

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 (1.75)

Project Rating:

| Project | Title | Average Score |
|----------------|---|----------------------|
| 0863901 | Roles of the Nuclear Receptor Cofactor LCOR in Placental Development and Gene Expression | Favorable (1.67) |
| 0863902 | Establishment of an Animal Model for Respiratory Infection with Influenza during Pregnancy | Favorable (2.00) |
| 0863903 | Immune responses to herpes simplex virus type 2 and Chlamydia muridarum in a murine model of co-infection | Favorable (2.00) |
| 0863904 | Integration Study of the Target Genes of PPAR gamma in Human and Mouse Placenta | Favorable (2.00) |
| 0863905 | Soluble KIT Receptor in the Pathogenesis of Preeclampsia | Favorable (1.67) |
| 0863906 | Post-Transcriptional Regulation of Fstl1 mRNA in Human Trophoblasts | Favorable (1.67) |
| 0863907 | Analysis of Functional Domains within NDRG1 | Favorable (1.67) |
| 0863908 | Immunity to MUC1 Tumor Antigen in Conditional and Transplantable in vivo Models for Ovarian Cancer | Outstanding (1.33) |

Project Number: 0863901
Project Title: Roles of the Nuclear Receptor Cofactor LCOR in Placental Development and Gene Expression
Investigator: Barak, Yaacov

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 research is proposed based on the PI's previous novel findings. The preliminary data for the proposed studies is very strong and the data obtained indicate progresses on targeted objectives. The specific aims were cohesive, investigators were strong, and approaches were state-of-the-art. Other strengths were the in vivo and in vitro models.

Weakness: None identified.

Reviewer 2:

In general, the project met many of the stated goals. The original hypothesis that LCoR is a key co-activator of PPARgamma-regulated genes in the developing placenta, did not reveal that this co-activator was crucial for gene expression of MUC1. In an effort to examine whether LCoR was critical for widespread gene regulation through PPARgamma, the PI employed the LCoR-deficient mouse and detected 100% neonatal lethality for a myriad of reasons. This was examined throughout the remainder of the project.

Reviewer 3:

The project met only a portion of the originally stated objectives, in part due to the ambitious nature of the original research plan. Several important strengths emerged from this research:

1. In studies from Aim 1, the PI successfully developed the use of the LCOR gene trap mouse mutant to aid in the clarification of the role of LCOR in vivo with particular emphasis of the competency of the placenta. These studies will form an important foundation for future studies of this transcriptional regulator and show how LCOR is linked to gene activation and/or transcriptional repression in a physiologically relevant model system. While this LCOR gene trap model supported a role for LCOR on Muc1 transcript levels within the placenta, these differences were quite modest, and despite the PIs claim that LCOR may play a critical role in Muc1 regulation, the role of LCOR likely falls to more important genes within the mouse placenta.

2. Studies in Aim 2 established an important perinatal lethality of the NCOR null animals characterized by modest reduction in fetal weights, enlargement of placental weight, and a corresponding shift in fetal weight to placental weight ratios. The preliminary data suggests the possibility of altered glycogen metabolism within the spongiotrophoblast compartment within the placenta; however, this observation is quite preliminary and would need a more extensive examination beyond H&E histopathology analyses to confirm and better characterize this putative placental defect and how it may contribute to enlarged placental size and function of the maternal fetal interface.

3. A great deal of progress was made within Aim 3 where the PI's lab really appears to be at its strongest with in vitro transfection studies. The PI demonstrates an important analysis of structural and functional domains with LCOR as well as analysis of promoter enhancer elements within the Muc 1 5' flanking sequence that may play a role in how LCOR may participate in the control of Muc 1 gene transcription. Perhaps an important highlight of these studies is identification of the putative role of KLF6 in the regulation Muc 1 through putative association with PPAR γ and LCOR. While intriguing, these studies currently are quite preliminary, but appear worthy of further analyses in the context of PPAR γ - and LCOR-dependent regulation of placental amorphousness and function.

Several important weaknesses dampened the enthusiasm for the outcomes assessment of the research plan:

1. A more extensive or convincing rationale would have been helpful for why the analysis of Muc 1 as a unique and novel target of PPAR γ and NCOR molecular mechanisms is necessary for our understanding of placental function. While a potentially interesting target, it remains unclear if Muc 1 has much relevance to biomedical issues or potential application to placental health. This is an important point of the completed research and, at present, it is difficult to generate high enthusiasm without a more vibrant argument for the importance of Muc

2. Overall, the PI describes research outcomes in terms that appear to lack qualitative accuracy or rigor. The text lacks a description of how statistical analyses were carried out. The PI references statistical differences that appeared surprising given the apparent large variance components for the means presented. An excellent example is in Figure 3 where modest changes are evident, with means characterized largely by overlapping standard error bars, but are reported as statistically meaningful. This may reflect a large sample size; however, this is not made clear.

3. In Aim 2, the observation of increased presence of glycogen cells within the spongiotrophoblast compartment is interesting; however, there is no data regarding this cell type beyond H&E staining. This is an important aspect of the histological analyses, yet is relatively poorly characterized. Additional analyses using immunohistological approaches would be more convincing to determine the identity of this lineage and how it may affect placental function.

4. While progress in Aim 3 is the most substantial, these studies are almost entirely dependent on reconstitution studies and over expression of a number of transcriptional regulators. The PI provides no evidence that the level of expression of (for example) NCOR or KLF6 is within any physiologically relevant level. I believe this to be quite important since over expression and

reconstitution studies like these can be easily over-interpreted and misleading without additional verification of the physiological and or pharmacological levels of expression. Lastly in Aim 3, concern was raised for the various use of different reporter constructs ranging from a putative full length Muc 1 promoter, to a series of enhancer fragments that is unclear, in interpretation, of the totality of this approach. Could these over expression approaches be useful in analysis of endogenous gene transcript levels to compare to the reporter gene studies? This may be particularly important since much of the basis for interest in NCOR is in the ability to modify the chromatin environment; use of the transient transfection brings into question the level of “chromatinization” on these templates within the transfected cell. These studies do have the potential to be quite interesting, but as presented, they appear superficial and lack a collective rigor necessary to form a discrete conclusion beyond the initial finding that PPAR γ and LCOR are important for Muc 1 promoter activity.

Overall, the data reflect that reasonable progress was made within the research plan and likely sets important foundations for future studies.

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 basic science project targets on transcriptional control of placental development. Although no immediate benefits will be generated for improving health outcomes, data obtained are important for the understanding of basic placental biology.

The investigators have generated interesting data to support their major hypothesis that LCOR is a co-activator for PPAR gamma-regulated Muc1 expression in the placenta.

They have planned to get the data published as a short-term goal and set a long-term goal to further decipher the diversity and specificity of LCOR and other transcription factors and co-activators in placental gene expression and development.

Reviewer 2:

Although this mouse project does not have direct or immediate consequences for human health, these fundamental studies highlight the role of PPAR gamma in placental development, acting through the co-activator LCoR.

Reviewer 3:

The beneficial impact of the outcomes thus far on this project is related to increased understanding of important molecular determinants of placental function/development at a very basic level. There is a likelihood that these studies and the studies that come next will inform how we might approach placental disease at the bedside or (perhaps more importantly) the intrauterine environment that may be critically linked to the fetal origins of disease; however, at present there is no direct application to improvement of health outcomes. Importantly, the PI acknowledges this. The studies planned for the future continue to address important basic

questions on the molecular determinants of placental function. Perhaps the greatest significance comes from the development of new mouse models of placental insufficiency and the determination of the molecular mechanisms involved in these unique phenotypes. It is hoped that these determinants may reflect important bio markers or “druggable” targets that may have future utility.

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 unclear whether the project leveraged additional funds or if any additional grant applications were submitted.

Reviewer 2:

No additional funds were leveraged from other sources for this project.

Reviewer 3:

The PI likely anticipates that these new data will contribute, in part, to a renewal of NIH funding or potential funding from other federal or private sources. However, during this funded year, no new funds were leveraged. The PI indicates anticipated application to new 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 authors planned to submit at least one paper to *Molecular and Cellular Biology* or the *Journal of Biological Chemistry*.

Reviewer 2:

The PI has indicated that at least one peer-reviewed publication would be submitted based upon the findings during the project, but none have been submitted to date.

Reviewer 3:

No new publications emerged from this data set as currently present. The PI anticipates these data to be incorporated into future publications.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

It is not clear whether any improvements were made to infrastructure. No new investigators were added, nor were any researchers brought into the institution to help carry out this research. No pre- or post-doctoral students were hired.

Reviewer 2:

The quality of the studies outlined in the project and the expertise of the PI in mouse genetics and PPAR γ biology enhances the overall quality/capacity of the institution. There were no apparent changes to infrastructure.

Reviewer 3:

The PI provides no evidence that the infrastructure of the institution was improved or that these funds were used to support scientific training at the pre-doctoral or post-doctoral levels. The PI does report the development of an important collaboration with a faculty member at McGill University.

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 investigator is collaborating with Dr. John White (the original discoverer of LCOR).

Reviewer 2:

No additional collaborations are mentioned in the Final Report.

Reviewer 3:

The PI reports a new collaboration with Dr. John White at McGill University, in Montreal. This collaboration focuses on the role of LCOR in the regulation of KLF6. This is an important aspect of the research outcomes described since this new discovery of the putative role for KLF6 in the regulation of PPAR γ and LCOR-dependent transcriptional mechanisms is critical to future research plans.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

None.

Reviewer 2:

1. The investigators should work to complete the studies outlined in Aims 2 and 3.

Reviewer 3:

1. As written, it is presently unclear that Muc 1 (a central model used in these studies) plays a crucial role during placental development. A more extensive or convincing rationale would have been helpful for why the analysis of Muc 1, as a unique and novel target of PPAR γ and NCOR molecular mechanisms, is necessary for our understanding of placental function.
2. A more comprehensive description of the use of statistical analyses in these studies would have added strength to the outcomes described.
3. The histological analyses of the important finding of changes in population of glycogen cells within the spongiotrophoblast layer of the mouse placenta, is relatively poorly characterized. Additional analyses using immunohistological approaches would be more convincing to determine the identity of this lineage and how it may affect placental function.
4. In Aim 3, good progress is recognized with the analysis of the Muc 1 gene promoter; however, the use of many different promoter fragments including very small segments of the Muc-1 gene promoter leave open to argument how to interpret these data. This reviewer believes there is important information in these studies. A more systematic approach to how these reporter gene studies are carried out and the data interpreted would be of great help.

Project Number: 0863902
Project Title: Establishment of an Animal Model for Respiratory Infection with
Influenza during Pregnancy
Investigator: Beigi, Richard H.

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 specific aims were: 1) determine the LD50 for aerosolized flu (PR/8) in pregnant and non-pregnant mice; 2) compare whole-body aerosol with nose-only delivery of flu in pregnant and non-pregnant mice by disease course and clinical signs; and 3) compare the deposition and dissemination of flu in pregnant and non-pregnant mice.

The overall project goals mirrored these aims, as well as to develop a collaborative group that would facilitate a comprehensive approach to study highly infectious diseases in an animal model of human pregnancy. On the whole, the project was able to meet the stated objectives or a reasonable variant thereof. Certainly, reasonable progress was made on the objectives. The research design and chosen methodology were appropriate for the objectives. The investigators were able to determine, at least within a factor of 10, the LD50 values for the virus delivered by aerosol inhalation in their exposure system. They were more or less able to show with inert microspheres that delivery of PM to the animals was comparable with aerosol in whole body exposures, and by nose-only exposure. Based upon this experiment they decided to omit the comparable experiment with the flu virus and go to the whole body exposure route, which is inherently less stressful and more closely models actual human exposures to the virus. They also used the data from the inert microspheres experiment to conclude that the lung deposition studies indicated the amount of the microspheres deposited and retained at 2, 24, and 48 hours in the lungs of pregnant and non-pregnant mice, did not differ significantly. This is a significant change in the research protocol, which originally proposed to do this experiment with aerosolized live virus, and involved some major assumptions that should be examined in future studies.

Strengths: The investigators have reviewed the relevant literature and make a convincing case for the need to better understand why pregnant women are a particularly sensitive and high-risk group for influenza-induced mortality. The experimental approach they have taken to investigate the interaction between influenza virus exposure and response in pregnant and non-pregnant mice seems to be a solid start to studying the underlying mechanisms responsible for these previous clinical observations.

This project supplied seed funding for a young clinical investigator in obstetrics, recently recruited to the state of Pennsylvania, to begin his laboratory research career in a productive collaboration in an area of high relevance to public health. A quick search on PubMed showed that the principal investigator has been a productive clinical investigator with 23 publications listed, but this seems to be his first laboratory-based research that will produce publishable data. The overall quality of the research is sound and the questions posed are important. This research group has the potential to make an impact in their chosen field as these studies continue. Substantial progress has been made on all of the specific aims.

Weaknesses: One of the major potential confounders in whole-body exposure experiments is an animal behavior called “preening,” where rodents lick each others fur during and after exposure. Thus, the virus may be ingested as well as inhaled in these experiments, and allowance for the ingested virus must be made in the dosimetry and deposition experiments. There is no indication that the investigators have accounted for this phenomenon, which may be contributing to (or responsible for) their conclusion that the virus has a lower LD50 (by 10-fold) by the aerosol challenge than the prior literature values estimated for the LD50 by intranasal inoculation. This putative additional route of exposure should be controlled for in subsequent studies of virus infectivity. A nose-only exposure study to test whether this phenomenon of preening is affecting the apparent LD50 for the virus would be a simple approach.

In addition, the comparison of viral load (and/or viral titers), by one route of administration, aerosol exposure , with literature values for another route of exposure (intranasal instillation) previously published by other laboratories, is subject to many potential errors. The investigators should calibrate their conclusion by determining whether the comparative parameters for intranasal instillation performed in their laboratory with their viral strain and their animals agree with the cited values from the literature. The assumption that the inert microspheres behave identically to live virus in aerosol exposures should also be tested experimentally in future work.

Viral deposition and retention should be evaluated in tissues other than the lung after whole-body exposures, especially in the gastrointestinal tract, liver, uterus, and the fetuses themselves. Virus particles may be swallowed after preening or after mucus clearance from the lung and nasopharynx. Dosimetry calculations should account for these alternative routes of exposure and loci of distribution.

The basic maxim of toxicology as attributed to Paracelsus is “the dose makes the poison.” That adage may also apply here; very high viral loads (LD50-LD99) are being used for these experiments. This might be part of the reason for the obvious fetal losses at the highest dose of virus used. The relevance of these findings for clinical use is questionable. Careful attention to appropriate dose-response experiments as this project proceeds is highly recommended.

The investigators identified an issue of non-pregnant mice among the animals purchased as timed pregnant and allocated to the pregnant group. They have shifted the dates of exposure to allow themselves to better ascertain pregnancy prior to virus exposure, which seems to be an appropriate response. The researchers also suggest the possibility of creating their own breeding colony to better control the overall process. They do not discuss the possibility of stress-related abortion in the mice that should have been pregnant, and should better monitor their animals in order to be able to rule out this possibility. They also mention cannibalization of dead or

moribund fetuses as a possible reason for smaller litter size in the infected animals, which suggests less than optimal monitoring of the mice might be occurring during critical experimental periods.

Reviewer 2:

The project has met the stated goals; the investigators have established a model of influenza challenge by aerosol in mice and have established the basis for the comparison of influenza effects in pregnant and non-pregnant mice. The data were partially developed in line with the original research protocol. The investigators could have gathered more information than just changes in body weight and survival to establish whether differences exist between pregnant and non-pregnant mice challenged with flu.

Reviewer 3:

The project failed to meet all of the stated objectives; however, three of the four objectives were met at least in part:

1) Develop a model of collaboration and infrastructure. This objective appears to be met as the project was developed and did move forward over the nine months of the award. The investigators did an excellent job of developing a collaborative project and executing preliminary studies in a very short time. Moreover, they developed presentations for three abstracts and submitted an R21 grant proposal after the end of this funded work and have plans to combine results from two out of three of the abstracts for a publication. For this objective, expectations were exceeded, which is a major strength.

2) Develop an aerosol delivery challenge model for inhalational influenza among pregnant & non-pregnant mice. This objective was mostly met, although some details regarding controls and endpoints are unclear. The investigators have made considerable progress in generating data supporting a model of aerosol infection in pregnant and non-pregnant mice; however, additional experiments must be completed to control for and validate the results. They can clearly infect mice and measure weight loss and survival; however, they failed to address other endpoints from the proposal, and the added endpoints of birth weight, number of live births, and pup survival are not clear indicators of virulence and cannot be compared to non-pregnant mice.

Original endpoints also included lung virus titers, pathology, and placental virus titers. None of these endpoints, which certainly will vary with aerosol and topically delivered virus and may vary in pregnant and non-pregnant mice, were addressed. These are obvious oversights, especially as they were noted in the proposal. No reason was given for this omission, although the shortage of pregnant animals may offer an explanation. In any case, it is not reasonable to omit established disease endpoints for the murine influenza model when developing a new model.

Finally, the investigators proposed to test BALB/c and C57Bl/6 mice for model development. It appears that only BALB/c mice were tested. The change was not addressed; however, the immediate testing of two mouse strains is excessive and the omission is valid. It is a moderate strength that parts of this aim were achieved and the omission of Bl6 mice, while deviating from the proposal, is warranted. Presentation of possible novel endpoints (birth weight, etc.) is a

strength, but requires further investigation. Omission of pathology and tissue titers is a weakness and should be addressed in the future. Providing options for improving availability of pregnant mice is a strength and will be necessary for future work. Proposed work with pregnant ferrets seems unlikely considering difficulties in getting flu-free ferrets, let alone pregnant flu-free ferrets.

3) Compare whole-body aerosol exposure to nose-only delivery of influenza. This objective was not completed. The investigators never compare topical (droplet via pipette) to whole-body aerosol, to nose-only aerosol delivery of influenza. The comparison of whole-body aerosol to nose-only aerosol delivery was explicitly stated as an aim; the comparison to topical delivery was not. This is critical to some conclusions the investigators have made. Instead of virus, the investigators compared deposition of 1mm diameter latex beads in the lungs. It is unclear why this method was used. It was not mentioned in the Strategic Plan and is a poor substitute for virus.

While the investigators saw no differences between whole-body aerosol to nose-only aerosol delivery of 1mm latex beads (data not shown), these results do not address delivery of influenza in aerosol particles with a Mass Median Diameter of 0.0025mm (http://www.bgiusa.com/agc/output_distribution.htm). With different breathing patterns in nose cone apparatus compared to chambers, there could be very different deposition of small particles as compared to large 1mm beads. Also, the large 1 mm particles will have a very different suspension time in the chamber compared to the small virus particles, making the comparison tenuous. In summary, there was no scientific rationale provided for omitting this aim, which is a major weakness.

4) Determine the lethal dose (LD50) of a A/PR/8/34 (aerosol) in pregnant & non-pregnant mice. This objective was partly completed. This aim was mostly complete as an LD50 value was determined. This needs to be clarified in regard to comparisons made. First, there is no mention of controlling for weights of pregnant and non-pregnant animals and weight can influence mortality in the mouse flu model. Second, they note that aerosol deposition results in a lower LD50 than topical delivery, but there was no specific comparison made and virus stock to virus stock variability in lethality of influenza viruses is well documented (e.g., DI particle content), so the investigators cannot rely on reported LD50 values. Much of this aim was completed, which is a moderate strength, but additional controls need to be run and questions about contribution of weight need to be addressed. Omission of topical delivery for comparison of LD50 values is a minor weakness.

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:

Likely beneficial impacts of this laboratory study at the present stage are small, but these studies could have future clinical implications with regard to recommendations vis-à-vis vaccination of pregnant women at risk of exposure to influenza virus. The bioterrorism implications emphasized by the investigators are highly theoretical and difficult to evaluate in this context

except insofar as the extensive potential funding sources for Homeland Security are relevant. In light of the project's very modest budget, this small, very early stage, has potential beneficial impacts.

Reviewer 2:

The data presented can be considered very preliminary to ascertain its likely beneficial impact. However, the investigators are addressing a very significant, yet poorly studied area of influenza research, which is the effect of flu infection during pregnancy. These studies could, in the future, provide alternative avenues for intervention.

Reviewer 3:

This project has great potential for improving health. As recently seen with the 2009 pandemic H1N1 virus, pregnant individuals were at increased risk of complication from H1N1 infection as compared to non-pregnant females. Broad assumptions are made to explain differences; however, there is no clear mechanism. These studies will provide the framework to address these questions. While significantly more work needs to be completed to provide a complete model (e.g., C57Bl/6 studies for using transgenic and KO mice for immunological studies), the work funded by this project is a beginning. One question is the rationale for starting with aerosol delivery. While aerosol more clearly represents what is seen in nature, there is abundant data using topical delivery of influenza in the mouse model. Starting with topical delivery is a logical first step followed by aerosol delivery. The current data do not show convincing evidence that aerosol is different from topical, let alone showing differences in pregnant and non-pregnant mice. Future plans involving the submission of the R21 grant and a manuscript for publication are logical next steps in 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:

The project leveraged additional funds (\$10,000) from the investigator's department, and the research group has also submitted a new NIH R21 grant application for additional extramural funding of this work. If the NIH R21 grant (\$275,000) is awarded, this would be an additional strength of the project.

Reviewer 2:

No funds have been leveraged yet, but the investigators have submitted and/or plan to submit applications to major funding agencies.

Reviewer 3:

The project did leverage support from the PI's institution and have already submitted an R21 application. This is more than sufficient.

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 not yet resulted in any peer-reviewed publications, but three abstracts have been presented at scientific meetings, and the results presented in these abstracts should serve as the basis for a full publication in the future.

Reviewer 2:

No publications have resulted yet, and I would add that perhaps a significant amount of additional work is necessary in order to produce data with significant impact.

Reviewer 3:

Three abstracts were presented and one manuscript is in preparation. This meets the milestones as proposed in the Strategic Plan.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

An interdepartmental collaboration has been established and is productive. Small equipment for data capture by remote monitoring was purchased and should expand the research capacity of the institution.

Reviewer 2:

This is a collaborative work. In this regard, the project has met the expectations.

Reviewer 3:

The project resulted in purchase of a device for implantation of transponders and non-invasive monitoring clinical disease (temperature) in animals. While not a relevant endpoint for the mouse model, this is an improvement in capacity for larger mammals such as ferrets. There does not appear to be recruitment of new investigators; however, that was not within the scope of the proposal. The project did appear to foster new collaborations with existing faculty, which is a major strength. No pre or post-doctoral trainees were 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:

The collaborative group whose CVs were available to this reviewer are all affiliated with the University of Pittsburgh, but in different departments and research units.

Reviewer 2:

I could not discern whether there was external collaboration from the information provided.

Reviewer 3:

As noted above, the project did support a new collaboration with existing investigators at the institution, although there does not appear to be new collaboration as a result, outside the institution. The investigators did present the work at meetings, which could ultimately result in extra-institutional collaboration, but that is uncertain at this point. The submitted R21 appears to be an extension of the collaboration established with this project.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

1. One of the major potential confounders in whole-body exposure experiments is an animal behavior called “preening,” where rodents lick each others fur during and after exposure. Thus, the virus may be ingested as well as inhaled in these experiments, and allowance for the ingested virus must be made in the dosimetry and deposition experiments. There is no indication that the investigators have accounted for this phenomenon, which may be contributing to (or responsible for) their conclusion that the virus has a lower LD50 (by 10-fold) by the aerosol challenge than the prior literature values estimated for the LD50 by intranasal inoculation. This putative additional route of exposure should be controlled for in subsequent studies of virus infectivity. A nose-only exposure study to test whether this phenomenon of preening is affecting the apparent LD50 for the virus would be a simple approach.
2. In addition, the comparison of viral load (and/or viral titers), by one route of administration, aerosol exposure , with literature values for another route of exposure (intranasal instillation) previously published by other laboratories, is subject to many potential errors. The investigators should calibrate their conclusion by determining whether the comparative parameters for intranasal instillation performed in their laboratory with their viral strain and their animals agree with the cited values from the literature. The assumption that the inert microspheres behave identically to live virus in aerosol exposures should also be tested experimentally in future work.

3. The assumption that the inert microspheres behave identically to live virus in aerosol exposures should also be tested experimentally in future work.
4. Viral deposition and retention should be evaluated in tissues other than the lung after whole-body exposures, especially in the gastrointestinal tract, liver, uterus, and the fetuses themselves. Virus particles may be swallowed after preening or after mucus clearance from the lung and nasopharynx. Dosimetry calculations should account for these alternative routes of exposure and loci of distribution.
5. The basic maxim of toxicology as attributed to Paracelsus is “the dose makes the poison.” That adage may also apply here; very high viral loads (LD50-LD99) are being used for these experiments. This might be part of the reason for the obvious fetal losses at the highest dose of virus used. The relevance of these findings for clinical use is questionable. Careful attention to appropriate dose-response experiments as this project proceeds is highly recommended.
6. The investigators identified an issue of non-pregnant mice among the animals purchased as timed pregnant and allocated to the pregnant group. They have shifted the dates of exposure to allow themselves to better ascertain pregnancy prior to virus exposure, which seems to be an appropriate response. The researchers also suggest the possibility of creating their own breeding colony to better control the overall process. They do not discuss the possibility of stress-related abortion in the mice that should have been pregnant, and should better monitor their animals in order to be able to rule out this possibility. They also mention cannibalization of dead or moribund fetuses as a possible reason for smaller litter size in the infected animals, which suggests less than optimal monitoring of the mice might be occurring during critical experimental periods.

Reviewer 2:

1. Provide a better description of additional methods to measure the effects of flu infection during pregnancy and change the challenge virus to the pandemic H1N1 strain.

Reviewer 3:

1. Weakness: Use of 1mm latex beads for Aim 3 instead of live virus.
Recommendation: Complete experiments as proposed with aerosolized virus in the whole-body and nose-only aerosol devices. Measure virus deposition (titration and immunohistochemistry) at 2, 24, 48 and 72 hours post infection. Compare to topical delivery. Do not compare pregnant to non-pregnant as you first need a baseline in normal mice. If you see differences, you may decide to focus your pregnant to non-pregnant in only one delivery model based upon those differences. If they are identical, then just use one delivery. Since the topical will not be identical to aerosol, you should compare this to aerosol in your studies.
2. Weakness: Omission of tissue titers and pathology.
Recommendation: Include these endpoints in all studies. These are established endpoints in the murine model of influenza infection; without them, you have not established the baseline of a murine model, especially when these endpoints are critical for aerosol models.

Generic Recommendations for Magee Womens Research Institute and Foundation

Reviewer 1:

On the whole, this was a good selection of a project for seed funding; anew investigator, competent team, potentially important topic, and modest investment of funds.

Reviewer 3:

Continue to support the collaboration and provide both project (investigator) mentoring and project support to enable publication and extramural funding of an interesting and important project.

ADDITIONAL COMMENTS

Reviewer 2:

Strengths:

- 1) The project employed a PI with ample experience in the vulnerability of pregnant women to infectious diseases.
- 2) A model has been established to perform aerosol administration of influenza to pregnant and non-pregnant mice.
- 3) Data suggests that differences may be very subtle but measurable.
- 4) A very modest amount of funds was requested.

Weaknesses:

- 1) The choice of viral strain is not the best, and the investigators should consider testing more than one strain.
- 2) There is very little indication and/or description of additional methods that will be used to determine whether differences exist between pregnant and non-pregnant mice, particularly in light of the fact that the number of pups produced is affected after influenza challenge (which is not unusual; it has also been observed in pigs).

Project Number: 0863903
Project Title: Immune responses to herpes simplex virus type 2 and Chlamydia muridarum in a murine model of co-infection
Investigator: Cherpes, Thomas L.

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 basically met its stated objectives. The design and methods were adequate in light of the objectives. The data were developed sufficiently to answer the questions. Changes were made midstream. Aim 3 was to examine the interaction between prior HSV-2 infection, using a survival intervention from Aims 1 and 2 to enable survival, and subsequent Chlamydia infection. However, the investigators did not observe any difference in the pathogenesis of Chlamydia infection between control mice and HSV-2 survivor mice. Thus, detailed investigation within Aim 3 were abandoned. This is considered rational as if there is no high level phenotype; mechanistic and detailed descriptive experiments were not warranted within Aim 3.

There was some ambiguity about whether or not the HSV-2 survivor mice used in Aim 3, were the outcome of CD4-style interventions that were the outcome from Aims 1 and 2. The other main change that was made in the protocol pertained to a new line of investigation not discussed in the proposal, namely the role of Th17 cells and IL-17 in HSV infection. The later reports provided contain extensive IL17/Th17 data and it is not entirely clear if these experiments were done using project-supplied resources. Nonetheless, the investigators have made novel observations concerning the roles of IL-17 in experimental HSV-2 infection.

Another major change from the proposal is that the source of adoptively transferred CD4 cells was changed from vaginally infected mice to ocularly infected mice. The stated reason, that the vaginal infection was not consistent, is not reassuring given the fact that vaginal infection is used in the readout phase of all of the experiments in the challenged mice. The investigators thoroughly executed the research planned and envisioned in their original Aims 1 and 2. The data presentations provided were adequate to evaluate the accomplished research.

Reviewer 2:

The project included three very ambitious aims that were well justified and of potentially great benefit to the understanding of immune responses to HSV infections. The first aim tested the hypothesis that activated/educated CD4 T cells adoptively transferred from an infected host soon after infection of the recipient would provide protection from disease and long-term survival.

This would be of great benefit potentially creating an improvement in the commonly used mouse model of HSV-2 genital infection. The second aim was based on success with studies in the first aim. The underlying hypothesis was that subsets of T cells and specific regulatory pathways involving a subset of cytokines played a key role in murine responses to HSV-2 challenge. The last aim was to capitalize on the success of the other studies to develop a dual challenge model examining the impact of HSV-2 pre-existing infections on chlamydial disease.

The progress reports were well written and communicated effectively designed and executed experiments. Substantial progress was documented for Aims 1 and 2, including several novel observations on the roles of CD4⁺CD25⁺ T cells, Foxp3⁺ Treg cells and IL-17 in protection against advanced HSV-2 disease following vaginal challenge. For Aim 3, limited data were presented to support the PI's conclusion that, in mice, HSV-2 infection does not change the course of Chlamydia muridarum disease progression and so additional work on this aim was not pursued. Unfortunately, the data presented do not include specifics on the primary outcome measure (upper tract pathology), nor do they have necessary power (a key group was listed as n=4) to fully support the abandonment of this research avenue.

Overall, the funding of this project led to substantial advancement in the understanding of the mouse model of HSV-2 infection and provided intriguing avenues for additional research and future grant applications.

There were a number of weaknesses, including a lack of publications, a lack of perceived commitment to the development of the co-infection model, and finally the lack of evaluations of latency and reactivation potential in the long-term survivors created through Aims 1 and 2. The PI indicates in the Final Report that a publication was planned for submission in the summer of 2010, but no related manuscripts were found upon a PubMed search. The PI also indicated concurrent support by a K23 award and a submitted R01 that had a pending funding decision. The R01 and future research directions appear to focus on the role of Tregs based on the preliminary data generated during this funding.

Reviewer 3:

This project proposed to identify how adoptively transferred CD4⁺ T cells impact survival after intra-vaginal HSV-2 infection (Aim 1), the role of regulatory cells among these CD4⁺ T cells (Aim 2), and how intra-vaginal HSV-2 infection impacts secondary infection with Chlamydia (Aim 3). Overall, the project accomplished the stated proposed objectives. Aims 1 and 2 yielded positive results that showed improved survival and reduced disease after the adoptive transfer of CD4⁺ and specifically CD25⁺ CD4⁺ regulatory T cells. Aim 3 showed only negative results in that HSV-2 infection did not significantly impact secondary Chlamydia infection.

Additional studies examined the role of CD4⁺ T cell differentiation subsets in the protection conferred by adoptively transferred cells including determining the role of CD4⁺ T cell IFN- γ production and IL-17 production on protection.

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 finding that IL-17 manipulations can alter the outcome of primary HSV-2 infection in mice could potentially lead to interventions that could benefit human health, although the pathogenesis of HSV-2 is quite different in mice (death or life after acute infection; no recurrences reaching clinical threshold or recurrent shedding) than in humans (very rare fatal acute infection; inevitable recurrences - at least at the virologic level). There are no likely changes in risk factors, services, incidence, death, diagnosis, etc. at this time, but there is the potential that in the long term an increased understanding of the roles of Treg and Th17 cells in HSV-2 infection might improve these parameters. It was stated that there were plans to publish papers and seek outside funding. No papers directly related to the Treg or Th17 findings are listed on PubMed as of the date of this evaluation. The PI is actively seeking NIH funding to follow up these results.

Reviewer 2:

Based on the current data, that needs to be published, it appears that at the minimum, the research team has enhanced the value of the mouse model of vaginal HSV-2 infection to allow for long-term survivors that may provide a critical opportunity to study latency and reactivation. This was one of the strongest goals of the original research strategy. A second benefit from the work is the data supporting the role for the CD4CD25 double positive T cell population as well as IL-17-based responses in the protection of mice from lethal outcomes following vaginal challenge. These findings provide important insights to the proper/improper responses and could lay necessary groundwork to evaluate clinical connections in vaginal lavages, etc. Because HSV-2 infects a disproportionately larger number of black females than males, this benefits an underserved population as well. Importantly, HSV-2 and Chlamydia represent two of the most common sexually-transmitted infections worldwide.

Reviewer 3:

The major strengths of this project lay in the impact HSV-2 intravaginal infection plays in human health and disease. Therefore, developing a more representative animal infection model, especially with a mouse model with excellent immunological tools, is a major strength.

Major weaknesses in the proposed approach and interpretation of the results are the relevance of adoptively transferred CD4+ T cells in human therapies to this infection, the lack of study of the antigen-specificity for the CD4+ T cell or CD25+CD4+ T cell response, and the use of immunological tools that do not allow discrimination of regulatory T cells from activated T cells that each express CD25 in the experimental system. Given that these studies were conducted in mice, and that transgenic mice that allow for the manipulation and identification of regulatory T cells based on Foxp3 expression are available, it is disappointing that these more specific reagents were not used.

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 was expected in the proposal. The PI has applied for additional funding to follow up the interesting results presented in the project reports.

Reviewer 2:

As noted above, the work led to the submission of an R01 focused on the role of Tregs in HSV-2 control and was partially supported by a concurrent K23 award from the NIH. . RePORT indicates that the R01 was selected for bridging funding as an R56.

Reviewer 3:

Although an R01 was not awarded, this research did lead to an one year R56 bridge award to the PI. Given the current funding climate, this accomplishment is very impressive. It is unclear whether the PI plans to apply for more sustained funding for this work.

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:

Papers were anticipated in the proposal but have not been published.

Reviewer 2:

The PI indicated that a manuscript was to be submitted in the summer of 2010. No licenses or patents were indicated.

Reviewer 3:

Five publications from the PI are found on PubMed during the funding period and thereafter. The PI is the senior author on two of these, and a middle author suggesting important contributions in three others. These publications are in good, but not excellent journals (*Journal of Immunology* and *Journal of Virology*) that represent articles that would be read by researchers in this field, but not likely read by a broader scientific audience. This level of productivity is good for the model and award period. However, disappointingly, none of the publications are directly related to the specific aims of the proposed project, intravaginal HSV-2 infection and CD4+ T cells.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

There was no listed support for pre- or post-doctoral students, improvements to infrastructure, or new hires. Of note, it is unclear how much money was spent on this project. The actual expenditures on this particular project are much more likely to reflect the more modest amount listed in the Final Report. For this modest sum, the detailed animal experiments represent a good value for the money and would not expect infrastructure, new hire, etc. milestones to be achieved.

Reviewer 2:

In addition to the enhancements of the mouse model of vaginal infection with HSV-2, the funding supported the training of three undergraduates.

Reviewer 3:

Three undergraduate students were supported by this award. There were no other students or trainees (pre- or post-doctoral students) involved.

There were no funds used for infrastructure improvements or recruitment of new investigators.

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 collaborations were anticipated or initiated.

Reviewer 2:

No outside collaborations are indicated. There are a number of groups working on similar modeling efforts and it would have been ideal to see some level of collaboration documented through this funding. Based on other publications, the group seems to have active collaborations with a number of other groups both in and out of the state.

Reviewer 3:

The project reports do not describe any collaboration with research partners outside of the institution or new involvement with the community.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

1. Clarify if CD25(+) cells that were transferred (or eGFP(+) cells from the special Foxp3-eGFP mice) were actually true Treg or just activated CD4 T cells. There is some evidence that CD4 T cells can non-specifically turn on FoxP3 after activation. The PI needs to work out whether the beneficial adoptively transferred CD4 T cells are really Treg or just activated day seven splenocytes from infected mice that could represent antigen-specific regular CD4s or bystander activated regular CD4s.
2. Clarify if CD4 T cells and/or Treg-like cells from vaginally infected donor mice behaved differently from similar adoptively transferred cells from ocularly infected mice.
3. Do IL-17R KO mice have a difference in HSV pathogenesis ?

Reviewer 2:

1. The main weaknesses surround the lack of data presented to indicate the PI and team evaluated the latency/reactivation potential in the long-term survivors created through the adoptive transfer studies. There are indications that the latent viral DNA burden was evaluated, but too little information is provided to indicate this was pursued effectively. The focus was clearly on the immunological outcomes, but the virology is crucial to support the utility of the enhancements in the model.
2. Another weakness is the lack of publications reporting the intriguing findings. The PI is strongly encouraged to publish the data because it has value for the field and could lead to opportunities for additional collaboration.

Reviewer 3:

1. The use of adoptively transferred CD4+ T cells is not practical as a treatment of HSV-2 infection. How can these results be used for more translational applications and the design of therapies for HSV-2 infection?
2. The antigen-specificity of the protective CD4+ and CD4+CD25+ regulatory T cells should be interrogated. This seems important for designing preventative and curative therapies for HSV-2 infection.
3. The use of CD25 to identify regulatory T cells is problematic because the most activated T cells will also express high levels of this marker. Instead, additional markers Foxp3+, CTLA-4, etc. that more specifically identify true regulatory T cells among CD25+CD4+ T cells would strengthen the interpretation of the results presented for Aim 2.

Project Number: 0863904
Project Title: Integration Study of the Target Genes of PPAR gamma in Human and Mouse Placenta
Investigator: Chu, Tianjiao

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 investigators proposed clearly designed experiments to differential display of the PPAR gamma-regulated placental genes with microarray technology and using PPAR-gamma-null mouse models as well as human placental cells treated with or without PPAR gamma agonist.

The project met, in essence, the stated objectives. Data obtained also answered the questions asked.

Weaknesses: It is not clear why the proposed ChiP on chip studies were not performed. Even though the microarray data as accomplished can differentially display the PPAR-gamma regulated genes, however, the ChiP on chip experiments will also provide data to further validate the targets identified. The research plan does not contain any functional analysis of the target genes identified.

Reviewer 2:

This project examined the gene expression profiles in the placentas of wild-type and PPAR gamma-null mice (and corresponding, and in human placental tissue and cultured trophoblasts). The mRNA expression data were collected by the PI's collaborators, and the PI conducted the bioinformatics mining studies in the current project.

1. For the most part, the project addressed the stated objectives outlined in the original proposal.
2. On the surface, the design and methods were adequate, although the project was quite limited in scope, significance, and impact of the work.
3. The analysis of the gene expression data was quite superficial, and while it addressed the original questions posed, the overall impact is limited.

Reviewer 3:

The PI made important progress in meeting the proposed aims of this grant. The strength of the grant was the preexisting array data sets from collaborator labs that were readily available for computational analyses by the PI. The PI was highly creative and innovative in the analyses,

which allowed for important and, for the most part, well-defined overlapping analyses of array data sets. It appears the PI largely met all of the proposed aims of the original grant.

In Aim 1, the PI analyzed several key mouse models of PPAR gamma null placentas, RXR null placentas, and mouse TSC treated with vehicle or PPAR gamma activators during two or four days of in vitro differentiation to examine overlapping target gene regulation. The results of these comparisons gave rise of a series of predictions based on array analyses that will help to predict important expression attributes for how PPAR gamma and potentially RXR contribute to placental gene expression.

The description of these studies would have been improved modestly by a more extensive explanation of how the placental tissues were dissected to avoid potential contamination by maternal decidua. In addition, enthusiasm was dampened modestly by a lack of validation of these gene targets to test the series of predictions using independent qPCR or western blotting approaches. The PI reports these types of studies as components of the future aims; however, these computational studies are largely best appreciated with elements of important validation beyond the direct computational analyses of array data.

In Aim 2, the PI assesses human trophoblast stem cells treated with vehicle or PPAR gamma agonists. It would have been prudent to describe the isolation and enrichment of these TSC. The array analyses also included TSCs treated with a PPAR gamma antagonist, p38. In general, these studies are appreciated for their importance; however, they largely reflect a single experimental replicate, and as such, it is difficult at present to determine their critical value. Further, the PI offers no description of potential off-target effects of the drugs used leaving open to question to specificity of these compounds. I am certain some have reliable specificity, but assurances for the selectivity of (in particular) the p38 inhibitor would have been useful and instructive. Again, consistent with Aim 1, initial validation of a limited number of genes (two to three) would have provided more confidence in the data analyses. That said, the PI did appear to accomplish the original goals set.

In Aim 3, the human and mouse array data sets were examined for overlapping gene expression patterns. Unfortunately, only a single gene was identified between analysis of the placentas of these two species. Clearly, additional work in this area is necessary and should be encouraged.

Overall, the PI has carried out an important and useful analysis of several array data sets examining the potential role of PPAR gamma on placental gene expression that will no doubt be leveraged toward important future studies translating these gene lists into important mechanistic elements for the control of placental function in mouse models of placental insufficiency and in human disease.

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 basic science project that addresses a significant perinatal biology question. It does not have immediate benefits to health outcomes; however, data obtained are important to understand the mechanisms of placental development.

The PI planned to further test the functions of the target genes identified in the mouse and human placenta.

Reviewer 2:

The project does not directly or immediately effect human health. However, PPAR gamma has been shown to be a transcription factor that is crucial for successful placental development, a thorough understanding of the likely targets of PPARgamma activity is germane to placental developmental biology. In theory, dysfunctional placental development and/or function is a hallmark of many human diseases in pregnancy such as preeclampsia and intrauterine growth restriction, among others. Thus, the study has, at least, modest scientific merit, although it falls short in mechanistic detail and in verification of the microarray data.

There is insufficient detail of future plans for this research project.

Reviewer 3:

As articulated by the PI, the computational studies carried out in the proposal were of a basic nature and were not anticipated to provide direct consequences toward human health. These studies do contribute greatly to potential identification of novel target genes involved in the translation of how PPAR gamma functions as a transcriptional regulator during placental morphogenesis. The other strength here is the overlapping data set analyses, effectively creating the opportunity to assess this key issue from multiple directions. Thus, the benefit of these studies was entirely consistent with the budget.

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 leveraged. They planned to further this research; however, it is not clear whether they applied for additional funding.

Reviewer 2:

The project did not leverage additional funds from other sources. There is no plan described for future submissions to continue this work.

Reviewer 3:

Unfortunately, no additional funds were leveraged thus far; however, I anticipate these studies will contribute to future grant applications from the investigators at the Magee Institute.

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:

Investigators plan to submit at least one paper based on the data generated.

Reviewer 2:

No peer-reviewed publications have resulted from this work. The PI stated that a manuscript will be submitted in 2010, but no details are given. This work does not appear to have been presented nationally or locally in abstract form.

Reviewer 3:

Unfortunately, the project has not resulted in any peer-reviewed publications at present.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

This project has made improvements to infrastructure, especially on developing analysis of microarray studies to the institute.

No new investigators or pre- or post-doctoral students were hired.

Reviewer 2:

There were no infrastructure improvements/enhancements, no new investigators added as a result of the project, and no new trainees.

Reviewer 3:

The studies do enhance the institutional capacity with the in depth analyses of these data sets using these computational approaches. There is no report of new investigators added or any trainee support.

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 external collaboration or new involvement with the community.

Reviewer 2:

No new collaborators have been brought into the project.

Reviewer 3:

No new collaborations are reported.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

1. The proposed ChiP on chip studies were not performed. These studies will provide data to further validate the targets identified.
2. There was no functional analysis of the target genes identified.

Reviewer 2:

1. The study was superficial and the gene expression analysis used only gene ontology descriptions, which are a reasonable starting place, but current bioinformatics capabilities allow for far more comprehensive analyses of these datasets.
2. There was no attempt to corroborate the microarray data with follow-up quantitative RT-PCR analysis of selected genes. This is a minimum requirement for subsequent publication.
3. While the aims outlined in the proposal were addressed by the analysis in the project, there was no clearly defined hypothesis tested.

Reviewer 3:

1. One important weakness was the lack of some preliminary validation of the array analyses using qPCR and or western blot analysis. In my view, this was a missed opportunity, particularly, for example, the single gene that demonstrated the overlapping expression pattern between mouse and human placental material.
2. Caution is suggested when assuming the pharmacological drugs used have high selectivity in their actions. Off-target effects are always an important caveat to these types of studies and how alternative actions could alter interpretation of the results.
3. This reviewer fully appreciates that populations of human TSC are highly precious; however, studies examining a single experimental replicate is very difficult to describe in terms other than highly preliminary. I am certain the PI and this research group seek to add more replicates to these analyses which will strengthen interpretation of the human TSC data markedly.

Project Number: 0863905
Project Title: Soluble KIT Receptor in the Pathogenesis of Preeclampsia
Investigator: Hubel, Carl A.

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 has completed the experiments as designed in Aims 1 and 2 to investigate the changes of soluble Kit (s-kit) concentrations in circulating blood samples in women who later developed preeclampsia, and those who had a normal pregnancy. However, Aim 3 of studying placental s-kit expression and trophoblast s-kit expression under normoxia and hypoxia was not complete.

One major accomplishment is the analysis of blood samples, which suggested that the PI's hypothesis is right on track: maternal plasma s-kit concentrations are reduced early in pregnancy in women who later develop preeclampsia as compared to women who had normal pregnancy.

That being said, the data obtained sufficiently answered the research questions posed in Aim 1 and Aim 2.

No changes were made to the original research plan.

Data presented indicate that the team has made acceptable progress.

The data are well in line with the research strategy.

One major weakness is that no data was presented for Aim 3.

Reviewer 2:

The project met its objectives. The investigators were able to show that soluble c-kit is lower in preeclamptic subjects prior to delivery in a cross-sectional study, as well lower in subjects destined to develop preeclampsia, even as early as 18 weeks of pregnancy. Finally, the investigators were able to show that the c-kit alterations were specific to pregnancy. A small weakness in the study is the lack of power for any subgroup analyses - for example, are these alterations more dramatic in early onset disease?

Reviewer 3:

The overall goal of this application is to investigate the use of a serum biomarker (sc-kit) as a predictor of impending preeclampsia. Three specific aims were proposed. The first directly addressed the overall goal in that it was to measure sc-kit (and its ligand SCF) longitudinally during pregnancy. This used archived serum samples from a previous program study, which is a significant strength. The second aim was geared to determine if the low sc-kit levels during preeclampsia would remain low 6 to 24 months postpartum. Correlations of the analytes with insulin resistance, BMI and CV risk factors in the ladies was assessed. Aim 3 focused on the potential origin of aberrant sc-kit levels by determining protein expression levels in normal versus preeclamptic placentae.

For Aim 1, it was found that sc-kit levels were statistically lower in the preeclampsia group than normotensive group at 34 weeks gestation. The relative fold difference was comparatively small (~1.3x); however, differences in other, more established biomarkers was ~5-6x. In women with just gestational hypertension (no proteinuria), there was no difference in sc-kit levels with normotensive control women at 39 weeks gestation. Collectively, this would indicate that lower levels of sc-kit could be used to differentiate clinical preeclampsia from gestational hypertension.

For Aim 2, it was found that there was no significant differences in serum sc-kit or SCF levels between non-pregnant women, irrespective if they had never been pregnant or were approximately one Th year post partum after a normal or preeclamptic pregnancy.

The goal of Aim 3 was to investigate sc-kit levels in placentae from normal vs. preeclamptic pregnancies and the effect of hypoxia on expression. It seems that these experiments were not attempted. The reason for this is not clear. Instead, the investigators attempted to characterize sc-kit production in HUVEC in culture, with and without PMA treatment. However, levels of sc-kit in the conditional media, even after 16x concentration, did not reach threshold levels of detection in the ELISA. It seems worthwhile to attempt this using placental samples by western blot or, if needed, IP plus WB as shown in Figure 3 for serum samples. To their credit, the investigators have recently received mutant mast cell lines that reportedly produce high levels of sc-kit. Although not an initial aspect of the current grant, these cells could be used as indicator cells to determine factors that may induce production (cleavage) of sc-kit.

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 investigated the potential importance of s-kit in preeclampsia. Preeclampsia complicates 5-7% of all pregnancies worldwide and currently remains a major cause of maternal, fetal and neonatal morbidity and mortality. Despite intensive investigations during the last 20 years, the understanding of this pregnancy-specific disorder remains limited. Thus, this project, although not to provide "bench to bedside" translation of research findings for health care, provided knowledge for the understanding of the pathogenesis of preeclampsia, which is an important contribution to this pregnancy disorder.

The PI applied for a U.S. patent and had initiated industrial collaborations on developing potential clinical tests using s-kit as a biomarker for preeclampsia.

Reviewer 2:

We don't have a predictive test for preeclampsia. The authors suggest that soluble c-kit may be included as one of the factors that may be used in an algorithm to predict preeclampsia. I believe the findings presented are extremely valuable to the preeclampsia research community in their quest to develop multiple markers to predict preeclampsia. The alterations in the c-kit pathway during pregnancy may also be responsible for defective endothelial repair noted in these subjects. This work should set the stage to evaluate the biological consequence of the low c-kit amongst subjects with preeclampsia.

Reviewer 3:

The most important impact of this study may be the eventual clinical implementation of the use of serum sc-kit levels to help predict impending preeclampsia. Conceptually, use of sc-kit may not be so powerful alone but, as with all predictive tests, the predictive power increases as more variables are added to the screen. Thus, it could be easy to see sc-kit being added to PGF, sVEGFR1, and s Eng screens to improve preclinical diagnosis of preeclampsia.

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 leveraged. The PI planned to apply for an NIH R21 application on this research.

Reviewer 2:

Researchers are planning to submit grants to NIH to study the biological consequences of the altered c-kit pathway.

Reviewer 3:

As of yet, no additional sources of funding have been applied for to continue or expand this project. Plans are to submit an NIH R21 Exploratory/Developmental Research Grant application in October 2010. It is not clear what the subject of the R21 will be.

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 are no publications yet; however, the PI planned to submit at least one major paper based on the data presented in the Final Report. The PI also filed for a patent on s-kit and preeclampsia diagnosis. The quality of the research is highly rated.

Reviewer 2:

A patent has been filed by Dr. Hubel and his team. The institute can potentially license this discovery to commercial partners.

No manuscript has been published by Dr. Hubel in this subject matter; however, there are plans to submit a paper in the *Journal of Clinical Endocrinology & Metabolism*, which should be well received.

Reviewer 3:

A manuscript entitled, "Reduced concentration of soluble c-kit receptor in the material circulation: an early marker for pre-eclampsia," was planned for submission to the *Journal of Clinical Endocrinology & Metabolism* in May 2010.

An invention patent was filed (November 30, 2009) pertaining to the use of soluble c-kit levels in pregnancy to diagnosis pre-eclampsia and eclampsia. Plans are underway to license the application and at least one unilateral confidentiality agreement with a pharma business has been executed.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

The project does not appear to have enhanced the infrastructure at the institution. A new technician has been trained to perform placental culture for Aim 3. No pre- or post-doctoral students were trained.

Reviewer 2:

Dr. Janet Catov, a trained epidemiologist and Dr. Augustine Rajakumar, a molecular biologist were added to the team to carry out the research.

Reviewer 3:

No funds were reported to be used to support new investigators or trainee-status researchers.

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:

Industrial collaboration was initiated. No other plans on collaboration is identified.

Reviewer 2:

The investigators should establish collaborations with investigators in the stem cell field who have expertise in c-kit signaling pathway and therefore may be able to provide valuable advice to test the biological consequence of this pathway in human preeclampsia.

Reviewer 3:

Collaboration with Dr. J.H. Butterfield at Mayo Clinic has been established. Dr. Butterfield provided a mast cell line that secretes sc kit. This collaboration could prove to become more fruitful in the future.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

1. Aim 3 is interesting and should have been tested.

Reviewer 2:

1. I would encourage the authors to evaluate the biological consequence of the altered c-kit in future studies. For example, is the low c-kit responsible for the insulin resistance of preeclampsia?
2. The authors should develop an ELISA kit that measures free SCF levels. It appears that the current kit only measures total SCF levels and therefore does not correlate with clinical disease.
3. The source of c-kit alterations and nature of regulation of c-kit should be explored in future studies.

Reviewer 3:

1. There is a central question that needs to be answered (placenta source of sc-kit?) and one that was proposed as a specific aim (Aim 2) was not attempted. The techniques and samples are in hand, so it was disappointing not to see at least some initial attempts. This is a relatively minor weakness in an otherwise well done project.

Generic Recommendations for Magee-Womens Research Institute and Foundation

Reviewer 2:

In summary, Dr. Hubel has performed quite admirably, and has demonstrated a potential biological role for dysregulated c-kit pathway in preeclampsia. These findings have important implications for the prediction and the pathogenesis of the disease. This work should lead to additional NIH funding to evaluate the biological consequences of the c-kit pathway in human preeclampsia.

Project Number: 0863906
Project Title: Post-Transcriptional Regulation of Fst1 mRNA in Human Trophoblasts
Investigator: Mouillet, Jean-Francois

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 objective of the proposed research was to examine the post-transcriptional regulation of Fst1 mRNA and its cognate (potentially) microRNA (miR-198) in human placental trophoblast. The study outlined two specific aims: 1) to correlate the expression of Fst1 and miR-198 in primary trophoblasts and HTR-8/SVneo extravillous trophoblast-like cells; and 2) to determine the half-life and mechanism of the proposed post-transcriptional regulation of Fst1 and miR-198.

1. Although a considerable amount of work was done during the project period, there were several experiments that did not yield the predicted results, and, in some cases, data were somewhat contradictory.
2. In general, the research design and methods were appropriate.
3. The contradictory data with regard to the expression of Fst1 mRNA in cultured trophoblast, yet absence of the Fst1 mRNA (by in situ hybridization) or protein (by immunohistochemistry) is problematic and perplexing. A more in-depth analysis of these observations would have strengthened the PIs conjecture that this was due to the inherent instability of the miR-198 and Fst1 transcripts. At this point, there is circumstantial but not direct evidence for this notion.
4. The data, while perplexing, did address (albeit indirectly in some cases) the objectives of the proposed research.

Reviewer 2:

There are many strengths to the proposal as described, with appropriate outcomes based upon the research plan, budget, publications, interactions with new collaborators, and increased ability to leverage this support into new NIH funding. The PI appears to have largely met the original objectives. These strengths include:

1. The research plan was nicely articulated and tractable with adequate preliminary studies in support of the proposed hypotheses. The study of microRNA modulation of placental function is a very timely and a highly important area of research.
2. The PI and this research group has a long history of using human primary trophoblasts as a model system providing the opportunity to investigate these issues in a physiologically relevant system.
3. The PI clearly met the goal of obtaining new federal grant support to the Magee-Womens

Institute. A new grant was awarded to this group in 2010 from NICHD under the grant title listed by the PI.

4. The PI states clear objectives for the future with incorporation of extravillous trophoblasts (EVT) into studies of the role of *Fstl1* on placental function and a more sophisticated analysis of mechanisms related to processing mechanisms for miRNAs in trophoblasts. These are likely to be very exciting and relevant studies.

5. In Aim 1, the PI provides compelling data for unique miRNA or usRNA processing of miRNA 198 within placental trophoblasts treated with hypoxic conditions. While some of the data presented regarding spatial expression of *Fstl1* and miRNA198 are difficult to reconcile at present, the PI provides a reasonable plan to move forward with these experiments.

6. In Aim 2, the PI sought to examine the 3'UTR of the *Fstl1* mRNA to characterize potential mechanisms related to the control of *Fstl1* mRNA stability. The approach was fairly standard and revealed that miRNA 198 is likely not involved in the post-transcriptional regulation of *Fstl1* mRNA stability. Despite the negative outcome, the PI successfully accomplished the analysis originally proposed.

While the strengths outweigh the weaknesses in this outcomes assessment, there are several weaknesses that are noteworthy (although considered minor):

1. Perhaps most importantly, the argument for the importance of the *Fstl1* target gene in the context of placental function was not particularly convincing since it is currently unclear exactly what *Fstl1* contributes to or regulates in the context of the human placenta. Reference to mouse models (if these have been reported) would have potentially strengthened the argument. That said, the outcome of these studies are listed as supportive to a new R01 grant to the collaborator Dr. Sadovsky where Dr. Mouillet is listed in the proposal as a co-investigator.
2. Unfortunately, no training at the graduate or post-doctoral level took place via this application.
3. It was unclear why Northern analyses is used here when there is the strong possibility that a qPCR approach for miRNAs may be considerably more sensitive. Future studies might consider approaches and techniques beyond the Northern Blot.
4. The PI has published one manuscript linked to the funded research in *Placenta*; however, in reviewing this manuscript, there is no evidence presented that related to the core elements of this grant on miRNA 198 or *Fstl1*. This manuscript does characterize hypoxia-regulated miRNAs linked to fetal growth restriction, so the link may be more indirect.

Reviewer 3:

The overall goal of this application is to determine the inter-correlation of microRNA198 (miR198) and expression of follistatin-like 1 (*Fstl1*) in trophoblast. miR198 was found to be differentially regulated by hypoxia in trophoblast. Since the genomic location of miR198 lies within the transcription unit of *Fstl1* and that there is a potential miR198 target site in the 3'UTR of *Fstl1*, the PI surmised that miR198 expression may be linked to expression of *Fstl1*.

Two specific aims were proposed. The first was to better characterize and correlate expression of miR198 with *Fstl1*. The second was to determine the potential role of the long 3'UTR of *Fstl1*, with its potential miR198 target site and several ARE sites, in regulating expression of *Fstl1* protein. For Aim 1 it was found that *Fstl1* mRNA is relatively well expressed in isolated primary villous trophoblast and some, but not all, trophoblast cell lines. However, in situ hybridization and immunoblotting only detected *Fstl1* expression in EVT; none was detected in

villous trophoblast. Isolated trophoblast miR198 was seemingly expressed, and expression increased when the cells were challenged with 1% O₂. Conversely, Fstl1 mRNA expression decreased under hypoxia.

In keeping with the hypothesis that miR198 may regulate Fstl1 mRNA, the PI determined that miR198 in a trophoblast cell line, but not in primary trophoblast, could decrease receptor activity harboring the Fstl1 3'UTR (Aim 2). Controls for this approach are a significant strength and provide strong support for the data in general. In that the HTR8 cells express high levels of endogenous Fstl1, it would have been reassuring to see if similar results were obtained in a trophoblast line with little/no competing endogenous target.

A series of Fstl1 3'UTR deletion mutants were constructed to begin to determine if regions are important for regulating stability of the mRNA. The data was inconsistent between different trophoblast cells used. This may be a reflection of several things, not the least of which is physical length of the 3'UTR. It may prove more beneficial to compare luciferase activity between full length 3'UTR vs. full length 3'UTR with site directed mutations in the putative AREs. Similarly, comparing luciferase activity of clone 6 (for example) vs. clone 6 with the three putative AREs mutated effectively controls for length of 3'UTR. As presented, the PI is comparing luciferase activity in a control clone with essentially no 3'UTR vs. clones with variable length 3'UTRs. This may yield heterogeneous results simply due to nonspecific instability of the 3'UTRs in the different cell types. How far to pursue this is uncertain. It appears that stability of endogenous Fstl1 mRNA did not seem to be significantly altered in primary trophoblast under hypoxic culture conditions, although the toxicity of actinomycin D is a concern.

Thus, this grant met its objectives to a large extent, but generated more questions than it answered. This is not a weakness as much as it is a testament to exploratory science.

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 proposal outlined basic science studies of the expression of Fstl1 and its miRNA (miR-198) in trophoblast under normoxic and hypoxic culture conditions. The work does not directly have immediate clinical benefit for patients, but it allows for a thorough understanding of the mechanisms by which hypoxia within the placenta can have negative consequences for fetal growth and well-being. Thus, the work is of fundamental importance in human development. Presumably, the PI will continue to work toward understanding the mechanisms of Fstl1 gene regulation and hopefully he will sort through the discrepancies in the data that were reported in the Final Report.

Reviewer 2:

The understanding of the role of miRNAs in controlling pathologies at many levels is of growing importance, particularly to our understanding of biomedical issues linked to placental function and the intrauterine environment. This reflects an important strength of this grant. As

acknowledged by the PI, the direct contribution of these types of basic studies are two or three steps removed from a direct contribution to improving health.

Reviewer 3:

The clinical significance of this project awaits further study. The interplay between the two genes in question, miR198 and Fstl1, is not so promising. However, defining the potential biological significance/function of Fstl1 in trophoblast and the potential roles of usRNAs expression in trophoblast are promising offshoots of this application. Of these, the unanticipated findings of usRNAs may prove to be the most significant strength of the grant.

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:

1. There was no expectation of a cost-match for this particular project.
2. The PI has indicated in the Final Report that a section of an R01 grant that has recently been reviewed (with a high priority score), but no details are provided. Thus, it is difficult to know for certain whether a full proposal has been prepared specifically related to this Pennsylvania funded project at the time of this review.
3. The PI has indicated that a manuscript will be prepared, but there is no evidence that a paper has been submitted. It is also not clear if any of the work has been presented in abstract form.

Reviewer 2:

There is clear evidence that the studies and outcomes described in this proposal are linked to the success of a new R01 application by this research group as determined and confirmed through the NIH RePorter database.

Reviewer 3:

The PI and associates submitted an NIH application in October 2009 that incorporated at least some of the findings from this CURE project. The PI of the CURE award is listed as co-PI for the NIH submission.

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 indicated that a manuscript will be prepared, but there is no evidence a paper has been submitted, nor have any abstracts been listed by the PI.

Reviewer 2:

The PI reports one manuscript recently published in *Placenta*; however, it is not clear what the connection is to the funded studies beyond the analysis of miRNAs on a more general level since review of this manuscript indicates no data associated with miRNA 198 or the control of Fstl1

mRNA stability.

Reviewer 3:

A manuscript entitled, “The expression of hypoxia-regulated micro RNAs in plasma of pregnant women with fetal growth restriction” with Dr. Mouillet as first author was submitted to the journal *Placenta* in February 2010. An additional manuscript describing the expression patterns of Fst 11 in the human placenta is planned. Based on data presented in the Final Progress Report, this seems to have a high likelihood of success. Collectively, these two submissions represent solid productivity for a one year project.

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 changes to infrastructure, no new investigators recruited to institute, and it does not appear that the project supported any students.

Reviewer 2:

There is no indication that institutional infrastructure was improved by this award.

Reviewer 3:

Funds were not used to support new investigators or trainee-status researchers on 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:

The PI has initiated an important collaboration with a key investigator who will add significant value to the project.

Reviewer 2:

The PI reports a new collaboration with Dr. Bino John, an assistant professor of computational biology at the University of Pittsburgh. This appears to be an important collaboration that will strengthen the computational component of these studies and future studies of miRNA modulation of key genes that affect placental function.

Reviewer 3:

The project has resulted in collaboration with Dr. Bino John in the department of computational biology at University of Pittsburgh. Given his expertise in unusually small RNAs, deep sequencing and bioinformatics, this collaboration will likely prove to be very fruitful.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

1. The seemingly contradictory data shown in the Final Report must be clarified. This will be critical for the project's funding success at the national level.
2. The mRNA turnover studies were inadequately performed. The PI saw no substantive decay of the Fst1 mRNA during the chase period. This could have been due to the lack of sufficient transcriptional suppression. A pilot experiment to assess the percent of transcription inhibition must be performed to clarify the half-life calculations.
3. The primary trophoblast cultures represent principally the villous component of the trophoblast of the placenta. Yet, HTR-8/SVneo cells represent the extravillous component of the placenta. The work alludes to the notion that perhaps it is EVT and not villous trophoblast that expresses Fst1. Thus, the two models are inconsistent. This should be addressed by the PI.

Reviewer 2:

None.

Reviewer 3:

1. Analyses of the Fst1 3'UTR reporter constructs should be revisited with comparisons made between similar length regions (native vs. mutant).
2. Whether stability of Fst1 is affected by its 3'UTR at all, needs to be determined before launching into more detailed and specific mapping experiments.

Project Number: 0863907
Project Title: Analysis of Functional Domains within NDRG1
Investigator: Sadovsky, Yoel

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 partially met the stated goals. The first aim was to make deletions in the NDRG1 protein, which was almost complete. However, the 2nd Aim was not completed mainly due to unexpected results on altered NDRG1 expression that was unable to regulate trophoblast cell proliferation. Despite this limitation, the project made reasonable progress towards the goals.

Overall, the research plan and design are clear and logical. Data obtained have suggested a role of NDRG1 in protecting trophoblast cells from hypoxia insult, although Aim 2 cannot be complete as originally planned due to cell proliferation data. However, they have developed alternative ways to test the function of this protein in trophoblast, which is apoptosis. This is very reasonable considering the proposed studies.

Reviewer 2:

This project made reasonable progress toward meeting the stated objectives of the research plan; however, not all of the objectives were met as articulated by the PI. The original research plan was ambitious and the PI had to overcome several technical challenges during the funded year. The initial hypothesis was interesting and reasonably well established by preliminary studies. Several clear strengths emerged from these studies:

1. The PI and colleagues established a series of structure-function mutants from NDRG1 using thoughtful analyses of potential domain structure and homology to other members of this class of protein.
2. Overexpression and siRNA-mediated knockdown techniques were established, which for the most part were reasonably well controlled.
3. The studies revealed that NDRG1 appeared to play a critical role in protection against hypoxia-induced cell death and promoted survival under stress conditions, mediated by CoCl and UV light.
4. Knockdown of NDRG1 appears to blunt cAMP-induced differentiation of BeWo cells in vitro.
5. Finally, stress appears to induce a nuclear translocation of NDGR1 using CoCl and hypoxic conditions suggesting that re-compartmentalization of NDRG1 to the nucleus may play a critical role in protection against apoptosis.

These strengths were offset somewhat by several important weaknesses that may simply reflect the possibility that the studies will require more time to fully complete and appreciate.

Nonetheless, these are important weaknesses to consider:

1. In Aim 1, several structural mutants were created; however, only a single mutant (deletion within the amino termini) was discussed in any detail. It may be more useful to examine the impact of the effects of the ΔN mutant in context with other mutants that did not appear to have an effect if tested in a similar manner as the ΔN mutant. Of concern was that the ΔHTH and the ΔC mutants maintained expression levels that were not consistent with other mutants (markedly lower). Importantly, variation in expression levels and potential activity of these mutants may reflect the effects of the deletions on folding or misfolding of the mutant protein(s). This is actually an important point since without evidence of appropriate folding of these modified proteins, interpretation of these studies, particularly in terms of loss of function, is difficult. It would have been appropriate for the PI to acknowledge this and provide at least commentary for how this issue could be overcome in the longer term.
2. In Aim 2, the nuclear localization studies provide important preliminary evidence of an interesting mechanism controlling intracellular compartmentalization of NDRG1 under stress conditions in trophoblasts. It was disappointing that additional progress was not made regarding some of the structure-function mutants. These studies have remarkable potential and simply adding two to three additional wells to test other mutants in these preliminary studies would seem relatively simple.
3. Given that this grant funded three full time individuals in the context of a very well-established laboratory, it is modestly disappointing that this group is not nearer to peer-review of at least some aspect of this important work.

Reviewer 3:

The primary goal of this project was to characterize unique structural and functional aspects of the NDRG1 protein. Previous data show that NDRG1 functions in human trophoblast to protect the cells from oxygen deprivation (hypoxia). Mechanisms of how this protein accomplishes this are not known. Hypoxic insult to trophoblast during pregnancy is thought to occur in a number of prevalent human obstetrical complications. Understanding intrinsic trophoblast mechanisms to minimize the effects of hypoxia could provide new avenues to improve placental function and fetal outcomes in these obstetrical complications. To accomplish this goal, two specific aims were proposed: Aim 1 was to generate defined mutant (deletion) NDRG1 proteins to assess effects on proliferation in an indicator cell line; Aim 2 was to determine specific functions of different NDRG1 domains in trophoblast.

For Aim 1, thoughtful *in silico* analyses of the NDRG1 sequence led to five specific deletion mutants being successfully produced and characterized. Since presence or absence of wild type NDRG1 did not alter proliferation in trophoblast cell lines, which was to be the primary indicator function, alternative functions for NDRG1 in the cells were sought. These included determining the ability of wild type NDRG1 to protect trophoblast from hypoxia-induced apoptosis and to enhance syncytium formation (differentiation) in trophoblast. Of the mutant NDRG1 clones generated, one seemed unable to protect trophoblast from hypoxia- and UV-induced apoptosis. It is not clear if this mutant, or any mutants, were tested in the differentiation assay. Collectively, these data support that the N-terminal domain of NDRG1, and may be responsible for directing the anti-apoptotic function of this protein in trophoblast.

Aim 2 progressed to show that NDRG1 cellular distribution was altered in hypoxic trophoblast. Several appropriate methods showed that NDRG1 preferentially localized to the nucleus when trophoblast were placed in hypoxic environments. Ultimately, the proposed goals of Aim 2, employing lentivirus constructs were not fully met. This is understandable given the unexpected results concerning the inability of NDRG1 to regulate trophoblast proliferation, as it seems to do in other cell types. Thus, a simple and convenient functional assay could not be used as planned. Furthermore, significant effort was needed to optimize methods for NDRG1 knock down and simultaneous over-expressions of mutant NDRG1 in primary trophoblast. These deviations in methods are appropriate given the data amassed and required significant time and effort to optimize.

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 basic science project was intended to generate information on trophoblast cell biology. Immediate benefits are not evident; however, knowledge obtained will explain how trophoblast cells escape from insult. Such information is important because placental insufficiency is a major manifestation of many critical obstetric problems.

The investigators have submitted an NIH R01 grant application to continue this research plan.

Reviewer 2:

The PI presents important and compelling evidence for the role of NDRG1 in trophoblast cell function, particularly regarding the role of this novel protein in protection from hypoxic injury in the placenta. Using genetic and molecular means to further our understanding of how cell stresses impact placental function provides an important basis for how we can and will deal with disease processes associated with placental insufficiency. These studies also provide the important basis for identification of potential targets for therapeutic intervention to enhance placental function in humans.

Reviewer 3:

Clinical benefits from this basic structure-function research project, like any basic research, await further development. Although not immediate, potential benefits would include assessments of how to maximize the ability of NDRG1 to protect trophoblast from insults promoting apoptosis. Given the prominent role of apoptosis in numerous obstetrical complications, further understanding of the mechanisms by which NDRG1 can inhibit apoptosis may provide potential benefits down the road.

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 leveraged. The investigators have submitted an NIH R01 grant application to continue this research plan.

Reviewer 2:

The PI states that the studies performed using these funds will contribute to a new R01 grant to the NIH; however, to date and based upon publically available databases, this application has not been approved for funding. The PI does report non-federal funding linked to a training grant for Dr. Jacob Larkin; however, in the context of this grant, Dr. Larkin does not appear to be involved with these studies.

Reviewer 3:

The PI and associates applied for an NIH R01 grant in July 2009 that included some aspects of the role of NDRG1 in trophoblast. This application is reported to have received a favorable score, but was not funded by NIH. A resubmission was planned for March 2010. In addition, the PI was able to secure a training grant from the American Association of Obstetricians and Gynecologists Foundation for Dr. Larkin to continue the studies.

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 publications on this project at the end of the funding period. There were plans to submit one paper on the domain analysis of NDRG1 protein.

Reviewer 2:

At present, no peer-reviewed publications have resulted from this project; however, as presented, there is a high likelihood that these data will contribute to a manuscript(s) from the Sadovsky laboratory.

Reviewer 3:

Submission of at least one peer-reviewed manuscript is planned. Progress data submitted suggests this has a high likelihood of being successful. This is good productivity for a one-year project.

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 improvement to infrastructure, no new people were added, and no pre- or post-doctoral students were trained.

Reviewer 2:

The PI provides no evidence that the infrastructure of the institution was improved. Funds did support training of one undergraduate and one post-doctoral fellow in the context of these studies, which is a strength.

Reviewer 3:

No out-of-state researchers were recruited, nor were major impacts on research capacity/quality reported. The project did involve one undergraduate and one post-doctoral trainee, although it is not clear the capacity or the extent of support that was made available for each trainee from this grant.

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:

It is not clear that there were any internal or external collaborations as a result of the research.

Reviewer 2:

The PI reports improved collaborations with the Center for Biological Imaging at the University of Pittsburgh.

Reviewer 3:

Collaborative relationships with the University of Pittsburgh Biological Imaging Center was enhanced, which should be quite fruitful as the project continues.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

1. Freshly isolated primary term trophoblast cells do not proliferate. How about using trophoblast cells from the first trimester.

2. Although domain mapping of the NDRG1 protein is necessary, the studies may be better designed to focus on first trimester trophoblast cell endovascular differentiation.

Reviewer 2:

1. The primary weakness that reduced enthusiasm for this grant was a lack of progress on structure function mutants developed in Aim 1, and how quickly the studies progressed within Aim 2. This is also reflected in the lack of publishable material from this application to date given such a well-established laboratory and the relatively large group of individuals supported on these funds. This may clearly reflect technical challenges overcome during the course of these studies.
2. Careful consideration of technical aspects of the structure function mutants is necessary since loss of function of these mutants may reflect an inability of a given mutant to fold properly within the cell. Analysis at this level may require the addition of biochemical expertise currently not present within this research group.

Reviewer 3:

None.

Project Number: 0863908
Project Title: Immunity to MUC1 Tumor Antigen in Conditional and Transplantable
in vivo Models for Ovarian Cancer
Investigator: Vlad, Anda M.

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:

Ovarian cancer is the most lethal of the gynecological cancers and is the fourth leading cause of death in women. Although significant progress has been made in treating the disease, the exact mechanisms of disease pathogenesis remain to be defined. Ovarian cancer lesions are thought to arise from normal ovarian surface epithelial (OSE) cells, which give rise to tumor cells that over-express a modified form of MUC1 that is recognized by the immune system. MUC1 has therefore become an attractive cancer vaccine candidate in ovarian cancer. However, the biology of MUC1 and its immunogenicity during the natural progression of OSE to ovarian cancer remains poorly understood. The goal of this project was to develop mouse tumor models of ovarian cancer to study MUC1 expression and immunogenicity during tumor progression. Three specific aims were proposed:

- 1) In Aim 1 the Principal Investigator (PI) set out to generate a conditional triple transgenic mouse expressing human MUC1 protein, a mutated KrasG12D protein, and a conditionally-deleted tumor suppressor (PTEN) The PI expected these triple transgenic (MUC1+/-LSL-KrasG12D/+PtenloxP/loxP) mice to develop MUC1 over-expressing endometrioid ovarian cancer.
- 2) In Aim 2, the PI proposed to develop a transplantable MUC1-expressing mouse tumor model by injecting an immortalized ovarian epithelial cell line (MKOSE) generated from MUC1 transgenic mice into the peritoneal cavity of syngeneic mice. As with Aim 1, the applicant expected these mice to develop MUC1-expressing tumors in the peritoneal cavity similar to human ovarian cancer.
- 3) In Aim 3 the applicant proposed to utilize both models to study MUC1 expression and immunogenicity during ovarian cancer progression. The applicant expected these mice to develop MUC1-expressing IgG antibodies and accumulate regulatory T cells (Treg) in regional lymph nodes and ascites.

Review of the Annual and Final Progress Reports show that excellent progress was made towards accomplishing the specific aims in the study. The PI completed Aims 1 and 2 to the extent that MUC1-expressing conditional transgenic mice were generated that developed ovarian tumors and that a transplanted MUC1-expressing ovarian epithelial cell line (IG10) gave rise to peritoneal tumors. In Aim 3 the PI demonstrated that despite the presence of high circulating

levels of MUC1 in the tumor-bearing mice, MUC1-specific antibodies could not be detected. The PI also assessed the frequency of Treg in the spleen and regional lymph nodes of these mice, and the presented data shows the presence of increased levels of Treg in tumor-bearing double transgenic mice. It is intriguing that the PI chose not to show the data from the triple transgenic mice, which best approximate human ovarian cancer. Notwithstanding, the PI's achievements during this one-year of funding is quite impressive.

Reviewer 2:

The research project focused on generating two in vivo mouse models that express the tumor antigen MUC1, a cancer vaccine candidate. Investigators planned to use these models to study how the immune system recognizes the antigen during ovarian cancer progression with consequences on future cancer vaccine design. The investigators have successfully completed the specific aims of the proposed study. Overall, the project has demonstrated reasonable progress to sufficiently meet the stated objectives.

Reviewer 3:

The PI outlined three specific aims. The first was to generate a murine model of ovarian cancer which expressed the human tumor associated antigen Muc-1. The second aim planned to develop a transplantable ovarian tumor following in vitro generation of a stable MUC-1 tumor cell line followed by injection into the peritoneal cavity of mice. The third aim was designed to initiate immunologic analysis to confirm relevancy of the system. The ultimate goal of the project, which will likely be pursued, was the establishment of a relevant murine model in which to develop a vaccine immunotherapy for ovarian cancer.

Overall, the project met all stated objectives. The design and methods were adequate and well described. Considering the timeframe of the described studies, the PI did a very conscientious job at achieving the goals. In this reviewer's opinion, all the stated goals were met and the basis has been laid to work on vaccine immunotherapy with future application to human translational studies. Interestingly, alternative strategy was initiated for Aim 2 since the desired in vitro tumor cell line was not initially obtained using the strategy described. However, transfection studies resulted in a murine tumor line expressing MUC-1 human antigen, which was successfully transplanted to murine peritoneal cavities. The data obtained was well described, inclusive of all findings, and supported the fact that the original goals set forth were achieved.

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:

These animal models of ovarian cancer will provide useful tools for studying the biology and immunogenicity of MUC1 and testing the efficacy of MUC1 as a vaccine target in ovarian cancer. Such studies could potentially lead to improved understanding of the role of MUC1 in ovarian cancer progression and result in the design of physiologically relevant therapy-based studies of ovarian cancer that can be translated to the clinic.

Reviewer 2:

The successful development of the novel triple Tg mice will be instrumental to future studies in several research areas such as MUC1 vaccine design and development, in vivo testing of new and improved Kras and Pten inhibitors, identification of MUC1 targets and their role in ovarian cancer pathogenesis, and the identification of new ovarian cancer biomarkers. Thus, the beneficial impact of the project is reasonable in light of the dollars budgeted.

Reviewer 3:

Ovarian cancer is a major clinical problem for women. Interestingly, similar to melanoma, ovarian cancer tends to be an immunogenic tumor which is amenable to immunotherapy strategies. Furthermore, the antigen system chosen by the PI was highly relevant. Recent publications, Dobrzanski et al., *Clinical Immunology* 2009 and Oei et al., *International Journal of Cancer* 2008 illustrate the relevancy of the MUC 1 antigen in terms of Th1 immunity and the humoral immune response. Additional literature exists describing the structure and function of MUC-1 as well as clinical studies which support the development of this murine model.

Progression to vaccine development by the PI utilizing the described model will be highly beneficial to the field. The PI is urged to move forward if funds become available to support the project. If any suggestion were made, inclusion of early detection of the disease and monitoring of the disease possibly using MUC-1 and circulating tumor cells in order to determine characteristics of patients responding and failing to both conventional and investigational therapies is warranted. In terms of future plans, significant fundamental goals have been achieved; the PI is ready to pursue vaccine studies.

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 leveraged additional funds during the award period. The Department of Defense Ovarian Cancer Research Program recently funded a grant application entitled, "Disease heterogeneity and immune biomarkers in pre-clinical mouse models of ovarian carcinogenesis." The investigator also plans to apply for funding to expand the research via the R01 mechanism from the NIH.

Reviewer 2:

The project was successful in leveraging additional funds through a grant application from the Department of Defense entitled, "Disease Heterogeneity and Immune Biomarkers in Preclinical Mouse Models of Ovarian Carcinogenesis" for the amount of \$839,080. The preliminary data generated in the current study provided solid support for this grant application and likely contributed to its success.

Reviewer 3:

It appears that a DOD grant has been obtained and the PI is applying for NIH funding. It is not clear what the overlap would be, but the PI has been successful to date at obtaining additional funds for continued 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 result in any peer-reviewed publications, patents or licenses. The investigator plans to submit articles to peer-reviewed publications in the future. No patents were filed during the project period.

Reviewer 2:

The investigators have indicated that the results of their study, reinforced with further studies that are currently in progress, will be incorporated in a manuscript that will be submitted for publication in 2010.

Reviewer 3:

It does not appear that the data has been published at this point. A note was made, however, that it is being prepared for submission. No patents are reported, nor are there any plans for commercialization.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

One post-doctoral student (Budiou) and one research associate (Brozick) were supported with health research funds. Funds from the project were also used to support a flow cytometer operator in the flow cytometry core, which serves multiple investigators at the grantee's institution. Together, these activities enhanced the capacity for research at the University of Pittsburgh and the Magee Institute.

Reviewer 2:

The project was important in enhancing the quality and capacity for research in the development of novel Tg mice, which can be used in the area of vaccine design and development.

Reviewer 3:

Funds were utilized to support research personnel as well as a technician in the flow cytometry division. Certainly this would enhance the overall quality of the service for the research center. A post-doctoral associate was supported as well as the technician who provided flow cytometric analysis.

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 investigator stated that the project led to collaborations with clinicians and basic scientists of the University of Pittsburgh Medical Center, and the immunology department; however, the names of these individuals and the nature of the collaborations are not described. The absence of community involvement is not unexpected since these are laboratory-based studies.

Reviewer 2:

The program did not lead to collaborations with research partners outside of the institution.

Reviewer 3:

No major collaborations were listed other than with local physicians and surgeons.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

None.

Reviewer 2:

1. The project has not generated any publications so far. Thus, the productivity over the past few years appears to be limited. It is recommended that the investigators publish the results in peer-reviewed journals and cite the Pennsylvania Department of Health in the acknowledgements.

Reviewer 3:

1. I feel the progress of the PI and associates was truly outstanding. I would recommend, however, once additional funding is obtained that the PI clearly evaluate CTL responses as well as the potential role of MDSC in immune suppression. Since levels of Tregs did not meet expected changes in the murine model, it is possible that MDSC have an increased role. In addition, since the PI has such a strong murine model developed with a defined antigen, evaluation of circulating tumor cells utilizing MUC1 expression could be very helpful to the field. This data could be easily translatable to human translational studies, and could provide an avenue for additional NIH funding through cancer prevention and cancer detection study sections, as well as SBIR funding if appropriate collaborations within business were initiated.

Generic Recommendations for Magee-Womens Research Institute and Foundation

Reviewer 3:

This project and PI should serve as an excellent example of the successful utilization and completion of research aims in a short, well defined period of time.