-arterialized form of revascularization and the factors which impact graft patency are multifactorial and not just limited to vascular wall contractility.

Reviewer 2:
The impact is small; no publications and only one diabetic patient recruited, which is frankly surprising. The funding was modest. The lack of any real data from the project other than verification of technical methods is disappointing, since the concept is important. The importance of the concept is underlined by the fact that NIH funding was obtained for this general line of research.

Reviewer 3:
Understanding the contractile properties of vascular grafts would constitute a translational approach to predict enhanced restenosis due to dysregulated contractile proteins/contractility as a function of arterial vs. venous grafts.

Prediction of enhanced restenosis due to dysregulated contractile proteins/contractility as a function of arterial vs. venous grafts will improve treatment for cardiovascular diseases.

Strengths: Health relevant goals

Weaknesses: Due to limited experimental results, it is not possible to determine the impact of the study on health.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
An NIH grant was awarded.

Reviewer 2:
Grant applications were developed and successful funding of an R01 project by the NIH was achieved. In this aspect, the investigator was extremely successful.

Reviewer 3:
An NIH/NHLBI grant was funded on 7/26/2010.

They plan to submit a proposal aimed at understanding the effects of anti hypertensive drugs on vascular remodeling in diabetic patients.

Strengths: An NIH grant was funded.

Weaknesses: None.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
There have been no peer-review publications published but the authors are planning to submit a manuscript. There have been no patents.

Reviewer 2:
None were developed. The experimental data and patient recruitment never progressed adequately to allow for adequate data to support any of these outcomes.

Reviewer 3:
Strengths: None.

Weaknesses: Failed to disseminate research data with only one planned manuscript.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
There did not appear to be any improvements in the institution infrastructure or addition of new investigators from this research.

Reviewer 2:
Methods were developed; although, it is suspected that these were likely in hand from collaborators in the project. The investigator has apparently left the state and is located at another academic institution. If the benchmark is adequate patient recruitment, then collaboration between the basic science investigator and clinical investigator collaborators was also of limited success. Thus, minimal impact on the grantee's institution.

Reviewer 3:
Strengths: Appropriate equipment exists to perform future research.

Weaknesses: No new investigators were added.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The research did not result in collaboration outside the institution.

Reviewer 2:
No.

Reviewer 3:
Strengths: None.

Weaknesses: No new collaborations were made or are planned for.

Section B. Recommendations

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.

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 nipride and nitroglycerin, and also the diabetic status, insulin vs oral agents and the level of glucose control at the time the specimens were harvested.

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 endoscopicly, 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 determing vein graft function. Not all harvested veins are perfect.

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 theses conduits.

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.

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.

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.

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.

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.

Reviewer 3:

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

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

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

ADDITIONAL COMMENTS

Reviewer 1:

There are major weaknesses in this grant.
The grant did not meet the stated objectives.
The hypothesis is that by studying the expression of contractile proteins in both the LIMA and saphenous vein grafts of both diabetic and non-diabetic CABG patients, these changes in smooth muscle contractility regulated by the expression of contractile proteins will predict restenosis and improve graft patency in both the saphenous veins and the LIMA. It is already well-known that eNOS expression is decreased in both saphenous veins and the LIMA of diabetic patients, as well as iNOS expression. In addition, it is already known that endothelial mediated relaxation is also impaired in both diabetic LIMA and vein conduits. The investigators have not shown that a relationship exists between contractile protein expression and conduit contractile function nor have they shown that this may influence graft patency.
In summary, as of the latest data, this study has not added to our understanding of the pathophysiology of conduit dysfunction in diabetic CABG patients and will have no clincial impact in this field.

Reviewer 2:

Inadequate subject recruitment, especially of needed diabetic patients, so that the overall

hypothesis of the project could not be tested. Need much better collaboration with the clinician-investigators so that subjects could be recruited. Since this is essentially a discarded tissue investigation of a tissue obtained at bypass surgery, the recruitment hurdles and ensuring adequate amounts of tissue are obtained for the analyses suggests inadequate communication and collaboration between the basic investigator, who is the Principal Investigator, and clinical colleagues. The technical experiments show the feasibility of the analyses and likely would have generated important and interesting data had the proposed project actually been able to have been performed as a result of adequate subject recruitment.

Reviewer 3:
Limited number of patients recruited.
Limited number of tests performed.
Due to limited experimental results, it is not possible to determine the impact of the study on health.
Failed to disseminate research data with only one planned manuscript.
No new collaborations were made or are planned for.

Generic Recommendations for Penn State University

Reviewer 1:
I do not feel that this study has taken into consideration the various limitations noted above and will not result in meaningful data that will impact clinical outcomes.

Reviewer 2:
The institution should review the progress on these projects at the one-year benchmark to independently evaluate if adequate progress is being made and if unexpected challenges are encountered. Evaluation of this project at the one-year time point would hopefully have identified the unexpected difficulty in recruiting diabetic patients. Enhancement of the infrastructure for recruitment and retention of clincial subjects in trials should be considered. Enhancing the interactions and communications between basic laboratory investigators and clinician investigators could also improve the quality of projects; it would certainly have likely overcome difficulties in subject recruitment and adequacy of sample material.

Reviewer 3:
The research team will need support for recruitment of sufficient number of patients to complete the study, as originally proposed.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strengths: The formula project met some of the stated objectives.

The research design and methods were adequate in light of the modest project objectives, and the modest budget.

The data were developed in line with the original research protocol, and were in some cases developed sufficiently to answer the modest research questions posed.

Changes were made in the research protocol related to the withdrawal from the research project of a collaborator with crucial technical expertise, leading to termination of one important line of research.

The extent of laboratory and clinical activities initiated and completed, and the number of subjects investigated, met some of the modest target goals proposed in the original strategic plan, but the more sophisticated assays (lipidomics and NMR spectroscopy) were not completed or technical obstacles were not overcome.

In some cases, sufficient data and information were provided to indicate that the project made acceptable progress, relative to the modest original goals.

Major weaknesses: Essential collaborations were established, and initiated, but not sustained.

The IRB was approved for only 20 patients and 20 controls. No information was provided about any consideration of power calculations in view of anticipated signal-to-noise magnitude of biochemical measurements or of biochemical test precision, or in view of those quantities of the population sample sizes that would be required for detection of significant differences with statistical power of 80% or greater.

The patient groups were not matched by sex. Autistic cases were nearly all male, whereas control cases were equally divided among males and females. Only some of the biochemical parameters

studied were shown to be not influenced by the sex of the subject in control cases.

No information is provided about the initially awarded budget. No information is provided about how much of the initial budget is represented by the reported expenditure over less than two years.

The grant was terminated early before the end of the first half of the full granting period.

The Principal Investigator appears to have retired and closed her laboratory, and terminated her research efforts.

No information is provided about whether attempts were made to transfer the grant to other investigators who might have extended the planned experimental work.

Reviewer 2:
Despite the modest results obtained by this study, the project did meet its stated objectives of investigating membrane fluidity and lipid composition differences in erythrocytes from individuals with ASD and neurotypical controls.

Overall, the research design and methods were adequate, although one might question whether the investigators took measures to prevent auto-oxidation of membrane lipids and/or proteins after collection of blood since oxidation can affect both membrane fluidity as well as lipid composition.

Another membrane parameter not addressed is lateral diffusion of membrane lipids and proteins. Although not proposed, this property of membranes may be strongly influenced by changes in cholesterol composition, as well as changes in 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.

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.

Reviewer 3:
This particular project was based on rather weak contradictory evidence that membrane fluidity may differ in children with autism vs. typically developing children. In fact, they failed to meet the stated objective of designing a diagnostic test for autism based on lipid membrane fluidity in red blood cells. However, it became apparent early on that membrane fluidity did not differ significantly between groups. As such, the investigators went in another direction and found that lipid composition, most notably cholesterol and GM1, were statistically different among the groups tested. However, these results were not terribly convincing to this reviewer. The grouping of cholesterol levels was much tighter among the values for the controls than for the widely variable levels found in the autism samples. In addition, their selection of "gender" matched cases included 11 females in the control cohort, but only one female in the autism cohort (as expected for autism). Overall, this project suffered from a weak hypothesis that resulted in some

fairly insignificant results which will not produce a viable blood test for autism. In general, there are conceptual and experimental design weaknesses throughout the project. In addition, there will be little beneficial impact as a result of this work.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strengths: This project addressed the possibility that investigation of red blood cells from autism patients might reveal biochemical changes in lipid composition or biophysical properties that could contribute to diagnosis or to diagnostic standardization, and perhaps to biochemical and mechanistic understanding.

The study found that red cell membranes from clinically-diagnosed autistic children exhibited modestly-reduced cholesterol content and modestly elevated GM1 content as compared to red cell membranes from control children.

Although not reported in either the published Neurochemical Research paper or in the final progress report, the FY10 progress report noted this finding: GM3 content in red cell membranes from children with autism was markedly lower than in red cell membranes from control children. It was disappointing that no follow-up was evident. These preliminary results might serve, if reproducible, as a blood test to distinguish autistic from non-autistic children.

Major weaknesses: The value of the completed research towards eventual improvement in health outcomes is likely low. The research led to no changes in clinically-useful risk factors, services provided, incidence or death from disease, or stage of disease at time of diagnosis.

No major discoveries, new drugs, or new approaches to prevention, diagnosis, or treatment are attributable to this incompletely performed research project.

There are no future plans for this research project. The PI has retired and closed her lab. No information is provided about the plans of listed co-workers or collaborators.

Reviewer 2:
As indicated by the PI in the final progress report, there were no direct changes in outcome, impact, and effectiveness attributable to the project. However, the results do generally indicate that additional studies are necessary to investigate the role of lipid metabolism in the pathogenesis and/or pathobiology of ASD.

Reviewer 3:
There was some potential for this project to produce a simple diagnostic test from blood to identify a subgroup, or even the larger idiopathic group, of autistic individuals. Unfortunately, this project did not produce a simple blood test for autism. Thus, the impact of this research is quite small on the improvement of health outcomes. The total dollar amount spent on this project

was, however, relatively small.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Leveraging of funds was apparently not expected. Additional funds from the Thrasher Foundation were cited in Acknowledgements of the Neurochemical Research paper, but were not cited in the final progress report.

The researchers are not planning to apply for additional funding to continue or expand the research. The PI has retired and closed her lab. The research project was terminated before completion of the second year of the four-year grant.

Reviewer 2:
There was no leveraging of funds or further applications for funding, due to the retirement of the PI.

Reviewer 3:
The PI stated in the original application that they were not seeking external funding. I'm not exactly sure why they did not even propose to apply for additional funding at the NIH R21 level. This is clearly another weakness in their approach to the project.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strength: One manuscript resulted from this grant.

Weaknesses: The manuscript is a modest one, with limited data of uncertain importance.

No further articles, licences, patents, or commercial development opportunities are planned.

The most promising data presented in one of the annual progress reports was not included in either the publication or the final progress report, rendering its reproducibility (perhaps without justification), suspect.

Reviewer 2:
This project resulted in 1 peer-reviewed publication, Schengrun et al. (2012) Cholesterol, GM1, and Autism. Neurochem. Res. 37(6): 1201-07.

Reviewer 3:
There is one published manuscript. There are some inherent issues I personally have with this manuscript as well (listed below), but it has apparently been peer reviewed.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Weaknesses: No improvements were made to infrastructure.

No new investigators were added or brought into the institution to help carry out the research.

Strengths: Funds were used to pay for research performed by a pre-doctoral student, who co-authored the single manuscript resulting from the research.

No information was provided about whether or not the results from this research eventually contributed to the doctoral thesis of the student, or whether the student remains enrolled or has graduated from her program of study.

Reviewer 2:
This project supported a graduate student, and therefore the research infrastructure at Penn State.

Reviewer 3:
No.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strength: The project did lead to collaboration with research partners outside the institution.
Weaknesses: Dr. Xianlin Han, then at Washington University St Louis, performed initial lipidomic analyses, but left the project after deeming the preliminary results unpromising in view of the assay expense.

Drs. Lin and Tian performed initial NMR experiments, but ceased experiments after failing to discern peaks for PS and PE, peaks that should have been detectable.

The clinical research involved the co-PI, but no other clinicians. The number of clinical subjects was small, and clinical studies never involved additional institutions.

Reviewer 2:
This project also facilitated some collaboration among faculty from different departments.

Reviewer 3:
There was some collaboration established with both Dr. Han and later with Dr. Fox to analyze phosphatidylethanolamine and phosphatidylserine - neither of which showed a significant change between control and autism groups.

Section B. Recommendations

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.

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.

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.

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

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.

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.

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.

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.

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.

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.

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.

ADDITIONAL COMMENTS

Reviewer 3:

1) Failed to produce an effective blood test for the early detection of autism.
2) Despite the fact that the results were published in a journal (impact factor 2.15) these results were not convincing and the sample size was sufficiently limited as to be unpublishable in this reviewer's opinion.

Generic Recommendations for Penn State University

Reviewer 1:

This project was a formula grant, that was not required to undergo initial peer review outside of the recipient institution. Had it undergone peer review with the budget and objectives presented in the documents provided, I would not have judged it of high priority for funding if I were evaluating it as part of the review panel. It would have been possibly appropriate as a pilot/feasibility proposal. Perhaps, the Penn State internal reviewers judged it in this light. But no information on this internal decision was provided in any of the documents included for review.

Formula projects should not be funded without peer review.

Reviewer 1:

As mentioned previously, this study was modest in scope and did not break new ground in the field of autism research. However, the studies proposed were well within the expertise of the PI and did provide support for training of a graduate student. It is suggested that future awards should be made with a goal of funding novel, high risk, and potentially transformative research.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Unfortunately, this project did not meet its objectives despite having a well-designed plan with worthwhile findings highly relevant to the treatment and prognosis of pancreatic cancer. The notion of identifying factors, in this case DNA polymerases, that prohibit greater efficacy of gemcitabine (considered the standard of care for pancreatic cancer patients) with the potential of targeting mechanisms that reduce the effectiveness of this drug is a relevant and powerful goal. Oddly enough, the one rather unlikely parameter that prevented this work from being completed was the recruitment of patients for blood draws before and after gemcitabine infusion. So no findings were presented or developed relevant to the main focus of this proposal. There were no laboratory and/or clinical activities to report, and the project managed to only to capture a few patients in comparison to the 50 individuals initially targeted for this project. Changes were made to encourage greater patient enrollment, and these alterations were reasonable though the investigator and colleagues did not attempt to reach out to other institutions to capture more patients.

The data that was presented in place of this project is discussed below in comments regarding the progress reports for each year. The data was applicable to the project in that in vitro assays were done to demonstrate the incorporation rates of dCTP base analogs, particularly dFdCTP, using 6 cell lines and 6 different DNA polymerases. Comments on the strengths and weaknesses of this work is also included in the below reviews.

Review of Plan and Progress Reports

Strategic Plan: It is stated that different DNA polymerases can alter gemcitabine incorporation yet it is implied that gemcitabine is efficacious when it is inserted into DNA. Perhaps this has to do with the replication fork, that certain polymerases can restore the replication fork even after incorporation of gemcitabine. Yet, the main tenant of this work is that changes in incorporation rates of gemcitabine are what determine efficacy.

One publication provided seems reasonable but does not address these newer polymerase isoforms to the extent that this proposal would require. The primary focus is DNA pol beta, delta, and zeta.

Using lymphocytes is reasonable in that obtaining blood samples is relatively easy and straight-forward. However, is the expression of these polymerases similar between lymphocytes and pancreatic epithelial cells? In addition, will delivery of gemcitabine to the blood be similar to that of pancreas cells? These are important issues to address to rationalize this work in lymphocytes. Are the DNA polymerase isoform ratios similar between lymphocytes and pancreatic epithelial cells?

Why not recruit from other centers, particularly if it's just for blood.

Were any of these Gem treatments in combination with other agents?

2009 Progress Report: It's difficult to believe that only one patient would have been enrolled in this Trial in a 9-month period. Why did this happen and what was changed on the IRB to include more patients?

Something of this nature would be difficult to predict, though a contingency should have been built into this project as an alternative in the event of low patient candidates and/or while the patients were being accrued. Recruitment at Penn State University would seem to be a potential limitation that should have been considered initially. It would seem reasonable to attempt to recruit at more high volume centers in the Philadelphia and Baltimore areas where several other institutions could have been petitioned for patient enrollment into this study, particularly since it only requires blood samples upon IRB approval. As an alternative, the PI did arrange to study the incorporation dynamics of base analogs into gapped DNA oligo substrates.

The data presented is in regards to base analog incorporation via base excision repair using DNA pol b and demonstrated that some base analogs were slightly less (~10-fold) efficient than the standard dCTP base, though these analogs (dFdCTP and araCTP) were far better (~100-fold) than other analogs (rCTP). Though well done and compelling regarding the use of these analogs, Figure 1 does not have error bars, bringing into question the number of times these data points were collected. It would have been much better for these data points to be substantiated with multiple runs and error bars so that statistical significance could be determined.

2010 Progress Report: Due to a lack of patient enrollment in this trial, the investigators in combination with clinical colleagues met to discuss improving the number of enrolled patients. The recommendations that they came up with are reasonable, though potential issues may be introduced. These included enrolling patients with (1) other cancers, primarily lung and breast, (2) combined therapies in addition to gemcitabine, and (3) different doses of gemcitabine initially proposed in this trial. In addition, it was determined that blood needed to be collected before and after chemotherapy infusion so as to avoid having patients make another trip back to the clinic for the second blood withdrawal. Also, improved communication from the lead PI and clinical colleagues was suggested in order to improve cancer patient enrollment.

Yet, it was not proposed to enroll patients from other institutions within the region. This would have more readily captured pancreatic cancer patients using gemcitabine in the correct dose range, and then a single adjustment (patients with combined therapy) to this protocol could have been proposed as a secondary method to improve patient recruitment. As proposed, the overall changes made to this protocol are not likely to greatly alter findings. However, it is conceivable that other drugs combined with gemcitabine or doses of gemcitabine may indeed alter incorporation rates leading to skewed data. Collection times after infusion are not likely to be much different than collection points initially planned for the next day, so this is a very reasonable alternative.

2011 Progress Report; Patient recruitment continued to be a problem despite making several changes to the IRB protocol and approach in the clinic. Without reasonable enrollment, the PI investigated the incorporation of dFdCTP at various concentrations for 8, 24, and 48 hours in blood cells isolated from cancer patients. It was not clear why these doses and time points were selected, and no rationale was provided. Likewise, it was initially stated that human blood cells would be used for this application, yet the data provided investigates dFdCTP incorporation into Hep2G (hepatocellular carcinoma) and A549 (lung cancer) cells. The statement that blood cells from two different cancer cell lines does not make much sense, as these cell lines are a homogenous population of cells. The data shown, though, is reasonable and a small advancement in the field. Additional work with more lines and/or true human blood cells administered more than one dCTP analog would have been an improvement to what was provided.

2012 Progress Report: In this progress report, a variety of work is highlighted involving the incorporation of dFdCTIP in six cell culture systems using six DNA polymerases. This detailed study is comprehensive and shows correlation with several important factors, many of which are significantly different in comparison. The two issues that need to be considered include the method for selecting colon, lung, and liver cancer cells over pancreas cancer cells and blood cells and why this study was not conducted earlier considering the issues with generating greater patient enrollment in this trial. Indeed, if this study was conducted earlier, additional work more strongly centered around pancreatic cancer cells could have been performed, including normal base analogs and variants thereof, as mentioned in previous work in prior years.

The data presented is compelling in that certain DNA polymerases seem better suited for the incorporation of dFdCTP, including DNA pol kappa and eta, although this may be line dependent and does not appear to be significant. Yet, DNA pol zeta did approach significance with a trend following three separate cell isolations and is a likely candidate for inhibition via dFdCTP. Since there are notable trends with these results, it would have been appropriate for the investigator to attempt another triplicate study to determine if statistical significance could be achieved in the second study or when combining both triplicate runs.

Admittedly, it is difficult to predict the lack of patient involvement in this study, as a blood draw before and after Gemcitabine infusion is a rather nominal request considering the potential prognostic value for patients. Perhaps if this were explained better to each prospective patient, it would have made a difference in enrollment. Likewise, reaching out to other institutions for recruitment would have been an ideal means of helping to circumvent poor patient accrual. Yet, the principal investigator is not responsible for making this happen, although moving more

quickly to the in vitro incorporation study would have allowed greater work to be done in this regard.

Reviewer 2:
This project did not meet stated objectives of the first aim, which was to evaluate the effect of Gemcitabine on DNA synthesis, because of a failure to accrue patient samples for evaluation. The major problem encountered appeared to be the fact that the protocol was overambitious with respect to the potential of accruing patients. It appears that this was due in part to the fact that the proposed correlative studies would not benefit the patients and would require a number of additional blood draws that were not medically indicated. In addition, it may be that this center did not have adequate patient volume to achieve the proposed goals.

The project did make acceptable progress in the second aim, which was to determine if specific DNA polymerases that participate in base excision repair, were able to incorporate gemcitabine derivatives into DNA. These studies, which were conducted in cell lines, provided evidence that translesion DNA polymerases, which have relatively low fidelity, are able to incorporate gemcitabine derivatives into DNA.

Reviewer 3:
The project overall was not very successful. The design did not adequately anticipate the difficulty in recruiting patients for this study. Therefore, the results are not clear or robust and it is difficult to make strong conclusions positive or negative. Overall progress was borderline.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The significance for improving health is obvious in that determining what suppresses gemcitabine efficacy can then be targeted in combination with gemcitabine. The latest wave of therapy for pancreatic cancer includes using a variety of drugs in combination with gemcitabine in both the preclinical and clinical settings. Recent findings have determined that various inhibitors including erlotinib (Her2-neu) and abraxane/nab-paclitaxel show improvements in a variety of outcome measures, especially survival. Findings from this study would have potentially offered yet another target that would help make gemcitabine a more powerful anticancer agent, offering yet another effective combined therapy for pancreatic cancer.
Even though this proposal was unsuccessful at recruiting enough patients to do this work, it is a worthwhile pursuit and still needs to be done. This particular investigator should be encouraged to pursue this project in a setting that will guarantee greater patient participation including developing collaborations at high-volume institutions to recruit 100 patients for this project. It is unclear if that is the goal of future work from this group, but it precisely what does need to be done for this particular cancer and a more immediate result of more efficacious combined therapies with gemcitabine.

Reviewer 2:
This project provides an incremental advance in our knowledge of the mechanism of action of Gemcitabine in treating pancreatic and other cancers. There are no stated future plans for this research.

Reviewer 3:
The project approached a scientifically very interesting question. However, it did not adequately test its primary hypothesis. There is no clear path forward.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
It is not clear if these researchers are planning to pursue this work further, nor is it known if these funds and the alternative project pursued were employed to garnish additional funding for future work. It is likely that a well-written proposal as an intra- or extramural grant would have served to generate additional funds for either of these two projects (the main focus: DNA polymerase levels in patients with pancreatic cancer or the alternative project: the difference of dCTP base analog incorporation through a cell- and DNA polymerase-dependent mechanism).

Reviewer 2:
The project did not leverage any additional resources.

Reviewer 3:
No leveraging specified.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
One publication (*Biochemistry* 2010, 49, 4833–4840; Impact Factor = 3.5) was produced from this alternative project which highlighted most of the work described in the annual progress reports. In general, the findings from this article demonstrate that the effect on DNA base incorporation, primarily dFdCTP, was due mainly to changes in activity level of DNA polymerases (kpol) with no differences in the affinity of the nucleoside triphosphates to the polymerase. Ligation efficiency was not affected by these nucleotide analogues, and base excision repair is capable of incorporating araC and dFdC into the genome. This is very reasonable *in vitro* data to support the tenants of the proposed clinical trial-style project. Even though this was not done, this publication can easily serve as strong preliminary data for a second attempt at garnishing funds for an evaluation of various DNA polymerases in human plasma of pancreatic cancer patients and how these proteins suppress the efficacy of gemcitabine.

Reviewer 2:
This project resulted in 1 peer-reviewed paper to date and additional data that may be incorporated into future manuscripts. There were no licenses, patents, or commercial development opportunities.

Reviewer 3:
There was one publication.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Based on what is provided, it is difficult to asses if improvements were made in the institutional infrastructure, considering the size and breadth of Penn State University. However, it is quite likely, even somewhat evident, that through the inability to recruit enough patients for this clinical and laboratory trial, collegial interactions were established and improved between basic research scientists and clinical oncologists. In fact, there were several meetings between colleagues in attempt to build a better program for accrual of additional pancreatic cancer patients. Even though this did not succeed, the ground work has been laid for future projects involving clinical trials, basic and clinical investigators, and the institutional IRB.

Reviewer 2:
There were marginal improvements in the infrastructure of the host institution. Essentially, the institution gained awareness that the process for developing translational clinical trials needs.

Reviewer 3:
They did purchase equipment to improve the overall infrastructure of Penn State University. No new investigators were brought in. The project did support a post-doc.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The unfortunate loss in this proposal was that outside institutions were not contacted in order to expand the patient pool for this trial. Considering the location of Penn State in Hershey, PA, there are several high volume medical and cancer centers within 100 miles of PSU including John Hopkins in Baltimore and University of Pennsylvania, Thomas Jefferson, and Fox Chase in the greater Philadelphia region. The number of pancreatic cancer patients being treated at these centers in combination with experienced staff accustomed to recruiting patients for a variety of clinical trials could have made all the difference in the world in regards to securing enough patient participation for this project. It is unknown why this avenue was not pursued, even with just one of these institutions. With so many high volume centers less than 2 hours away, it is

possible that many pancreatic cancer patients make the trip to these places for their treatment and follow-ups. Indeed, even reaching out to these institutions in order to capture patients that are close in proximity to PSU could have increased patient involvement.

Reviewer 2:
There was a modest attempt to reach out to other individuals for recruitment of patients. Overall, this effort was modest at best. This group would have benefited from reaching out to other high-volume centers that see pancreatic cancer patients in an effort to identify patients willing to participate in the correlative studies.

Reviewer 3:
The clinical trial was unable adequately to accrue patients to test its primary hypothesis.

Section B. Recommendations

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.

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 (eg. 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.

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.

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.

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.

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.

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.

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.

Reviewer 3:

1. Failed to test its primary hypothesis adequately.

2. Failed to result in new grants.

ADDITIONAL COMMENTS

Reviewer 2:

The major strengths of this project were the in vitro studies with cell lines that provided evidence that the mechanism of action of gemcitabine includes incorporation into DNA by base excision repair DNA polymerases.

One major weakness of the project was the failure to enroll patients in clinical trials.

Additional weaknesses included the fact that no additional resources were garnered to carry the

project forward and that there were no specific plans to further this line of research.

Reviewer 3:
Failed to anticipate problems leading to poor trial accrual.

Generic Recommendations for Penn State University

Reviewer 1:
The obvious recommendation to Penn State would be to push this idea forward by requesting that this project be completed as initially proposed. PSU should take an active role in contacting other neighboring institutions for their involvement in this project. Garnishing 100 enrolled patients, with plasma samples both before and after gemcitabine infusion, would be a huge achievement and provide the samples necessary for a full evaluation of these DNA polymerases. The lead investigator should be encouraged to complete this study and also consider looking into potential inhibitors of specific DNA polymerases that could be used *in vitro* as well as for a preclinical assessment. The notion of improving the effectiveness of gemcitabine is a more immediate means of enhancing the survival and quality of life for pancreatic cancer patients. In fact, this approach should also have ramifications for other cancers that respond modestly to gemcitabine therapy. Yet keeping this work focused on pancreatic cancer is a strong feature of this proposal, and should be embraced due to the need for this type of research in the field and the growing emphasis on combined therapies with gemcitabine as potential new targets are identified.

At the very least, the investment already made to publish *in vitro* findings regarding DNA polymerase and various components of base analog incorporation is a very strong stepping stone for the advancement of this proposed but yet unfinished clinical study. Indeed, should the Institute step out at this point and not continue to fund this work in some capacity, this would mean that funds spent previously on this project were not followed by a strong initiative to finish this study. Additional support would also provide this investigative team with the ability to be competitive on a national level with this project, and the spawned scientific questions that arise from such work. It would serve the Institute (and the scientific/medical community at large) well to see this project through to completion as the contribution to cancer research and patient outcomes could be rewarded with an effective therapy that combines gemcitabine with a specific DNA polymerase inhibitor.

Reviewer 3:
It was commented that this trial's failure led to changes in oversight that will provide more supervision of trials to insure that adequate patient numbers are available to achieve trial goals.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The project did not meet its stated objectives in terms of the number of community health providers to enroll and train as community-based researchers. It also was not able to meet its objectives in terms of the number of participants to enroll in the project. The research methods were adequate for the intervention group; however, no explanation was provided about who the control group was or how they were included in the project. The proposed statistical tests were not presented in the discussion of the results achieved. The intervention participant data provided were applicable to the project objectives; however, very little was provided about the control group, other than number and mean age.

Reviewer 2:
Strengths: The project partially met the stated objectives.

The team's use of consistent, ongoing meetings and education appear to have been effective for meeting the first aim. The team appeared to stay active and made impressive progress in the first 6 months to establish research forms and protocols in a timely manner (would have made a stronger case if there were some reporting of the percent of participation by team members over the 2.5 years of the project). Assessment of the strengths and weaknesses of the collaboration was explored by survey, discussed by team members and this information informed the one publication from the project.

The third aim also seems to have been adequately met; however, the data to support or refute was limited and not clearly defined. It appeared the embedded health professionals were able to recruit and maintain on average 34 community-dwelling older adults in the project and to complete required evaluations and data collection.

Weaknesses: The second aim was not met (to test if the project would reduce falls/injuries and increase percentage of flu immunizations for elders). The sample size was small and did not meet the goals of the proposal. Only 170 older adults out of the 400 originally targeted were enrolled; and only 5 out of the planned 16 community research/interventionists were hired (role to assist with recruitment, collect research data, complete home safety evaluations and provide

recommendations to improve home safety). Additionally, a lack of clarity on recruitment/enrollment numbers and resulting data collection makes evaluation of the second aim difficult (a flow diagram may have been useful). Overall 230 were contacted, 170 consented, 165 completed baseline data, 116 had in-home safety assessments, 86 completed follow-up measures, 41 completed flu surveys and 33 participants withdrew from the study. It is not clear why the wide range in numbers on assessments and no specifics provided related to reason for attrition. Additionally, this reviewer was unable to find any discussion on comparison/differences in intervention and control groups (realize is a crossover design).

Unconvincing evidence provided for the choice of the specific fall prevention intervention (limited evidence to support the effectiveness was provided in the proposal, and no discussion of alternative approaches that were considered such as strength training, medication reduction, education, etc.).

Process evaluation was not specifically included; for example, it is unclear if measures such as treatment fidelity for the CHAs were assessed. This type of information would seem appropriate to more fully address the "effectiveness of using health professionals….as research coordinators" (Aim 3).

The fourth aim was not met; however, it is potentially a more long range outcome that may be met over a longer period of time. It may also be that expecting immediate employment opportunities in research related jobs in a rural setting were unrealistic.

Reviewer 3:
This was a very ambitious project which appears to have been carried out reasonably well, although not fully. Of the four major stated project objectives listed below, very good progress was made in meeting numbers 1, 3 and 4. The specific health care outcomes data collected did not support the hypothesis described in Objective 2.

1. Establish an effective health research coordinator network (Rural Embedded Assessment Community Health [REACH] Network) in central Pennsylvania, that will both represent the health needs of these communities and also facilitate community based translational research and educational programs aimed at improving the health of this population.
2. This study will test the hypothesis that using the REACH network to provide in-home interventions to make the home safer will reduce the risk of falls and fall-related injuries compared to a control group not receiving the intervention. Likewise, the availability on in-home influenza vaccinations will translate to a higher percentage of immunized elders, compared with elders not offered in-home vaccination.
3. Determine the effectiveness of using health professionals embedded in their rural communities as research coordinators. Traditional health professionals (nurses) and EMTs will be trained in the conduct of clinical research. The effectiveness of this approach will be tracked and compared to a hypothetical, centralized model.
4. Determine whether the use of distance learning techniques for training in the good clinical practice of research has enhanced immediate employment opportunities or alternative career pathways for the trainees.

Objective 1 clearly required the most time and energy by the investigators to create, and appears to have taken enough lead time and energy to perhaps not permit full development of the strategies necessary to achieve Objectives 2, 3 and 4. No analysis of comparative effectiveness to a centralized model was offered for Objective 3 other than assertion, and no actual survey data was provided to independently assess this outcome. And while it appears that the training in Objective 4 was accomplished, there is no data regarding alternative career pathways provided. The time allowed for the project may not have been sufficient to influence this outcome. Generally, the research design and methods were adequate in light of the project objectives. The data were developed sufficiently to answer the research questions posed in line with the original research protocol. The research protocol was changed to adopt a crossover design which was reasonable in view of subject recruitment and developing continuity of relationships with rural residents. Using this design, sufficient numbers of clinical activities with sufficient numbers of subjects were performed to demonstrate acceptable progress in meeting the project objectives; although, less than half the expected number of participants were enrolled. The data and information provided were applicable to the project objectives listed in the strategic research plan.

The project investigator speculates that recruitment of the originally planned numbers of nurses and EMTs and better administrative oversight of these individuals would have resulted in successfully recruiting the target number of participants, which is likely true. However, since nearly half the desired population was recruited and failed to show any significant difference from controls at all, it is unlikely that the health care outcomes desired would have been achieved even with the full number of enrollees. It is likely that one of the target interventions chosen was superfluous in a population already effectively saturated with the desired preventive health outcome (influenza vaccination). It may also be that the strategy chosen to address the public health problem identified (home modification for falls) did not have a large enough effect size to make a detectable difference in outcomes for a geriatric syndrome known to have multifactorial causes. There is significant literature in the field of falls published by Mary Tinetti, et al, which demonstrates that multifactorial interventions over time are necessary to actually be effective in falls prevention rather than a single, focused intervention.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The principal investigator identifies major factors impacting the health of rural elderly. The results of the intervention tested in this project did not demonstrate that the type of intervention undertaken would have a significant impact on the health of the participants. Their intervention did not appear to lead to a decrease in falls. To continue pursuing the use of local RNs/EMTs as researchers and reducing falls and improving immunization rates among the elderly, different intervention methods would appear to be needed. Given the increasing pressures on healthcare professionals, asking them to assume additional duties as community researchers may become increasingly difficult.

Reviewer 2:
Strengths: This project has the potential to positively impact research capacity and translation of programs in rural communities and as a result improve health of older adults. The impact of this study was limited; however, given the budget and the novel collaborative approach the positive outcomes are promising and appropriate.

The feasibility of using community-based healthcare providers (already living and working in the communities) to provide health services was supported by this project. Finding delivery models that are effective in translating interventions are critical to meeting health needs in rural communities and at-risk populations and this project has contributed to the current knowledge on this topic.

Weaknesses: The specific intervention to decrease fall risk and fall injury did not show significant improvement in community health. While this project used a community-based participatory research approach, the intervention components seemed to have been chosen by the academic partners prior to formation of the REACH collaborative (perhaps outcomes would have been stronger if the intervention was from the community members).

The limited success in hiring CHAs (community healthcare providers) and the resulting low enrollment of older adults is a major weakness of the study that greatly limits any discussion of impact and effectiveness of the program. The final report did not provide reflection on what might be done to improve enrollment of CHAs this in the future.

Reviewer 3:
Successfully establishing the REACH Network did appear to effectively use rural nurses and emergency medical technicians (nurse/EMTs), and team these workers with Penn State University cooperative extension staff to perform detailed assessments in the homes of elderly individuals. The data collected on these individuals is well-described but not very well analyzed in presentation. Community engagement and input is asserted to have been highly successful as assessed by the collaboration survey created to assess the network's level of community engagement, which was aimed at members' involvement, effectiveness of leadership and communication, network participation, community support, and barriers. It would have been most useful to have the actual data collected by this instrument described and analyzed for significance, themes and construct validity so that this assertion could have been substantiated. The proposed innovative mobile data acquisition device for facilitated and enhanced site data input (the HomeFast survey application for the iPod Touch) did appear to be successfully piloted. Overall, this is a promising infrastructure design for strengthening rural and small town research and health education at a relatively modest cost. However, the key requirement for successful translational clinical research that merits the cost of infrastructure development is the ability to improve health outcomes, and the project failed to do so. There might have been a more successful outcome if the investigators had engaged the community's input when *choosing* the health care outcomes to be targeted for improvement, rather than selecting these in advance.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The researchers were not successful in applying for additional funds during the project, although there is expressed intent to continue seeking funding. The questionnaires and training materials developed for this project could contribute to successful development of additional funding. The empirical results of this project, however, may make it difficult to obtain funding for continuing the expansion since benefits in terms of improved health of the participating population was not demonstrated.

Reviewer 2:
Strengths: At the time of the final report, no additional funds had been received. However, leveraging had been attempted (3 grants were submitted, 2 not funded, and 1 was pending a decision). The investigators intend to continue to explore funding opportunities.

Based on findings and experience from this project; the investigators are expanding their use of this research/delivery model to address health needs of newly discharged patients and individuals needing other in-home support.

No weaknesses identified.

Reviewer 3:
A proposal for a similar concept was submitted to the Center for Medicare and Medicaid Services as part of a Healthcare Innovation Challenge grant and was received favorably, but was not funded. Two additional grant applications to potential funding opportunities were written during the grant reporting period for funding to continue or expand the research, one of which was not funded and one of which was pending review at the time of the final report. The authors appear to intend to apply for additional funding in the future.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The project led to a publication in the American Journal of Community Psychology in 2013. An additional publication was proposed assuming outcome measurements were adequate. From the results provided in the final report, it may be more difficult to get a publication, although journals are becoming more receptive to publishing negative results. For the results to be publishable, more detail needs to be provided on the methods employed and the control group enrolled.

Reviewer 2:
Strengths: The project resulted in one peer-reviewed publication. The article provides qualitative description and provides insights (from 5 stakeholder perspectives) into the strengths and barriers related to the development of an academic-community partnership.

Weakness: No publication on effectiveness of intervention on health outcomes (fall risk and injury).

Reviewer 3:
One peer-reviewed publication was created and another is planned with the outcomes of the study, Louis D. Brown, Theodore R. Alter, Leigh Gordon Brown, Marilyn A. Corbin, Claire Flaherty-Craig, Lindsay G. McPhail, Pauline Nevel, Kimbra Shoop, Glenn Sterner III, Thomas E. Terndrup, M. Ellen Weaver. Rural Embedded Assistants for Community Health (REACH) Network: First-Person Accounts in a Community–University Partnership. *American Journal of Community Psychology*. Published online May 1, 2012.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
A Ph.D. from Australia was brought to the University to assist with research. In addition to students, one undergraduate and one pre-doctoral, were engaged in the project. More explanation of the roles of these individuals in the project would be helpful.

Reviewer 2:
This project enabled the institution to apply for other funds using a similar approach (embedded health community healthcare workers targeted to participate in research studies as well as deliver an intervention in the community aimed at improving health).

Reviewer 3:
No new investigators were brought in and only very minor equipment was purchased with other funding to permit continuing study supporting the research project. One pre-doctoral student and one undergraduate student were funded by the project. However, the demonstrated proof of concept has enabled their institution to apply for other funds using a similar approach. The institution is also piloting a study using EMTs to follow up with 5 patients recently discharged from the emergency department as described in future plans. The investigators have expanded into EMS research in the community of Pennsylvania paramedics focusing on airway safety and simulation (R18 grant from AHRQ, 3 year grant ending May 2015; Terndrup, PI). Also during this time frame and reportedly because of this success, the institution participated in several prehospital trials with EMS (IMMEDIATE and RAMPART, both of which had primary efficacy manuscripts published, JAMA and NEJM).

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
A strength of the project was the collaborative activities engaged in with community leaders and community health professionals. The weakness in terms of the small number of participants, both community-based professionals and elderly populations, has been discussed. There is expressed interest in continuing to develop research partnerships with individuals outside the University environment. Such collaboration could strengthen the ability to implement change in a community when the evidence warrants such changes. Transforming research into community implementation is an increasing focus of much public policy at this time.

Reviewer 2:
One of the major strengths of this project is the collaborative nature of the research and intervention planning. The project required the development of a community advisory board (interdisciplinary members from a number of community sites within 2 counties) that also collaborated with a scientific advisory board.

Reviewer 3:
Collaborations were developed with the members of the REACH advisory board, the Visiting Nurses Association, Lewistown Hospital, senior advocacy groups in the Lewistown area, and local emergency medical services personnel. As noted above, several successful EMS trials were begun, some completed and others are in progress. (e.g. CARES registry, ProTECTiii trial). New involvement with the community included relationships with Fame EMS, Lewistown, PA; Visiting Nurses Association (VNA) of Central PA; Lewistown Hospital, PRN (a healthcare staffing organization based in Lewistown), and The Learning Center in Lewistown, PA. The project's community board included advocates for elder care in the Lewistown area, including hospital, primary care, nursing, Aging Association Agency, and other community health activists.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:
1. Inadequate information is provided about the control group included in the research. Authors should provide more explanation about who the control group is, how they were selected, and more information on how they compare to the intervention group.

2. Lack of alternative strategies when inadequate enrollment was being observed. Authors need to provide descriptions of alternative actions that could be undertaken in case the projected enrollment does not progress at the intended speed. What could be done to increase

enrollment when it became evident that the current strategy was falling far short of desired results?

Reviewer 2:

1. Recruitment and enrollment of study subjects into the study fell far below the projected goals of the study. This was evident from the first annual report. While rural older adults are recognized as being challenging to reach, the lack of reflection by the investigators on the potential causes and alternative strategies to remediate the issue during the study weakened the study and the yearly evaluations. Additionally, the limited success in hiring CHAs (community healthcare providers) brings up questions of the feasibility of this approach.

   Recommendation: Include discussion of recruitment challenges when approaching difficult-to-reach populations and provide detailed recruitment plan to meet the known challenges in future proposals. Include alternative recruitment strategies in future proposals and provide transparent problem solving and changes to recruitment during a research project (if numbers are falling short of projected goals).

2. The specific intervention to decrease fall risk and fall injury did not show significant improvement in community health. While this project used a community based participatory research approach, the intervention components seemed to have been chosen by the academic partner prior to formation of the REACH collaborative.

   Recommendation: Perhaps outcomes would have been stronger if the intervention was developed by the community members.

3. Measures of process evaluation were not included in the study plan which limits further understanding of program feasibility and transferability of this kind of model to other populations or communities.

   Recommendation: Consider including process evaluation measures in future studies.

Reviewer 3:

1. Selection of the target health outcomes and interventions prior to community involvement. Recommend using the well-designed community network established to perform a local needs assessment of the community's health care status to permit identification of needs which are not already being met.

2. Inadequate time allotted for development of community network and recruitment of nurses/EMTs. Recommend allowing one entire year to establish network and target health care interventions prior to planned start of recruitment of health care professionals and participants.

3. New design of nonvalidated survey instrument for collection of key outcome measures regarding community infrastructure success. Recommend validation of survey instrument or use of an established survey instrument. In either case, the data collected should be reported.

4. Apparent attrition of health care professionals participation in the project is not explained, does not appear to have been anticipated, and a retention strategy for these key project personnel does not appear to have been included in study design. Recommend creating a strategy to follow these individuals' career paths, allocating more resources for recruitment, including a retention strategy as part of the plan, and initial over-recruitment to allow for expected attrition.

5. Choice of health care outcomes intervention used in this project was not supported in literature as effective for falls. Recommend very careful literature review for potential health care interventions in the future to assure that a proven successful strategy is chosen.

Generic Recommendations for Penn State University

Reviewer 1:
Monitor progress carefully and work with the investigators to identify strategies to improve research design and implementation to achieve desired results. The proposed study identifies important issues and a creative intervention strategy to address those issues; however, implementation of the proposed strategy was inadequate to provide conclusive or even supporting evidence of the value of the intervention.

Reviewer 3:
I think this project was a good selection for funding and the process for carrying out the review has been effective.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The project had three specific aims and seven stated objectives. The first aim and its three stated objectives were met by successfully preparing and properly refolding recombinant forms of each of the six Duffy-Binding Like (DBL) domains of the surface antigen VAR2CSA from the human malaria parasite, P. falciparum. The second aim was to determine which of the DBL domains interact with each other to possibly form binding pockets for adherence to the placental-specific receptor chondroitin-4-sulfate (C4S). This stated objective was met and the results indicated that DBL1x and DBL2x each bound to DBL3x, but DBL4ε, DBL5ε and DBL6ε did not interact with each other or with either DBL2x or DBL3x with measurable affinity. Together, these data suggest that DBL1x, DBL2xl, and DBL3x form an interacting unit in VAR2CSA, and the stated objectives were achieved. The final specific aim had three stated objectives which were to 1) crystalize the interacting DBL domain pairs in the presence or absence of the C4S oligosaccharide receptor; 2) clone larger fragments of VAR2CSA in mammalian cells; and 3) express ^{15}N-labeled DBL domains and perform preliminary NMR analysis on a DB2x, DBL3x, and their mixture. In summary, protein crystals were formed only for DBL2xL, but were not useful for diffraction analysis. This objective was only partially met. The objective to express larger fragments of VAR2CSA in eukaryotic cells was attempted but not completed due to insufficient funds. Conditions for the expression of 15N-labeled DBL2xl and DBL3x domains were optimized and sufficient amounts of purified [15N]-DBL2x and [15N]-DBL3x were obtained to determine their structures in solution by NMR spectrometry, thus meeting this objective. In summary, a large majority of the stated objectives were met.

The data presented were sufficient to answer the questions posed where the objectives were met. The methods used were appropriate to obtain the needed data. The only change in the research protocol was to use bacculovirus expression versus the original proposal to use a mammalian expression sytem for the larger VAR2CSA fragments. This objective was approached but not completed; however, the switch in expression systems was justified based upon the reduced cost and the suitability of the bacculovirus expression system for this purpose.

The investigators made very significant progress on this challenging project, and obtained a large amount of relevant preliminary data to approach the stated goal of establishing an assay to identify small molecules capable of interfering with the binding of VAR2CSA to CS4. To

proceed with this work further grant funding will be necessary.

Reviewer 2:
The project had 3 objectives: (1) to clone and express individual DBL domains of VAR2CSA; (2) to measure interactions between these domains; and (3) conduct X-ray crystallization and NMR studies of the domains. The investigators completely met the first two objectives, and partially met the third.

Aim 1was a genetic engineering exercise, and the investigators used appropriate and successful methodologies. They also were able to incorporate alternative methods when the methods they initially proposed encountered obstacles, such as when one expressed protein formed aggregates. They should be commended for using a new bioinformatic tool to redesign the construct successfully.

Aim 2 was to study the interactions between the domains with a variety of biophysical methodologies. Evidence using five assays was presented for a strong interaction between DBL2x and DBL3x. There was some evidence also suggesting that DBL1x also binds to DBL2x. In contrast, the investigators did not find any interactions between DBL3e, DBL4e and DBL6e with any other domains. This data is important in light of recent evidence from Denmark suggesting that the N-terminal (DBL1X-DBL2x-DBL3x) is the VAR2CSA domain responsible for C4S binding. In aggregate, the data support the investigator's hypothesis that multiple domains are needed to the C4S binding site.

Aim 3 was the most difficult of the aims. Crystallization studies are always high-risk, high-gain enterprises. The investigators describe significant effort at attempting to obtain crystals and crystal structures, but were unsuccessful. They did, however, obtain interesting structural data using NMR.

Overall, the productivity was excellent.

Reviewer 3:
It appears that ~ 75% of the total funds for this grant were used towards the purchase of a microscope that had little to do with the actual proposed research. Although parts of Aims 1 and 2 were achieved, the major aim (Aim 3) of obtaining diffraction quality crystals was not met. Although the PI was able to produce some of the target DBL domains, and obtain some preliminary SAXS and NMR data, these data do not allow one to address the actual stated problem which was to understand the interaction between VAR2CSA and chondroitin 4-sulfate (C4S). In fact, although progress was made, it appears that little new knowledge was gained as the structures of DBL domains were already known and the obtained structural data is only of low resolution and adds little to the understanding of interaction with the target.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Up to 200, 000 newborn deaths occur each year in Africa as a result of malaria in pregnancy. The adherence of the malaria infected erythrocytes to the placenta via VAR2CSA is largely responsible for this complication in pregnancy. A method to prevent or to release cytoadhered parasites from the placenta would begin to address this disease.

The research is at an early stage, and practical progress will not be made until the assay for interference of binding is in place and the binding domains on VAR2CSA are mapped at high resolution.

Reducing the impact of malaria in pregnancy would greatly reduce the risk for loss of the newborn for women without antibodies to VAR2CSA.

The PI is seeking additional funding to continue pursuing the research objectives as yet unmet. These would describe the structure of the VAR2CSA binding domains and identify small molecule inhibitors that may be used to interfere with the binding of VAR2CSA to the placenta.

The major strength of this project is the potential impact of successfully obtaining a small molecule that would affect the release of parasites from the placenta. Otherwise this is a very challenging, high risk project which, although utilizing very sophisticated technologies, is likely to lead to obtaining only a partial picture of the adhesion-receptor binding phenomenon. Whether the data obtained by this biophysical and crystallographic approach is sufficient to accurately guide future progress toward the practical goal is yet to be determined.

Reviewer 2:
Many millions of women experience malaria during pregnancy, with adverse effects on both the moter and developing fetus. Efforts are currently underway to develop a vaccine against VAR2CSA to protect pregnant women from malaria. Dr. Gowda's results will help inform this vaccine effort.

The investigators are hoping to use the data they have obtained as preliminary evidence to help them obtain NIH funding to continue this work. This is a very good plan.

Scientifically, the only weakness is that the investigators have focused solely on the 3D7 VAR2CSA protein. The gene coding for VAR2CSA is highly diverse, and there are probably hundreds if not thousands of different VAR2CSA variants that are expressed by malaria parasites in the field. Some effort to extend this work to other VAR2CSA variants is needed in order to have an impact on clinical malaria.

Reviewer 3:
Understanding the interaction between VAR2CSA and C4S could be important for eventual

design of vaccines against malarial illness.

The project made no major discoveries that will impact this goal. Only 25% of the allocated funds were used in direct pursuit of the aims. This is a major weakness.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Funding on this topic came originally from Burroughs Wellcome Fund in 1997 and subsequently from the NIH from 1999 to 2009. During this period some funds were obtained from CURE, but recent NIH grant applications have not been successful.

Four grant applications have been submitted, two were not funded and two are being prepared for resubmission. The investigators continue to actively pursue funding to continue this project, which is a strength.

Reviewer 2:
The investigators have not yet leveraged additional funding for this work, but are planning to apply for additional funding from the NIH. The exciting preliminary data they have generated with this proposal will increase their likelihood of success.

Reviewer 3:
Attempts to leverage the project for additional funds from the NIH have not succeeded thus far.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
No publications were accepted or submitted during the period of this grant, but were preliminary and were mainly intended for supporting the grant applications to the NIH.

Publications were not expected until further funding is obtained to finalize results and draw appropriate conclusions. Plans are to publish the data when additional results are obtained.

Only two manuscripts have been published since 2008 relative to this research topic from the PI's lab, one of which is a review. The slow pace of publication speaks to the difficulty of the project and is a weakness in applications for additional funding. This is a weakness in the project; however, less than $80,000 of the Health Research Grant funds were spent on the project, and additional results needed for publication will require additional funding, as stated by the PI.

Reviewer 2:
They have not published any of their results. The investigators should consider writing and submitting a paper based on the results of Aims 1 and 2; it seems that they have enough data and such a publication might improve their odds of being funded by the NIH.

Reviewer 3:
No peer-review publications have been generated from the studies.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The largest portion of the funds from the Health Research Grant ($217,486) was spent on a Delta Vision Elite Imaging System fluorescent microscope. This microscope is useful for live cell and fixed cell imaging, as well as for the tissue histology.

The PI was able to recruit two specialists to join the project, and assist in accomplishing the stated goals. They also increased the research capacity of the lab.

Infrastructure enhancement was both a strength and a weakness. The purchase of the microscope is reported as being extremely beneficial to the institution (a strength), but it is unclear how it was used in this project (a weakness).

Reviewer 2:
Most of the funds were used to purchase a state-of-the-art microscopic imaging system, will be very useful for future research efforts. The grant also paid for a small part of a post-doc's salary (7 months). It does not appear that students were involved.

Reviewer 3:
Major weakness: A substantial amount of funds ($217,486) (~75% of the total award) were used to purchase a portion of a fluorescent microscope. It appears that this expensive purchase had little, if anything, to do with achieving the aims of this particular grant application. From that standpoint, it does not appear to have been an appropriate use of the funds provided for this project. Had that substantial amount of funding been used for the actual objectives of Aim 3, which are the most difficult to achieve, it seems likely that the PI would have been able to get much farther in obtaining diffraction quality crystals, entities that would certainly also have improved the chances of securing extramural support for this research program.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
A new collaboration was established with an investigator from Denmark to obtain reagents needed for the proposed studies to enhance grant applications. Adding an investigator to the research team who has specialized reagents needed for the research is a strength.

Reviewer 2:
Yes, importantly, the investigators have established a collaboration with Dr. Ali Salanti from the University of Copenhagen who is involved in the VAR2CSA vaccine effort and who has offered to share useful reagents.

Reviewer 3:
No collaborations outside of the institution were made as a result of the studies.

Section B. Recommendations

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 milleux 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.

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.

Reviewer 2:
Generate and study VAR2CSA constructs other than the 3D7 variant in the future.

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.

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.

ADDITIONAL COMMENTS

Reviewer 3:

Overall, progress was incremental and did not substantially advance the research towards the goal of understanding how multiple tandem DBL domains interact with C4S. While the PD was able to obtain crystals of a single DBL domain, DBL-2x, of the six originally targeted, these were not advanced to the point of obtaining sufficient diffraction for structural studies.
Little new knowledge was obtained during the support period.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strengths: The project met all the stated objectives. The research design and methods were quite appropriate. The data obtained were in line with the original research protocol, and were developed sufficiently to answer the research questions posed. There were no changes made to the original research protocol. Sufficient data were presented and published to illustrate the fact that the project met all its objectives. The data and information provided were relevant to the project objectives tested in the strategic plan.

Weaknesses: The relevance of the work submitted for publication (and later published in the *European Journal of Immunology*) was not directly applicable to the strategic research plan, nor was it discussed in the progress report.

Reviewer 2:
The investigator developed the original grant application into 2 specific aims.
In Aim 1, he intended to generate melanoma-specific cytotoxic T lymphocytes (CTLs) by inserting MART-1/Melan-A-specific-TCR into human iPS cells and differentiate them to T cells ex vivo by culturing iPS in "Notch signaling" culturing conditions. In Aim 2, he intended to characterize in vitro and in vivo the functionality of these ex vivo generated CTLs. The proposed aims were extremely innovative and the investigator succeeded in accomplishing the specific aims and in publishing the results in Cancer Research.

Strengths. The investigator generated ctl mart-1 specific from human ips and demonstrated the functionality of these cells ex vivo and in vivo in a mouse model. The investigator accomplished the proposed aims and proved that his hypothesis is correct.

Weaknesses. None.

Reviewer 3:
Strengths: The project met the stated objectives. The *objective* of this project was to generate and characterize highly avid melanoma-specific cytotoxic T lymphocytes (CTLs) from human iPS cells. After completion of this study, they generated and characterized MART-1/Melan-A-specific CTLs from iPS cells by TCR gene transduction and stimulation with *in vitro* Notch

ligand, and tested the therapeutic efficacy of human iPS-derived MART-1/Melan-A-specific CTLs in a murine melanoma model (Figures 1-4 in the final progress report).

The research design and methods were adequate in light of the project objectives.

The data were developed sufficiently to answer the research questions posed. And, the data were developed in line with the original research protocol.

No changes were made to the research protocol.

The data and information were provided sufficiently to indicate the fact that the project met its objectives or made acceptable progress.

Weaknesses: The description (source, name, etc.) of human iPS cells used in this study was not clear.

The clinical potential of this approach may be limited to only one subtype of melanoma at a time since it has to be antigen-specific.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Strengths: The significance of this project for treating cancer is very high. Progress has been made by other investigators using adoptive transfer of tumor-specific cytotoxic T cells, but there are difficulties in generating large numbers of these cells from patients. This project investigated the possibility of generating melanoma-reactive cytotoxic T cells from induced pluripotent stem (iPS) cells, and studied their cytotoxic effectiveness *in vitro* and *in vivo*.

A sophisticated approach was used to transduce human iPS cells with genes of melanoma-specific T cell receptor, and to culture the transduced stem cells *in vitro* with Notch stimulation so that they would differentiate into highly avid melanoma-specific T cells. The investigators were successful in generating highly reactive MART-1/Melan-A-specific cytotoxic T cells derived from human iPS cells. The transduced human iPS cells were cultured on OP9-DL1/DL4 cells which express Notch ligands DL1 and DL4. The results showed the generation of mature $CD8^+$ T cells expressing MART-1-Melan-A. It was also found that T cell receptor gene-transduced iPS cells differentiated into $CD8^+$ T cells *in vivo*. The process took six to ten weeks. These cells responded to antigen stimulation, proliferated, and secreted cytokines. They also underwent a recall/memory response when re-stimulated with antigen, and were cytotoxic to MART-1/Melan-A-expressing target cells. The MART-1/Melan-A-specific cytotoxic T cells were adoptively transferred to mice implanted with melanoma cells the next day or six weeks later. Melanoma tumor growth was suppressed and survival extended. These data support the hypothesis that adoptive transfer of tumor antigen-specific T cell receptor gene-transduced iPS cells can induce tumor antigen-specific T cell persistence and tumor protection. Evidence of T

cell infiltration into tumor masses was shown.

Reviewer 2:
Strengths: The analysis performed by the investigators is a proof of principle that functional tumor-specific CTLs can be generated starting from human iPS cells.

Weaknesses: Although the proposed approach is innovative, its clinical translation remains highly questionable due to the teratogenic potential of iPS cells despite a differentiation process in vitro. In addition, it is clear from clinical trials that highly functional tumor-specific CTL can be easily generated from peripheral T cells by gene transfer of TCR or chimeric antigen receptors. Finally, the "stemness" of iPS generated T cells versus peripheral T cells still remains to be demonstrated.

Reviewer 3:
Strengths: The significance of this project for improving health is that they can induce human iPS cells into functional antigen-specific CTL cells with preventive effect in a murine melanoma model.

Consider any major discoveries, new drugs and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. In the final progress report, they state that "we developed a novel approach for generating therapeutic Ag-specific T lymphocytes from iPS cells." And based on the results of this project, they would file an application for a U.S. patent-Generation of Ag-specific T lymphocytes by programming iPS cells.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
No leveraging of funds occurred, nor was this expected. The researchers submitted a NIH R01 grant in October 2012. This research project did not receive funding from any other source during the project period when it was supported by the health research grant, nor were applications submitted to continue or expand the research. A NIH R01 application based on the research project was submitted in October 2012 to carry out follow-up studies.

Reviewer 2:
Strengths: The investigator submitted a RO1 on October 2012 which is a follow up study based on the data generated.

Weaknesses. None.

Reviewer 3:
Strengths: The researchers submitted an NIH R01 for additional funding to continue or expand the research.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
One manuscript on *in vivo* programming of tumor antigen-specific T cells from pluripotent stem cells to promote tumor surveillance was published in *Cancer Research* in May 2011. A second manuscript on transgenic expression of survivin which compensates the OX40-deficiency in driving Th2 development and allergic inflammation was submitted to *European Journal of Immunology* on 12/22/2011 and published in 2013. The Cancer Research paper was categorized as a Priority Report and contained 4 of the figures from the progress report. This paper illustrates that antigen-specific T cells can be generated from pluripotent stem cells *in vivo*, and is a major accomplishment. The second paper showed that survivin transgenic mice exhibit an increased antigen-driven Th2 lung inflammation, and that constitutive expression of survivin reversed the defective lung inflammation even in the absence of OX40 co-stimulation. These results suggest that OX40 costimulation engages survivin during antigen-mediated Th2 responses. These two published papers are high quality. However, the second paper was not directly related to the original research aims.

The research project did not result in commercial development of any research products.

Reviewer 2:
Strengths: The investigator generated data that resulted in a very good publication in cancer research in 2011.

Weaknesses: A second manuscript is ready for submission, but the topic of this manuscript seems unrelated to the funded research. However, this is not necessarily a weakness since it proves that the investigator is productive.

Reviewer 3:
Strengths: One manuscript published, one submitted, and a third in preparation for submission to Journal of Immunology in May 2013.

Based on the results of this project, the researcher are planning to file a U.S. patent application: Generation of Ag-specific T lymphocytes by programming iPS cells.

Weaknesses: The researchers provided two peer-reviewed published articles. But, they are not the results of the proposed experiments of this study although they used a similar technical approach.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
This project expanded the scientific base for cancer research at the institution. There was no recruitment of out-of-state researchers. Funds were used to pay for research performed by a post-doctoral student and two pre-doctoral students participated in the project.

Reviewer 2:
Strengths: The grant was used to train 2 pre-docs and 2 post-docs. The project also extended the knowledge of the institution on the plasticity and potential clinical use of human iPS cells.

Weaknesses: None.

Reviewer 3:
Strengths: Funds were used to partly pay for one post-doc (25%).

There were two new pre-doc students and one post-doc added to help carry out this research.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The research project led to collaboration with Richard A. Morgan, Ph.D., at the National Cancer Institute. However, little information is presented in this regard.

Reviewer 2:
Strengths: The investigator established collaboration with Dr. Richard Morgan who provided the mart-1 specific tcr.

Weaknesses: The investigator should attempt to establish collaboration with investigators in the field of iPS cells to study in detail the potential for malignant transformation of T cells generated from iPS. Finally, an accurate evaluation of the insertion of the transgenes into T cells generated from iPS should be performed. All these aspects are crucial for a potential clinical translation of the proposed approach.

Reviewer 3:
Strengths: This project enabled them to collaborate with an investigator outside of their institution.

Section B. Recommendations

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.

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.

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.

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

Generic Recommendations for Penn State University

Reviewer 2:
The investigator is developing a very interesting area of research and he is scientifically productive.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The project is in the process of meeting its original objectives. They have developed a new cannula system for their pediatric LVAD system but have not yet been able to extend the LVAD implant to 8 weeks.

They have shown that these cannulas are biocompatible for the short period (35days) that they have been implanted and have completed modifications and specifications for arterial and apical cannulas and their connectors.

The data collected and the information obtained was applicable to the objectives that were listed in the original plan.

Reviewer 2:
The primary objective of this project was to take the Penn State Pediatric Ventricular Assist Device (PVAD) to the next stage of development. The PI and co-investigators have met this objective in that they have completed the design and fabrication of the cannulae and connectors. A particular strength of the study was the fact that they used expanded polytetrafluoroethylene (ePTFE) in the construction of their cannulae components. Polytetrafluoroethylene is extremely stable and resists biodegradation. It is also anti-thrombogenic and minimally activates platelets and inflammatory responses. Thus, their design represents an important improvement over devices that are presently in use. Moreover, the data were developed sufficiently to address the proposed research question.

A secondary objective was to perform studies of 8 weeks duration in juvenile sheep (n=2) to further assess the biocompatibility of the system. Thus, effects of the PVAD on thrombogenicity (via assessment of indices of clot formation and blood coagulation) were determined.

Biomarkers of renal ischemic injury were also identified as potential biomarkers for renal ischemic injury. In one of these animals, the objective was met. No clinical evidence of thromboembolism or end organ damage (e.g. kidney) was found. Necropsy results indicated excellent biocompatibility. Minor renal infarcts were found of uncertain origin. The second

animal study was not completed; this sheep developed respiratory dysfunction post-operatively and was subsequently euthanized.

Since the loss of the second sheep was not offset by the addition of a third animal, the investigators only partially met the criteria of this secondary objective. While the results of the one successful study do suggest that the new cannulae are suitable for surgical use, a minor weakness is that the investigators were not able to demonstrate that their one outcome was reproducible. For example, due to biological variability, it is not clear that the magnitude of the clotting and coagulation responses would be consistent.

Reviewer 3:
The research team completed the design of the apical and arterial cannulae for the Infant PVAD. Prototypes were manufactured and reasonably tested *in vivo*. Even though one animal was euthanized, the findings of the animal experiment indicated that the new cannulae are suitable for surgical use; additionally, the risk of thromboembolism was found to be low with reduced levels of heparin anticoagulation during testing. No significant issues were demonstrated with the resulting cannula design or connectors. The second objective to investigate biomarkers for renal ischemic injury was also performed with promising results as more sensitive measures of device embolization. The only criticism is that a true chronic animal study was never achieved; perhaps one more attempt would have been successful. It is well-known, however, that chronic animal studies are very challenging and death during such testing is common for a wide range of reasons that quite frankly may have nothing to do with the device intervention.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The project will help to develop and implement VADs for infants requiring a bridge to cardiac transplantation which is currently an unmet need in congenital open heart surgery.

This will have a major impact in the improvement of health outcomes in a variety of congenital heart lesions that cannot be corrected by surgery.

The authors plan to continue testing the use of these new cannulas and extend the time that they are implanted to 8 weeks.

Reviewer 2:
The beneficial impact of this project was the advances that were made on the Penn State Artificial Heart Program that have led to the development of smaller ventricular assist devices that can be used in children and infants (the primary objective of the project). This is an area of need as the types of devices available for this purpose are limited. In this regard, only one PVAD is presently available commercially for providing cardiac support of greater than one month. Using material such as ePTFE, the investigators have provided evidence that their devices minimize the risk of clot formation and stroke, which bodes well for the safe implantation for

longer periods of time, and is indicative of an important advance in the development of these devices (a major strength of the project). This development also has clinical relevance for prolonged cardiac support that may be necessary in infants with congenital heart defects; especially where heart transplantation is required in the face of long waiting periods.

The future plans for this research project are to secure additional funding to initiate pre-clinical trials that ultimately lead to clinical testing of their devices.

Reviewer 3:
The purpose of this research is to advance the Penn State Pediatric Ventricular Assist Device (VAD) to the next stage of preclinical development. The Pediatric VAD is a blood pump, which is intended for infants less than 9 months of age, provides mechanical assistance to the native myocardium that is weakened by congenital or acquired heart disease. The VAD is designed to provide such mechanical circulatory assistance for several consecutive months, as a bridge to heart transplantation, or as support leading to recovery of native heart function. There are few treatment options available for the pediatric population, and this PVAD fills a significant and growing need in this country. Advances in the pump design and ancillary components were achieved. Additionally, progress was made in the use of the biomarkers to provide early insight into thromboembolism in end organs. It was not clear if the cannulae design, as implemented in the animal studies, will require any modifications or adjustments for clinical use in patients. It was not clear if these modifications, which are usually expected, would represent a drawback of the design and its translational implementation (i.e. shape change, lengthening or shortening of cannulae, etc.)

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The authors have received 1 NIH-sponsored grant and have applied for additional external funding

Reviewer 2:
In the strategic plan, the investigators indicated that additional funding is being sought for more extensive, pre-clinical studies leading to clinical trials. In addition, they state the project will be considered successful, in part, by the success of obtaining additional NIH grant awards that further fund the development of the Infant VAD and lead to FDA-approved clinical trials. In this regard, a NIH Grant titled “Novel cannulae system for pediatric ventricular assist devices” was submitted on 12/5/10, but was not funded. However, in July 2011, the investigators, along with Minnetronix, Inc., were awarded a Small Business Innovation Research Grant from the NIH to develop a portable pneumatic driver for PVAD (“Pre-clinical testing of a VAD for uni- and bi-ventricular support in infants”). Total funds of $2,984,501 were requested and $746,084 was awarded. The investigators are also pursuing industrial partner support. Thus, the investigators have met Criterion 3, which is a major strength of the outcome of this project, as only $76,264 in research funds were spent.

Reviewer 3:
The research team was successful in leveraging additional funding to continue the long-term objective of translating this device to the clinical realm. An SBIR grant was awarded for the portable driver system. The team submitted an R01 and other SBIR applications. Unless this reviewer is incorrect, an R01 was recently awarded to support preclinical testing of the PVAD. This team has a history of success in partnering with industry representatives and in the translation of ventricular assist devices from the bench to the bedside for adult and now pediatric patients.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The authors have published 1 peer-reviewed paper and others are anticipated. They do plan to file for patents and licences.

Reviewer 2:
One peer-reviewed publication related to the project was submitted: Cooper TK, Zhong Q, Nabity M, Rosenberg G, Weiss WJ (2012). Use of urinary biomarkers of renal ischemia in a lamb preclinical left ventricular assist device model, *Artificial Organs*, 36(9). It reports the results of the one animal study that was performed in this project that was related to effects of implantation of the PVAD device on clinical markers of ischemic tubular damage. The authors concluded that evaluation of urine biomarkers of renal ischemia are potentially valuable as sensitive real time monitoring of renal damage caused by blood pump devices.

The PI indicates that the project has not resulted in any licenses, patents or commercial development opportunities at this time. However, he also states that commercial partnerships to further develop the PVAD system are actively being pursued. Evidence for this contention is the fact that the investigators have recently partnered with Minnetronix, Inc. to obtain funding from a Small Business Innovation Research grant from the NIH to develop a portable pneumatic driver for the PVAD that may, in turn, lead to commercial development of this device.

Reviewer 3:
One publication resulted from this research effort. It is not clear if any domestic or international presentations were delivered to further disseminate results to the scientific and medical community. An industrial partnership was established. Based on the amount of data generated from the animal studies and the proprietary aspects of the cannulae design, an expectation of one publication seems reasonable.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
No improvements to the infrastructure, no new investigators brought into the institution, no funds for post-doc fellows.

Reviewer 2:
The project enhanced the quality of research at the grantee's institution as it contributed to a primary goal of the Penn State Artificial Heart Program of developing small ventricular assist devices for use in children and infants via advancing the development of the technology required to build them.

It is not clear if improvements to the infrastructure were made using funds from this overall formula grant. However, only $76,264 was spent on the project in question and most of this appears to have been used to cover the technical costs of developing the PVAD and conducting the animal studies.

No new investigators were brought into the institution to help conduct the research.

It does not appear that any funds were used to pay pre- or post-doctoral students as the key personnel were made up of Penn State faculty, a bioengineer, a mechanical designer and an animal study manager employed by the university. The use of this group of investigators is another strength of this project because these key personnel appear to represent a long standing, experienced team that is capable of providing the necessary expertise to successfully conduct the research project.

Reviewer 3:
The infrastructure and resources at Penn State Hershey are perfectly suited to conduct the research as proposed in the research project. The project leveraged the existing excellent pool of internationally renowned experts at Penn State who have decades of experience in the development of ventricular assist devices. No additional internal or external researchers were required to execute the objectives as set forth in this project. The team fostered a successful industrial partnership for the SBIR award. In an effort to train a technically-skilled workforce and the engineers of tomorrow, the inclusion of a doctoral student or post-doctoral fellow would have added value to the project. This team has a history of success in partnering with industry representatives and in the translation of ventricular assist devices from the bench to the bedside for adult and now pediatric patients.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
No partnerships outside the institution.

Reviewer 2:
Mary Nabity, DVM, PhD, from the Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, was a co-author on the publication resulting from this project. Her research focus is urine proteins as early makers of kidney disease.

The investigators did not indicate if they are planning to collaborate with research partners outside of the community as a result of this research project.

Reviewer 3:
The project leveraged the existing excellent pool of internationally-renowned experts at Penn State who have decades of experience in the development of ventricular assist devices. No additional internal or external researchers were required to execute the objectives as set forth in this project. The team fostered a successful industrial partnership for the SBIR award. There was no new involvement that was sparked by this project with the surrounding community.

Section B. Recommendations

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.

2. The authors still must determine the biocompatibilty 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.

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.

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.

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.

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.

ADDITIONAL COMMENTS

Reviewer 1:

The strength of the project was the ability to complete the modifications and specifications required to develop and test the arterial and apical cannulas and their connectors.

Generic Recommendations for Penn State University

Reviewer 1:

This is an important project with very relevant clinical applications. There is an urgent need to develop VADs that can support infant hearts for cardiac transplantation. The results of this proposal will go a long way to providing a clinical solution to the problem of infant pediatric support for those hearts which are not surgically repairable and must be transplanted. These initial results are promising and the authors should be encouraged to continue and complete this project.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The project has largely met and achieved its stated objectives. New information has been generated regarding the changes of branched chain amino acide (BCAAs) and the potential role of their metabolism in obesity and obesity-related diseases. There is a detailed and well written report of the finding and accomplishments from the project. There are several recent publications related to alterations of BCAAs in obesity and important funding has been obtained through an R21 grant from National Institutes of Health NIDDK Division (Diabetes, Digestive and Kidney Diseases) and an R01 was submitted and is in the review process at the time of the final progress report.

Reviewer 2:
It appears that the proposed work was done and the specific aims were completed. The stated performance measures were to have two peer-reviewed publications and submission of an NIH grant on this topic. As reported, there was one paper published and a grant application submitted to be reviewed summer of 2013, outcome unknown.

There were some changes made to Specific Aim 1 based on some reviewer feedback. This appeared to be a reasonable and not substantial change from original intended experiments. The purchase of the LYNX tunnel mouse cage washer for the Penn State Hershey animal facility was also described in the final progress report, but not presented in the application.

Reviewer 3:
Objective one: This objective was revised. The original objective of determining whether the "energy wasting" phenotype of BCATm KO mice is due to elevated BCAA elevation or BCKA depletion (or both) has not been met. This objective was to have been met using BCATm, BDK and E2 knockout mice. In lieu of that goal, the investigators sought to develop a BCATm floxed allele in order to develop tissue-specific knockouts in order to determine tissue-specific contributions to the phenotype. These mice have been generated but not yet bred with muscle-specific Cre mice to knock out BCATm in muscle. Thus, no data is available in support of aim one. The revised objective is more objective than the original, and most certainly worthwhile, but may not be a fully appropriate substitute for the original objective. It may have been more appropriate, and provided more continuity with existing literature, to first directly address the

original goal of identifying whether BCAA or BCKA modulate energy expenditure using the global knockouts originally proposed, in order to provide the rationale for generating tissue-specific knockouts.

Strengths: Progress in developing tissue-specific knockouts.

Weaknesses: No data. Revised goal should have built on data from objective as stated, rather than replace it.

Objective two: This objective, to determine the underlying cause of elevated BCAA in obese animals was fully completed, and the data persuasively indicated failure to activate BCKDC, resulting in markedly greater increases BCKA vs BCAA, with the BCKA appearing responsible for the phenotype, at least in Obese Zucker rats. However, it is not clear if these data will extrapolate to other models that more closely mimic human obesity.

Strengths: Goal addressed; conclusive, important data provides clarification of controversy in important area of metabolism; identification of therapeutic target.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:
The potential benefit of the project is substantial. Study of the metabolism of BCAAs and their role in obesity, diabetes and metabolic disease is considered to be a "hot topic" at this time and a better understanding of the detailed metabolic pathways, mechanisms, and relationships with energy balance, insulin sensitivity and hepatic and systemic BCAA metabolism are likely to lead to new insights and potentially new therapeutic options for these important and prevalent conditions. For example, a next step for the PI and his research team is to investigate the role of the BCKA kinase enzyme activity in regulating satiety and energy balance in the hypothalamus.

Reviewer 2:
The PI and his team appeared to have generated substantial data that contributed to a published paper and an NIH grant application. The work itself contributed to the research field of obesity mechanisms by helping clarify the role of branched chain amino acid metabolism in obesity and development of diabetes. This work may provide a new potentially druggable target to help induce a benign futile cycle for increased calorie burn to help reduce the problem of obesity.

Reviewer 3:
Strengths: Provides potential resolution of BCAA paradox in obesity (BCAAs leading to favorable phenotype, while elevated BCAAs in obesity serve as "metabolic signature" of adverse phenotype).

High impact in providing potential rationale for utilization of dietary leucine in sub-groups.

High impact in providing potential therapeutic target for T2DM in obesity.

Weaknesses: None noted.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:
New funding was obtained in 2012 for a R21 grant on using adipose tissue transplants to treat a disorder of BCAA metabolism from National Institutes of Health NIDDK Division (Diabetes, Digestive and Kidney Diseases). In addition, a R01 grant was submitted to the NIH in 2013. The current funding status of this application is unknown. A future application is planned to determine whether dysfunction in BCAA metabolism is involved in the pathophysiology of metabolic diseases in humans with insulin resistance and type-2 diabetes mellitus (T2DM).

Reviewer 2:
One NIH grant application is in the process of review, scoring, and awaits final resolution. This is a very useful outcome for a funded one-year project via this program.

Reviewer 3:
Strengths: One NIH grant submitted based on this project.

One additional submission planned to examine potential for alloisoleucine as a biomarker for partial BCKD dysfunction in T2DM.

Weaknesses: None noted.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Yes, the project has resulted in several important publications during the project period related to the topic of the research on branched chain amino acids including a paper on BCAA metabolism and obese Zucker rats. Importantly, the principal investigator has plans to follow up these observations in polygenic models with intact leptin signaling pathways. While not all the publications on BCAAs (18 discovered in a literature search) may be directly related to the project per se, this nonetheless represents a very good level of research productivity.

Reviewer 2:
One published paper: She P, Olson KC, Kadota Y, Inukai A, Shimomura Y, Hoppel CL, Adams SH, Kawamata Y, Matsumoto H, Sakai R, Lang CH, Lynch CJ: Leucine and protein metabolism in obese Zucker rats. PLoS One 2013;8:e59443

Reviewer 3:
Strengths: One paper published in PLoS One.

One invention reduced to practice and patent disclosure filed on BCKD kinase as a therapeutic target for T2DM in obesity.

Weaknesses: None noted.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:
A portion of the funding was expended to contribute to the costs of a LYNX tunnel animal cage washing system that is stated as a benefit to researchers from sevral departments using rodent models at the animal facility of the Hershey Center for Applied Research and the Penn State Cancer Institute.

Reviewer 2:
The leucine metabolism project mostly benefited the PI. The LYNX tunnel cage washer benefited ~65 investigators in multiple departments at Penn State Hershey.

Reviewer 3:
Strengths: Infrastructure: Contributed to purchase of tunnel cage washer for animal research facility.

Weaknesses: No graduate or post-doctoral trainees supported.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:
Collaborations with colleagues at the Institute of Innovation at Anjinomoto, Inc. in Japan are listed as collaborators/partners on the project. In addition, Dr. SH Adams from the Western Human Nutrition Research Institute and the University of California, Davis is a co-author on a recent publication in PLoS One on BCAA metabolism in Zucker rats and a manuscript on BCAAs in humans in the American Journal of Physiology.

Reviewer 2:
There was collaboration with a Japanese company Ajinomoto that provides special diets and nutriceuticals.

Reviewer 3:
Strengths: One collaboration (Ajinomoto).

Weaknesses: Association with Ajinomoto not a true collaboration; they are a source of material used in this research. No apparent collaborations with Ajinomoto scientists listed.

No other collaborations listed.

Section B. Recommendations

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.

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.