

**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 (1.90)

Project Rating:

Project	Title	Average Score
0990101	Refinement of the Appropriate Animal Model for Respiratory Infection with Influenza in Pregnancy	Favorable (2.33)
0990102	Relationships among PPAR gamma Activity, Hypoxia, and Differentiation in Human Placenta	Favorable (2.00)
0990103	Hypoxia Inducible miR-210 as a Potential Therapeutic Target in Renal Cell Carcinoma	Outstanding (1.33)
0990104	Role of miR-424 in the Differentiation and Function of Placental Trophoblasts	Favorable (1.67)
0990105	Epigenetic Analysis of Human Aneuploidy	Favorable (2.00)
0990106	Antigen Expression and Adaptive Immunity in Human Endometriosis and Endometriosis-Related Ovarian Cancers	Favorable (1.67)
0990107	The Impact of Age on the Nematode Germline	Favorable (2.33)

Project Number: 0990101
Project Title: Refinement of the Appropriate Animal Model for
Respiratory Infection with Influenza in Pregnancy
Investigator: Beigi, Richard

Section A. Project Evaluation Criteria

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

Strengths: The principal investigator (PI) intended to establish the respiratory infection of an animal (ferret) model to determine the effect of influenza virus infection on pregnancy and its immunological response mechanism.

Although the first experiment was not well-controlled, the ferret model could be used for the purpose of the project. In this regard, it was expected that the future experiments would produce meaningful results.

The PI found pre-exposure of the ordered ferrets to the virus and stopped the progress of the experiment without wasting time and effort.

Weaknesses: The data for the examination of aerosol characteristics of novel 2009 H1N1 influenza were not provided.

In the experimental analysis (Figures 1 and 2), no statistical data are shown. Since smaller numbers of ferrets were used than proposed and no uninfected control ferrets (two of them were dead) were included, it is difficult to draw convincing conclusions. It is unknown whether some of these ferrets were immune to the virus by the pre-infection with the virus.

The PI proposed to perform RT-PCR to measure the virus titer in the upper respiratory tract and also use blood and tissue samples in the first set of animal experiments, which have not been done yet.

It is unfortunate that the PI could not perform the second set of animal experiments, that involve multiple virologic and immunological assays, due to the problems with reinfection of the ordered ferrets and late Animal Care and Use Committee (ACUC) approval.

Reviewer 2:

The investigators failed to meet the stated objectives, primarily due to problems acquiring influenza-naïve ferrets for the proposed studies. Aim 1 proposes to “further develop and refine a model of collaboration and infrastructure...” Their continued efforts support success with this programmatic aim. Aim 2 proposes to “demonstrate and compare the disease course of aerosolized novel 2009 H1N1 in both a late gestation pregnant and non-pregnant ferret model of influenza infection.” They were able to execute one of two proposed studies addressing this aim, using reduced virus doses and numbers of animals. Aim 3, “to evaluate and compare the pathogenesis and immune response elicited by aerosolized novel 2009 H1N1 in both late gestation pregnant and non-pregnant ferrets,” was not addressed.

For Aim 2, the investigators provide data on weight gain (or an absence of weight gain) comparing influenza-infected pregnant and non-pregnant ferrets. However, as the investigators noted, the original strategic plan proposed to test aerosolization conditions for the pH1N1 initially to determine starting concentrations. Performance of these studies was not noted, and the data were not provided. Groups of three ferrets (pregnant or not pregnant) were infected with two doses (virus titer/volume and exposure time not provided) and tracked for weight and clinical symptoms. The investigators note that pregnant ferrets demonstrated “a febrile response and had clear influenza clinical illness that manifested as profuse nasal congestion/copious mucus production.” This data was not shown. Weight gain was presented in a graph, showing a failure to gain weight by influenza-exposed pregnant ferrets, while influenza-exposed non-pregnant ferrets gained weight without regard to virus exposure or dose. The data are presumably the average percent of starting weights for the three individual animals, but this is not clear. Also, it is surprising that one group of animals lost and then gained 20% of their starting weight over two days. Presentation of individual values, error bars, or other statistical analysis would help explain this phenomenon. I raise these specific points to emphasize that the investigators provided very little data from the completed animal study and provided only limited description of the experiment and the outcomes. This is a major weakness.

Related to this, the investigators provide no rationale for forgoing testing for virus shedding from nasal swabs or washes. This is critical data, since it is possible that the non-pregnant ferrets were not infected. This could be explained by differences in respiration rate, tidal volume, or physiological differences in the respiratory tract microenvironment. This is a critical question and a major weakness in deviating from the original protocol.

Finally, they provide no rationale for forgoing the original optimization experiments. While difficulties acquiring naïve ferrets are understandable, the changes to the experimental protocols and limited description of the results are considerable deficiencies. These deficiencies make interpretation of the results and drawing of conclusions to address the proposed questions difficult. It is possible that the project met some of the objectives of Aim 2, but the data do not show this. Aim 3 was not addressed, since Aim 2 was not completed.

Reviewer 3:

The objectives of the project were to: 1) develop and refine a model of influenza infection in pregnancy using the ferret; 2) demonstrate and compare the course of disease in an aerosol challenge model of influenza infection using 2009 pandemic H1N1 influenza virus in pregnant

and non-pregnant ferrets; and, 3) evaluate and compare the pathogenesis and immune response in ferrets challenged via small particle aerosol.

Due to a number of issues with obtaining animals that were influenza seronegative, they did not complete the objectives of the project. The research design was less ambitious than would be expected, with no goal to characterize the virus load in the respiratory tract via nasal aspirate collection, a standard for characterizing influenza infection in the ferret model. The only endpoint reported was weight changes, and these data are not indicative of any difference between pregnant and non-pregnant animals. There is mention of differences in clinical disease, but no objective clinical scoring was reported. Overall, the data was lacking and reflected the lack of understanding of the ferret model of influenza and influenza in general. A comment was made about the relevance of the aerosol route of challenge being more replicative of human infection. Actual human infection with influenza in humans is most likely by close contact with fomites or ocular injection or inhalation of large-particle aerosols after close contact with infectious aerosols from infected individuals when coughing or sneezing. In addition to the deficiencies in the design and endpoints, the deaths in sham-inoculated animals introduces variables to the results that are difficult to explain.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

Strength: The animal model of aerosolized influenza in pregnancy will be greatly useful for evaluating increased susceptibility among pregnant women and conducting its mechanism/treatment studies.

Weakness: The results provided are not convincing for the establishment of the model yet.

Reviewer 2:

The potential benefits of this proposal are quite large. The 2009 pandemic impacted a large proportion of pregnant women in comparison to other populations. Development of an animal model for influenza infection in pregnant females will provide significant opportunities to determine whether this is a unique event associated with the 2009 virus or if this is a previously unrecognized at-risk group. These data could impact vaccination recommendations. The animal model will also provide opportunities to study viral mechanisms of susceptibility to disease. This is a strength of the proposal.

The investigators propose to submit federal grants to acquire funding to continue this research, although details on the goals of the pending proposals were not provided.

Reviewer 3:

The characterization of influenza infection in pregnancy is an important comorbidity factor in human influenza. The mechanism(s) of enhanced pathogenesis during pregnancy or other complicating health conditions are critical to epidemic and pandemic influenza control. While

the outcome of this project has significant implications to the field, the design, which lacks robust endpoints that measure the virology, clinical disease, and immune responses leaves these results almost anecdotal at best. No new information was obtained from these studies, since they were lacking standard endpoints for flu in ferrets; and the lack of animals for part of the project left the results deficient in even the modest objectives.

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:

Strength: The PI plans to submit a proposal to the NIH based on the results of the future study.

Weakness: The current data are not enough for an NIH application. As noted by the PI, the proposed experiments need to be fully completed for its application.

Reviewer 2:

No additional grants were submitted. The original proposal mentioned submission of an R21, however no mention of this proposal was given in the progress report. Preliminary data from aerosol chamber optimization and demonstration of the ferret infection model are sufficient for submission of future grants. As noted above, the investigators propose to submit an R-type grant (R01, R21, or similar) and noted other “upcoming internal funding opportunities.” They mention “influenza-related research in pregnancy” and other “pathogens of high importance” but provide not details on the goals of these proposals, or specific aims. Most notably, the investigators fail to describe how they will leverage the existing data derived from this grant to strengthen future proposals. This is a weakness of this final report.

Reviewer 3:

No new funds we obtained related to these studies. Additional data would be required to make this project fundable by other sponsors. The lack of robust endpoints and thorough examination of clinical and viral loads in infected ferrets to compare replication in pregnant vs. non-pregnant animals, is problematic. Additionally, the deaths in sham-infected animals points to technical issues with delivery of aerosol or restraint of ferrets for these studies, decreasing the importance of even the limited amount of experimental data.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

Strength: The PI plans to complete the proposed experiments and then prepare for a manuscript submission/publication in a peer-reviewed journal.

Weakness: The current data are not enough for the submission. The proposed experiments need to be fully completed. Perhaps, a couple of other experiments need to be done for its publication.

Reviewer 2:

The investigators did not report any peer-reviewed publications, licenses, patents, or commercial development opportunities resulting from or filed from the project. The investigators plan to present at a national meeting and then prepare a manuscript for publication once they complete a second ferret experiment. The meeting is certainly appropriate; however it is unlikely they will be positioned to submit a research manuscript for peer-reviewed publication from two animal experiments, particularly if the data are as limited as presented in this report. Additional studies will be needed, suggesting that additional funding will be required. This is a weakness, as a publication would cement the collaboration and model strengthening and grant proposals.

Reviewer 3:

No publications were produced in the project, and the lack of data would prevent publication at this point in time.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

Strengths: The project involved the efforts of three faculty members/research and a director, and thus increased their collaboration.

The development of a new animal model has increased the quality and capacity for research at the institution.

Reviewer 2:

There were no improvements to infrastructure noted; however the project and burgeoning collaboration are foundational for expanding a program based upon pregnant ferrets as an animal model for infectious diseases. This is a strength of this proposal. It is of interest to this reviewer, other pathogens that could be modeled in the ferret.

New investigators were not brought to the institution to contribute to the research, and funds were not used to support post- or pre-doctoral trainees.

Reviewer 3:

The project did combine previously untapped capabilities in the institution and could serve as a stimulus for additional studies. A lack of experience with influenza may be a deficit for further influenza related programs, and it would be helpful to enlist a collaborator with influenza expertise - present at this institution, but untapped. Funds for this project could be seen as adding to the validation of the aerobiology core at the institution, but the issues with challenge and sham challenge call that validation into question. No new investigators were recruited to support this project, and no funds were used to train students or fellows. The program, if further developed,

has value to the institution, since it brings together infectious disease and other capabilities (pregnancy medicine). That potential may be the most important outcome of the program, since the data were not forthcoming.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

Strength: The study was presented at a national meeting and opened a productive dialogue between the group and NIH/FDA.

Weakness: No specific collaboration was identified.

Reviewer 2:

The project generated interest from investigators outside the institution. The investigators mention interest specifically from the FDA and the National Institute of Allergy and Infectious Diseases (NIAID). They plan to encourage these potential collaborations as well as their existing internal collaboration. This is a major strength.

Reviewer 3:

It is not clear if new collaborations outside the institute will come from this project, although there is certainly potential for that to occur.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

1. The data for the examination of aerosol characteristics of novel 2009 H1N1 influenza were not provided, although the experiment was said to be done.
2. In the experimental analysis (Figures 1 and 2), no statistical data are presented. Also, the PI proposed to perform reverse transcription polymerase chain reaction (RT-PCR) to measure the virus titer in upper respiratory tract and in blood and tissues, but this was not done.
3. It is unfortunate that the PI could not perform the second experiment that involves multiple important virologic and immunological assays. The results need to be provided.

Reviewer 2:

1. Design of experiment 1: The investigators did not test for virus shedding or test for seroconversion in inoculated ferrets. Without this, it is impossible to confirm infection; and the differences in pregnant and non-pregnant ferrets may be unrelated to influenza exposure. This data needs to be collected for all future experiments, otherwise they will not have shown they have a model for influenza infection in ferrets (pregnant or otherwise). This is a major

weakness, although they still have funding to complete a second experiment where they can test their hypothesis.

2. Presentation of data from experiment 1: The investigators did an insufficient job of presenting experimental data from the sole ferret experiment. The ferret infection model provides a variety of opportunities to measure and describe disease including, but not limited to, activity, fever, virus shedding (nasal, rectal), and seroconversion. The investigators showed percent weight only and did not provide statistical analysis of those data. This was a major weakness of the report, since there was variability in the weights and no direct evidence of infection presented. The data could be improved by providing data for individual animals and/or statistical analysis of the groups.
3. Failure to describe goals of future grant submissions: The investigators need to describe briefly, the long-term goals and aims of proposed grants. While the assertion of planned proposals is comforting, they failed to provide clear direction for the work. This would be similar to the one-page summary provided to NIH where one describes the problem, the goal of the proposal and the aims to address the goal.
4. Data generation for a manuscript: This is related to Recommendations 1 and 2, but more specifically, the investigators should not attempt to address Aim 3 until they confirm the infection model in Aim 2. Also, tools for immune response analysis in ferrets are very limited, so the investigator should pose a very specific question as opposed to proposing gene expression microarrays.
5. Delineation of Aims 2 and 3: The original proposal states that viral loads and necropsy will be assessed in Aim 2, however the investigators propose this is the goal of Aim 3 and state that Aim 2 is completed. As mentioned, the single experiment does not complete Aim 2. Also, there is no mention of the proposed optimization of the aerosol chamber. Was this done? The dose of viable virus aerosolized is a key component of the model. These points need to be addressed and Aims 2 and 3 clearly defined.

Reviewer 3:

1. To address the issue with obtaining animals, it is possible to challenge animals that were seropositive for H1N1 pandemic influenza with another influenza subtype to salvage a very limited resource (pregnant female ferrets).
2. To address the clinical score, virology, and immune parameters samples could have been collected for virus load (nasal aspirates) and immune characterization serum and nasal wash Ab or cytokine production by PCR.
3. Issues with the aerosol challenge system (including sham animals) must be addressed for these studies to proceed. Perhaps more detailed spray factor studies would be helpful for this deficiency.

Generic Recommendations for Magee Womens Research Institute and Foundation

Reviewer 2:

Clarify the goals of the final progress report to provide instructions on the description of future (proposed) grants and provide recommendations for data reporting.

Reviewer 3:

An important aspect of this project is influenza pathogenesis using a rational model. Additional expertise with influenza in the ferret may have helped with the overall design and endpoint collection. There is influenza expertise at the institution (Dr. Ted Ross), and collaboration with that laboratory may be helpful in further experiments, if planned.

ADDITIONAL COMMENTS

Reviewer 3:

The project did not characterize aerosols prior to challenge despite that having been a goal in the beginning of the program. There was a lack of complete endpoints for the few animals that were used in the project, and no virus loads were measured in animals through the time after challenge. No immune parameters were measured in these animals. Sham challenged animals were lost in this study, indicating issues with the aerosol challenge or some other husbandry practice for these animals.

Project Number: 0990102
Project Title: Relationships among PPAR gamma Activity, Hypoxia, and
Differentiation in Human 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:

The research team completed the proposed research work. The principal investigator (PI) is knowledgeable about the statistical analysis methods used in the research project, and there was good support from other team members. The research team identified up/down regulated genes for the research aims specified.

There was no calculation of sample size and power at the study design stage. There was little interpretation of the differential gene expression results.

Reviewer 2:

The overall goal of the project was to examine gene expression profiles in primary human term trophoblasts during differentiation, hypoxia, and PPAR ligands using historical data sets. In Aim 2, quantitative real-time polymerase chain reaction (qRT-PCR) was used to verify the microarray data. In addition, a goal was to examine gene expression profiles in placentas from pregnancies complicated by fetal growth restriction (FGR).

In general, the project met the stated aims of the grant. The PI discovered, interestingly, that the gene expression profiles in cultured trophoblasts were quite dissimilar from placentas from FGR pregnancies.

A strength of the proposal is the strong scientific collaboration between the PI and co-investigator (Sadovsky), who has vast experience in placental biology, especially in hypoxia and placental insufficiency syndromes.

A weakness was the rather simplified gene ontology tools used. Although cluster analysis and the other methods used are appropriate, additional information may have been gleaned from gene regulatory network analysis, for example.

Reviewer 3:

The researchers performed the proposed analyses. Four sets of microarray data were analyzed along with qPCR data.

For microarray analysis, the use of the robust means analysis (RMA) algorithm for normalization is standard and useful. The use of limma software for statistical analysis is also a commonly used and effective method. The investigator was also careful to adjust for multiple testing using appropriate methods for false discovery rate estimation in the initial microarray analyses.

Unfortunately, the previously existing microarray data used by the investigator did not contain sufficient biological replication to produce trustworthy results. The first set had only technical replication. Only one sample of each type was used with five technical replications per sample. This permits a statistical analysis that can identify differences between two samples, but differences between two samples exist between any two samples. Thus, there is no reason to believe that differences between two samples are due to the fact that the two samples were of different types (e.g., treated trophoblast stem cells vs. control). Multiple independent treated samples and multiple independent control samples are needed, rather than simply technical replicates created from single samples.

The second data set (the differentiation experiment) had no replication at all. Thus, the investigator used estimates of variability across technical replicates from the first experiment to determine statistical significance in the second. This is very likely to lead to an overstatement of statistical significance, given that technical replicates are much less variable compared to truly independent biological replicates. Furthermore, even if biological replicates had been available in the first experiment, there is no way to verify that the variability in the first experiment matched variability in the second.

The analysis of the third and fourth experiments looks reasonable because these experiments included biological replication, albeit minimal. Unfortunately, no significant differences were identified in the fourth experiment. Results from the third experiment comparing 20% to 0% hypoxia are likely the most trustworthy of all results produced.

The overrepresentation analysis was done with the standard approach of using Fisher's exact test. It is difficult to know how meaningful these results are, given that they are based on the analysis of unreplicated data, as discussed above. Furthermore, the investigator did not seem to use an explicit method to adjust for multiple testing in the overrepresentation analysis other than the use of a rather small significance threshold.

The analysis of the qPCR data produced results that suggest that *in vitro* conditions are quite different from *in vivo* placenta conditions, but more similar to FGR placenta than normal. This is somewhat useful information.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

This project contributes scientific knowledge of placental development, which may help to understand birth outcomes such as low birth weight. However, this project is exploratory; the immediate impact on health research is likely to be small. There are no identified weaknesses for this aspect.

Reviewer 2:

Inasmuch as this was a basic science study, there is little direct and immediate benefit to patients. It is important, however, that we better understand the nature of gene expression changes in the setting of placental hypoxia and differentiation during fetoplacental development. It is quite intriguing that the PI found such stark differences in gene expression in cultured trophoblasts versus placentas collected from FGR patients.

A potential weakness of the study is the apparent lack of control for gestational age at the time of placenta collections. The cultured trophoblasts were prepared from term placentas, while the FGR placentas are of varying gestational ages. It is possible that this is also a source of the gene expression differences. Future studies should consider this variable.

Reviewer 3:

Studying molecular genetic mechanisms that play an important role in fetal growth restriction (FGR) is a worthy undertaking that could eventually lead to treatments that prevent FGR. However, the data used for this study is not likely sufficient for making meaningful and important discoveries. A considerably more extensive investigation with well-replicated experiments would be needed to make better progress. Thus, I think the likely beneficial impact is minimal. Not much money was budgeted for this project, so perhaps the minimal output is not surprising.

If researchers undertake stated plans to study micro RNA and protein levels, I would encourage them to consider generating better data sets. Certainly this will cost more than the current project, but there are ways to make progress without too much expense. For example, one well-replicated data set from a well-designed experiment would be much better than four data sets that have minimal or no replication. Better high-throughput data would give better gene candidates for follow-up 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:

No leveraging of funds and no future plan for additional funding are discussed in the final report.

Reviewer 2:

It does not appear that the project leveraged additional funds, however the PI has indicated that future NIH applications will follow.

Reviewer 3:

The documents mentioned using this work to obtain preliminary data for an NIH grant application, but it does not appear that any additional funds were ultimately sought.

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:

Only one manuscript was submitted for publication. There were no plans for further attempts at publication, filing of patents, or commercial development.

Reviewer 2:

The project resulted in the submission of one peer-reviewed manuscript to *Placenta*, a major journal in the field. The paper reports on the differences in gene expression profiles between cultured trophoblasts under various hypoxic conditions. It is not stated whether this paper has yet been reviewed and accepted.

Hopefully, future manuscripts will include a discussion of the interesting and potentially important finding of major differences in gene expression profiles in cultured trophoblasts compared to placentas from FGR pregnancies.

Reviewer 3:

One paper has been submitted. No other publication plans were noted.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

This is another weakness of the project. There were no reports of infrastructure improvements, new investigators or involvement of students; but this is a small, exploratory, secondary data analysis project.

Reviewer 2:

The project addressed an important problem in perinatal medicine; that is, how does uteroplacental hypoxia during pregnancy alter trophoblast gene expression and differentiation? Less clear, is the rationale for using PPAR agonists as an exogenous inducer of trophoblast differentiation.

No direct improvements appear to have been made to infrastructure at the institution. However, the collaboration of the PI with co-investigator Sadovsky, a noted expert in placental biology, will likely enhance the research efforts of the PI and provide greater likelihood of success in extramural funding.

Reviewer 3:

One bioinformatician and one programmer received training. It is possible that these people and the example analyses produced could be useful to others at the institution.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

None. This project is exploratory and is not yet ready for clinical applications.

Reviewer 2:

The project involved a collaboration with co-investigator Sadovsky, a leading expert in placental biology, including differentiation and the role of hypoxia in trophoblast development.

No other extramural collaborations appear to have been formed from the project.

Reviewer 3:

The project did not lead to any collaborations.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

1. A careful sample size and power analysis should be conducted before a study begins. For microarray experiments, there are available tools for this type of study planning.
2. A research team should be multidisciplinary, including clinicians, who can interpret and understand the meaning of data analysis results.
3. The home institution should provide more guidance and support to a junior investigator and include the individual project in its overall organizational research effort.

Reviewer 2:

Pay greater attention to the gestational age of the specimens used from FGR pregnancies and placentas used to prepare cultures of trophoblast. It is challenging to obtain sufficient numbers of human placentas from pregnancies of first or second trimester; nonetheless, many of the features of FGR, including hypoxia and/or preeclampsia and other placental insufficiencies, occur prior to the third trimester (the time when cultures were prepared in this project). If this cannot be done easily at the PI's home institution, perhaps there are collaborative opportunities or some well-annotated rationale for the use of term cultures in studies such as this one.

Reviewer 3:

If researchers undertake stated plans to study micro RNA and protein levels, I would encourage them to consider generating better data sets. Certainly this will cost more than the current project, but there are ways to make progress without too much expense. For example, one well-replicated data set from a well-designed experiment would be much better than four data sets that have minimal or no replication. Better high-throughput data would give better gene candidates for follow-up studies.

ADDITIONAL COMMENTS

Reviewer 1:

The strengths of the project include: 1) the PI and the research team possess adequate knowledge and skills to carry out the proposed data analysis; and, 2) all the research aims were explored and analysis results produced.

The weaknesses of the project include: 1) there is a lack of sample size and power consideration during the study design phase; 2) there is a lack of careful interpretation of the results and discussion of the meaning and impact of the results; and, 3) this project is weak on all other metrics, such as impact on health research and clinical application, improvement of organizational infrastructure, and staff education.

Reviewer 2:

This was an interesting and potentially important bioinformatics study of gene expression patterns in placental development and differentiation, especially in the setting of hypoxia. The PI used historical data from trophoblast cultures grown under various conditions, that is, hypoxia (0% O₂ vs. 8 or 20% O₂) and peroxisome proliferator-activated receptor (PPAR) gamma agonists. Then, hypoxia *in vitro* was compared to FGR placentas. It was interesting that the gene expression patterns for cells grown in hypoxia were dissimilar from the patterns noted in FGR placentas. Although the PI did not specifically address the implications of this finding, this may indicate that we have been misinterpreting the devastating role of relative hypoxia (i.e., short term, oscillating) in placental differentiation. This could have important implications for future studies and for our understanding of the role of oxygen in fetoplacental development.

Project Number: 0990103
Project Title: Hypoxia Inducible miR-210 as a Potential Therapeutic Target in
Renal Cell Carcinoma
Investigator: Huang, Xin

Section A. Project Evaluation Criteria

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

The overall goal of this project is to determine whether miR-210 inhibition is useful in controlling the growth of clear cell renal cancer alone or in combination with chemotherapy. This is an ambitious goal, and the project did a good job in meeting these objectives, especially in understanding the mechanism underlying the inhibition of renal cancer cell growth when miR-210 is inhibited. The research design and methods were logical. In fact, the delay in achieving the completion of all the aims was that there were more data generated than could reasonably be handled in the time frame of this grant. This applies in particular to Specific Aim 2, where the goal was to identify miR-210 target genes in renal cell cancer. Overall, outstanding progress was made. This conclusion is based on: 1) understanding the effect of miR-210 inhibition on the proliferation of renal cancer cells; 2) the generation of an exhaustive list of potential miR-210 target genes; and, 3) the identification of the p53-p27 pathway that inhibits the cell growth of renal cancer cells that lack miR-210 expression.

Reviewer 2:

MicroRNA biology and biochemistry is a very complex field, and a blueprint on which we all agree is not available.

Reasonable progress has been made, and a high- impact paper was published.

The grantee studied the biology of miR-210 in the most relevant context. Indeed, miR-210 is a direct hypoxia-inducible factor (HIF) target; in fact it is one of the most reliable, based on the literature. Additionally, if there is a tumor type where HIF is extremely relevant, it is renal cell carcinoma (RCC).

The connection between miR-210 and p53 is novel and of great interest. I am confused by the lack of p21 induction despite p53 phosphorylation. p27 induction may be a secondary effect related to cell cycle arrest, rather than a direct consequence of p53. I recommend a larger number of p53 targets be interrogated, as well as chromatin immunoprecipitation (ChIP) and luciferase based experiments.

The data developed were sufficient to answer the research questions posed, and the connection with p53 is indeed interesting and of potential impact.

A major finding that supports the hypothesis that miR-210 may be a therapeutic target is the impact on cell proliferation, as well as the sensitization of cells to chemotherapeutic agents upon miR210 inactivation. The weakness is that the experiments seem to have been performed in one RCC cell line. It would be of interest to test additional cell lines, such as 786O cells.

Reviewer 3:

The specific aims of this proposal were to: 1) determine the function of miR-210 in ccRCC with *VHL* mutations; 2) identify miR-210 target genes in ccRCC; and, 3) elucidate the mechanism of cell death caused by miR-210 inhibition in ccRCC. Although the investigators did not specifically reach all of the objectives, they made significant progress towards achieving these goals. For example, they identified that miR-210 is essential for growth of a ccRCC cell line; they demonstrated that decreased growth correlates with decreased clonogenic survival, but not with increased apoptosis; and they found that decreased expression of miR-210 results in increased activation of p53 as demonstrated by increased phosphorylation of p53 on serine 15. Significantly, the investigators found that down-regulating miR-210 sensitized a ccRCC cell line to Doxorubicin and Camptothecin.

At the same time, a number of weaknesses with regard to the current data affect determining the impact of the studies. First and foremost, the studies were performed with limited scope, lending only minor significance to the conclusions drawn. For example, only one ccRCC cell line was used in the studies. How widely applicable are the findings to other cell lines? Only one approach to modulate miR-210 was used (knockdown). Can complementary experiments be done with overexpression to demonstrate specificity to the proposed effect of miR-210 expression? What about the timely issue of HIF1a versus HIF2a? The investigators previously demonstrated that miR-210 is a HIF1 target gene. Can HIF2a also activate miR-210? This is highly relevant, since a significant proportion of ccRCCs only express HIF2a; and in fact, a recent publication argues that HIF1a may be a tumor suppressor in ccRCC (Shen, et al., *Cancer Discovery*, 2011, 1:222-235). Of note, RCC4 cells, which the investigators have used, are known to be HIF1a and HIF2a positive. Thus, broadening the scope of the studies will enhance the potential impact.

A second weakness is the lack of defined target genes. This was a stated objective that has not yet come to fruition but will be a major determinant of the measured success of the project.

A third weakness is the inadequately explored relationship between miR-210 and p53. The preliminary data presented suggest p53 phosphorylation increases with miR-210 knockdown. Surprisingly, levels of p53 do not seem to be regulated by phosphorylation as is the case for wild-type cells. Also surprising is the lack of increase in p21 expression, generally regarded as the canonical p53 target gene. This suggests that p53 may not be transcriptionally active even though it is phosphorylated. So is it causing cell cycle arrest but without inducing p21 expression? Also, p27 is not to this reviewer's knowledge a p53 target, as stated by the investigators. Thus it is unclear how p27 and p53 are connected in this setting. In sum, while provocative, these data need to be developed further.

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:

Currently, most therapeutics for renal cell cancer result in a modest improvement of survival. This project attempts to exploit the importance of the micro RNA, miR-210, as a therapeutic target for renal cell cancer. If successful, targeting either miR-210 or a target gene of miR-210 would provide a new approach to treat renal cell cancer as a monotherapy or to enhance the effectiveness of chemotherapy. Therapeutics based on microRNAs are still in their early days, and the investigator is laying the foundation to exploit this technology as it is being developed for the treatment of renal cell cancer.

Reviewer 2:

As far as impact on improving health, therapy-wise, we do not yet have any drug which has been developed based on miR targeting in cancer. However, since *in vivo* inactivation of miRs is coming closer to becoming a reality due to development of relevant reagents, it is entirely possible that this limitation will be overcome in the future.

Diagnostic-wise, these small transcripts are currently being detected in blood in many tumor types and potentially in the urine in genitourinary malignancies; therefore, a deeper understanding of these may provide novel non-invasive or minimally invasive diagnostic and prognostic tools.

The applicant anticipates an R01 application once an additional paper is published.

Reviewer 3:

The likely beneficial impact of the project is modest. As with many “screen” projects, the impact is highly dependent on the results of the screen. It is possible the investigators will discover an excellent novel therapeutic target. Conceptually, however, miR-210 is hypothesized to inhibit the expression of genes thereby leading to renal carcinoma. Thus the miR-210 targets would be presumed to be tumor suppressors. If valuable targets are identified, the great challenge would be to reactivate them. It seems more likely that targeting miR-210 itself is more likely to be successful. One strength, therefore, of this project is using ccRCC as a model system in which to investigate the impact of miR-210 inhibition. It is not clear how the investigators would go about doing this, but they would be wise to consider this as a future direction. One weakness at this point is that the dollars budgeted for this project outpace the progress to this point.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

The project did not appear to leverage additional funds, but the investigator indicates that he intends to apply for additional funding in the future.

Reviewer 2:

The stated goal is to apply for an R01 after an additional manuscript is published. Based on the strengths of the progress achieved to date, I consider this a realistic goal.

Reviewer 3:

No additional funds have been leveraged. The investigators plan on applying for an R01 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 research has not yet resulted in publications, licenses, or commercialization; but after the analysis of the miR-210 target genes, they should be in a much stronger position to do so.

Reviewer 2:

A high-quality paper in *Molecular Cell* was published and is being highly cited in the field. Once the deep sequencing data (SOLiD™) are finalized and a few more targets validated, I anticipate another high-quality paper to be submitted. However, these deep sequencing data will primarily address VHL-specific miRs in a more general fashion, rather than being miR-210-specific.

Reviewer 3:

There are no publications on this project at this time.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

The investigator states that his research has increased his institute's awareness of renal cell cancer. They have also gained a significant amount of information on next generation sequencing that they will share with their colleagues in the Magee-Womens Research Institute and Foundation.

Reviewer 2:

Two post-doctoral fellows were supported.

Reviewer 3:

Two new researchers were recruited to Pennsylvania to perform the research. Additionally, as a foray into renal carcinoma, the investigators expanded the institute's scope to cover a new disease. Also, utilization of next generation sequencing technology improves the quality and capacity of research.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

The project has not yet led to collaboration with research partners outside of the institution or involvement with the community.

Reviewer 2:

The researchers are not planning to begin any collaborations as a result of the research.

Reviewer 3:

No new collaborations resulted from this project.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

None.

Reviewer 2:

There are many miRs that would conceivably get altered as part of VHL loss, and miR-210 is certainly a leading one, but unlikely the only one. As designed in this project, the deep sequencing approach from VHL-positive versus VHL-negative cells will yield targets that are not only miR-210-specific. Multiple HIF-regulated miRs seem to exist, and there are also HIF-independent effects of VHL loss (which may certainly involve other miRs).

A more miR210-specific approach should be added to complement the forthcoming data. Overexpression of miR-210 in VHL-positive cells for example, followed by Ago IP and deep sequencing may be valuable. I realize how costly this approach is, and additional funds would certainly be required. Therefore an additional grant, such as an R01, that the applicant states he will pursue, would be paramount.

The applicant needs to consider carefully how he will identify the miR-210-specific targets versus targets of other miRs that will likely be present in the RNA-induced silencing complex (RISC).

Reviewer 3:

1. Expand the research to include more tumor cell lines, and modulate miR-210 by additional means. Move into an animal model. Obtain clinical samples to increase the relevance of findings.
2. Identify targets of miR-210 to uncover the mechanism.

Project Number: 0990104
Project Title: Role of miR-424 in the Differentiation and Function of
Placental 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 project partially met the stated objectives. For the first aim, the inability to knock down miR-424 did not allow the investigators to assess the impact of miR-424 on trophoblast function. Although the investigators did show that partial knockdown of miR-424 released fibroblast growth factor receptor 1 (FGFR1) from the putative silencing, the effect was very limited. In addition, the restriction to a single target, FGFR1, diminishes the impact of these results. In the absence of substantial knockdown of miR-424, it is likely that the miR remaining continues to exert silencing effects. In the second aim, the investigators demonstrated that the cluster of miRNAs associated with miR-424 may be co-regulated with miR-424 by hypoxia; however the mechanism is not clear. In the mouse, unlike the human, hypoxia upregulated the two miR under study, possibly due to the mixture of cells being examined. Overall, the project had limited success in meeting the stated objectives.

In a situation such as this, where a lot depends on the a specific action (i.e., the knockdown of miR-424), the project might have been better served had the investigators determined the feasibility of this key step before embarking on the experimental protocols. The research design and methods beyond this step are appropriate for the proposed project.

Because of the failure to achieve miR-424 knockdown, insufficient data was obtained to answer the research questions. While the association between miR-424 and hypoxia is strong, the role played by miR-424 is still not clear.

The investigators made a variety of innovative changes both in trying to identify appropriate miR-424 targets and in the procedures used to try and knock down miR-424. The alterations were clearly described and explained, and the alternative strategies attempted were quite reasonable.

The data obtained and provided in the reports was sufficient to assess the progress of the project. The data and information provided were clearly applicable to the goals listed in the research plan.

The strengths of the project relate to the results showing increased support for miR-424 effects on trophoblast cell targets, the definition of targets, the development of new methods for probing miR expression and function, and the demonstration of miR cluster co-regulation.

The weaknesses include the continued inability to knock down the primary target, although the investigators indicate recent progress with morpholino antisense oligos. In addition, the *in vivo* model appears to have been much too broad and complex a target for adequate analysis.

Reviewer 2:

The proposal investigated the role of miR-424 in regulating MAP2K1 and FGFR1 in the placenta. miR-424 was shown previously by the investigators through a microarray screen to target these two genes and was shown to be highly expressed in the human placenta. The hypothesis stated that the decreased levels of miR-424, as might be observed in the setting of placental hypoxia, could lead to increased expression of target genes (i.e., FGFR1 and MAP2K1). The aims included an examination of the impact of miR-424 on cell function in cultured human term trophoblasts and a study of the miR-424 cluster genes in human and mouse placentas.

The team underwent a thorough evaluation of the hypothesis and specific aims, but the data obtained did not conclusively support all the predictions. For example, when inactivating mutations were introduced into the miR-424 gene, there was no resulting upregulation of MAP2K1. However, one of two introduced mutations did lead to de-repression of the FGFR1 gene and resulted in elevated luciferase expression. The lack of statistical testing does dampen enthusiasm a bit, but overall, the data do support the reciprocal nature of miR-424-FGFR1 interactions.

Reviewer 3:

The project effectively addressed all stated objectives. Experimentation was performed to investigate the role of miR-424 in the regulation of trophoblast cell function. The investigators investigated the regulation of miR-424 expression, identified miR-424 targets, and provided insights into the biological role of miR-424 in placentation. The investigators also made important strides in developing an animal model, which can be utilized in future investigations.

The experimental design and methodology was adequate to address most of the objectives. The investigators had to improvise regarding some of the objectives, especially the knockdown/silencing of miR-424. They were very resourceful and implemented new cutting-edge technologies when proposed methods were not successful and provided alternative future experimental approaches to address their technical challenges.

The experimentation was experimentally sound and will provide a solid experimental basis for future research. The researchers addressed each question that they proposed in their original

experimental plan. Their efforts provided important new insights into the role of a miR in the regulation of trophoblast cell function and especially its responses to stressors.

The main changes introduced into the experimentation were in regard to the implementation of new techniques and methodologies to address the experimental questions. The investigators provided a strong rationale and logical arguments for adjustments to their experimental plan.

The expected outcome of the proposed research was to generate a quality research publication and sufficient preliminary data to support a competitive NIH proposal. The investigators generated data for at least one publication and maybe parts of additional reports and demonstrated the feasibility of a more extensive grant application to investigate the biology of miR-424 and other miRs forming the cluster on the X-chromosome as regulators of trophoblast cells during injury.

The data generated by the research was directly relevant to the investigators' stated objectives.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

The significance in the impact to health is unclear. The investigators have made some progress towards characterizing the effects of hypoxia-mediated changes in miR expression; however, the inability to knock down the primary target limits the impact of this research. Nevertheless, this work is examining some fundamental questions and has the potential for significance.

The value of the research completed is that it provides a foundation for further studies in a new but extremely complex field. The knowledge to be gained eventually will define a new area of research into hypoxia in pregnancy.

Future plans are to follow up this work with other attempts to knock down or overexpress miR-424 and to develop transgenic mouse models lacking miR-424. Given the hypoxia/miR association which has been strengthened by the current work, this appears to be appropriate.

Reviewer 2:

This was a basic biomedical science investigation, and no direct benefit to individual human patients was examined or elucidated. However, the work did address an important issue in perinatal/placental biology; namely, the role of miR-424 in fine-tuning the expression of key genes in placental development. The study of the role of hypoxia in regulating miR-424 has potentially important implications for the pathobiology of preeclampsia, in which placental insufficiency frequently leads to hypoxia and compromised uteroplacental blood flow heralding intrauterine growth restriction.

The future plans outlined in the proposal and final report suggest a two-pronged strategy. First, the team will alter the levels of miR-424 in primary human trophoblasts cultured from term placentas. This will employ morpholino antisense oligos and lentiviral vectors expressing an shRNA system. Second, the team will use a mouse model with a targeted deletion of miR-424.

Reviewer 3:

The executed project is a basic science project. It is several steps away from directly improving human health. The investigators have identified a novel mode of cell regulation that responds to placental injury. Understanding the details of this mode of trophoblast cell regulation will lead to the identification of targets for therapeutic intervention. Such efforts will culminate in improvements in fetal and postnatal outcomes.

The quality of pregnancy is critical to our entire healthcare system. It is evident that exposures in utero impact postnatal health and the development of disease. Susceptibility to cardiovascular disease, obesity, cancer, etc. can all be traced to events transpiring during fetal development. The placenta is at the core in creating the environment in which the fetus develops. Thus, improving diagnostics and treatments of placental-related maladies is crucial to improving the health of our society.

Eventually the research will lead to more effective diagnosis of placental insufficiencies and the development of therapeutics that specifically target pathways controlling placental responses to injury.

As stated above, the investigators are at an early stage in the process. They are performing fundamental basic research that will lead to translational efforts and eventually to the clinic. miRs have proven to be key regulators in many tissues. The investigators have established an experimental framework for evaluating the involvement of miRs in trophoblast cell development and placentation. These efforts will lead to advances in the derivation of new diagnostics and therapeutics for treating placental insufficiency.

The future plans of the research are to further investigate the biology of miR-424 targets and other members of the X-chromosome miR cluster. These efforts will include the utilization of molecular and cellular approaches that the investigators have expertly developed. Additionally, it is critical that the investigators expand their approach to include *in vivo* analyses using animal models. The researchers provided essential preliminary data on miR-322 expression and analysis of the X-chromosome miR cluster in the mouse. Development of a mouse model will enhance the scientific impact of their research efforts. In order to accomplish these important tasks, the investigators need continued funding for this most important 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:

No additional funds have been obtained thus far. The investigators are planning future grant submissions based in part on this work.

Reviewer 2:

The proposal did not leverage additional resources from extramural sources for the project. The PI did indicate that future NIH applications are anticipated.

Reviewer 3:

Based on the information provided, I am not aware of any leveraging of funds.

The researchers are planning to submit an NIH grant application based on their accomplishments. The data generated represents impressive preliminary data that should lead to a competitive NIH grant application. Additional preliminary data, especially focused on *in vivo* analyses with the proposed mouse model, will enhance success for future funding.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

No publications have resulted from this work at this point; however, the investigators indicate that they plan to submit a manuscript based on their research in this project.

Reviewer 2:

No peer-reviewed publications have been submitted or published, though the PI indicates that manuscript submissions will be forthcoming.

Reviewer 3:

The researchers' progress was appropriate. The accomplishments will contribute to at least one outstanding report and will also form the basis of a highly competitive grant application to the NIH.

The researchers are planning to submit at least one manuscript based on their research efforts. This is realistic progress for a one-year award. No licensing agreements, patents, or commercial development opportunities are anticipated.

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 infrastructure improvements, addition of new investigators, or funding of research by pre- or post-doctoral students.

The investigators have developed techniques in a relatively new field at their institution which may be of significant help to other researchers at the institution.

Reviewer 2:

No infrastructure improvements were carried out in the project. It does not appear that pre- or post-doctoral students were supported by the project.

Researchers within the Magee-Women's Research Institute and Foundation will be able to employ many of the experimental tools developed in the project, such as lentiviral-driven expression of shRNAs in primary human placental trophoblasts.

Reviewer 3:

The only identified personnel associated with the project were Dr. Mouillet, Dr. Sadovsky, and Ms. Jennings.

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 work on this project has stimulated a new collaboration with Dr. Coyne in the Department of Microbiology and Molecular Genetics at the University of Pittsburgh.

Reviewer 2:

Dr. Carolyn Coyne, a professor of microbiology and molecular genetics, has become an active collaborator with the PI and his team during the project and moving forward.

Reviewer 3:

The research involves an effective collaboration between Jean-Francois Mouillet and Yoel Sadovsky at the University of Pittsburgh. It is not apparent whether additional collaborations have been established.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

1. Might it be possible to perform placenta-specific knockdown in the mouse model using the lentiviral strategy which confines vector expression to the placenta? Would an shRNAmir for miR-424 or other targets prove useful?
2. Laser capture microdissection might have been appropriate in the mouse model to distinguish between the responses in different cell types.
3. The investigators have indicated that they have a recent study in which miR-424 was neutralized using morpholino antisense oligonucleotides, addressing the major weakness in the current study.

Reviewer 2:

The proposal is solid, overall, and helps us understand the nature of miR-424 expression in human trophoblast. However, little attention is paid to the functional role of its proposed targets (i.e., FGFR1 and MAP2K1). The proposal already suggested that although in silico target scanning revealed a miR-424 binding site in MAP2K1, this did not yield meaningful functional consequences in trophoblasts. Moreover, inasmuch as target scanning did reveal a potentially interesting site for FGFR1, nonetheless, the PI and his team did not demonstrate or attempt to demonstrate directly, an effect of miR-424 on FGFR1 function in the trophoblast. This should be addressed in the future to make relevant the observations in the proposal.

Reviewer 3:

The investigators have made tremendous progress in identifying roles for miRs in trophoblast cells and placentation. They have established the foundation for a novel experimental approach and thus significant momentum in their research. There is a high probability that the research will be rewarded with NIH support; however, in the interim it is imperative that this research project continue to receive financial support.

ADDITIONAL COMMENTS

Reviewer 2:

The strength of the proposal was the employment by the interactive team of investigators of some novel and comprehensive techniques to express miRNAs in human primary trophoblasts. Inasmuch as these cells are quite difficult to transfect with foreign DNAs, these methods may prove invaluable for this work and other projects within the institute.

Project Number: 0990105
Project Title: Epigenetic Analysis of Human Aneuploidy
Investigator: Peters, David

Section A. Project Evaluation Criteria

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

The objective of the study was to determine methylome of placental DNA from euploid and aneuploid pregnancies.

The project has met some of the stated objectives because the methylome of placental DNA were obtained. The strength is that the area of research (i.e., epigenetic regulation of development and the relationship between aneuploidy and DNA methylation) is important both for basic and applied science.

However, the core of the objective was not met because of the following reasons:

- 1) The model (i.e., samples from different pregnancies) was not the most appropriate, because the epigenome would be influenced by many other factors besides aneuploidy. Indeed, the researchers have pointed this out as well.
- 2) The methods employed were unable to produce high levels of results that were consistent with each other.
- 3) The sample sizes were small.

Reviewer 2:

The stated objectives were largely accomplished. However, bioinformatic analysis of the data collected using the Illumina HumanMethylation27 microarray platform revealed unexpected but potentially highly significant findings. The first was that significant differences existed for the apparent detection of methylation in uncultured CVS samples versus those prepared from cultured CVS samples. Secondly, the principal investigator (PI) discovered that fetal gender has a profound effect on placental DNA methylation profiles. Confirming and understanding the full significance of these two factors is critical for future efforts to characterize the methylomes of normal versus aneuploid tissues, regardless of the analytical platform used to monitor methylation.

A third unexpected finding was the lack of corroboration between the patterns of DNA methylation detected by the microarray and a mass spectrometry-based approach. This spurred the authors to develop an alternative procedure utilizing mCpG-sensitive restriction nuclease digesting in conjunction with high throughput DNA sequencing. This has the potential to enable genome-wide characterization of DNA methylation with single base-pair resolution while avoiding the known potential for false-positive results associated with microarray approaches.

Reviewer 3:

The overall goal of determining the differences in DNA methylation patterns engendered by aneuploid states in placental tissues failed. Specific Aim 1 of using a genomics approach to screen for initial changes seemed to succeed, but upon verifying these in specific tests in Specific Aim 2, they were not verifiable. These are really related aims, where verification of the genomics approach data is always linked in the same aim. In the end, no meaningful data to apply to the objective of the grant was garnered. The investigators did provide evidence that culturing of cells changes the epigenetic state of the cells and that there are marked differences between males and females. However, this data suffers from the same verification problems as the other data. The investigators have since moved to more modern approaches to this genomic analysis involving deep sequencing.

The overall weakness was the failure to reach the objective or provide meaningful advancement in the field.

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:

Significant benefits are expected through successful elucidation of the stated goals of the project; because, if the linkages between DNA methylation and aneuploidy are determined, then DNA methylation profiling could be developed as a method to determine aneuploidy and other problems in pregnancy.

This reviewer is unable to determine the full impact of the studies at this point, because of the lack of consistent and comprehensive data.

Reviewer 2:

The data obtained revealing that sample handling (cultured vs. uncultured samples) and fetal gender have profound effects on the levels and genomic distribution of CpG methylation are highly significant for this research field, and for understanding normal and pathological human development in general. With further efforts, the work is likely to have a significant impact on our understanding of the role of DNA methylation in human development.

The group intends to develop further their own approach to analyse genomic methylation profiles and apply it to the study of aneuploidy.

Reviewer 3:

The weakness of this study is that it will have no meaningful impact to the field. The failure to verify the data of the genomic analysis makes the data from the study unuseable. The investigators have since moved to more modern approaches to this genomic analysis involving deep sequencing.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

The researchers were able to do the proposed research without leveraging additional funds. The researchers plan to apply for additional funding to continue research in the proposed area.

Reviewer 2:

Although no grants were submitted prior to the final report, the PI indicates that an R21 application will be submitted in the future. This is a rational plan.

Reviewer 3:

The weakness of the study was that no additional funds were garnered. The researcher does plan on applying for an R21 grant 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:

There were no peer reviewed publications yet because the data produced were preliminary.

Reviewer 2:

No papers were submitted during the term of the project, but the group has certainly laid the groundwork for a major publication to alert the research community to the potential biases that sample handling and fetal gender can have on methylation profiling.

Reviewer 3:

A weakness of this study is that no definitive measures of success, such as peer-reviewed publications, licenses, patents or commercial development opportunities, were garnered. The author does cite possibilities of contract work through NIH, but nothing concrete was shown. The author stated that part of the final milestone would be publication of the data, but this did not materialize.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

No improvements in the institution's infrastructure were made. There were no new investigators that were brought into the institution, and no pre- or post-doctoral students were employed.

Reviewer 2:

Direct improvements to infrastructure were not proposed originally. However, the experience gained through this project is likely to have significant impact on how the core facility at this institution counsels investigators in the design and execution of microarray analyses.

Reviewer 3:

The principal investigator states no enhancement of research at the institution was garnered. This is a weakness.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

There were mentions of improved dialogues between the institution and NIH, and FDA, however, this reviewer is unable to determine to what extent the relationships were strengthened or led to any solid collaborations.

Reviewer 2:

According to the final report, discussion of their findings has elicited interest in future collaborative work with scientists at NIH-NIAID/NIHCD.

Reviewer 3:

A weakness of the study was that no collaboration outside the institution was involved.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

1. The model: This study requires a model system where only the differences in chromosome numbers can be tested. For example, identical twins, cloned mice or pigs, etc.
2. The method: There are more sensitive methods becoming available for the study of DNA methylation.

3. Data analyses: Because of the extensive amounts of data produced, a computational biologist and a systems biologist would improve the proposal.
4. Sample size: Larger sample sizes would improve the proposal.

Reviewer 2:

None.

Reviewer 3:

1. The overall weakness was the failure to reach the objective or provide meaningful advancement in the field. The main failure was in the genomics technique used. It is difficult to determine if this was inherent in the technique or part of the application of the technique in the PI's laboratory. The PI has since moved to deep sequencing based technologies, but reports he is developing them himself when these are already available from vendors. I would encourage them to use those already available and use his expertise in the biology to ensure success and that an important biological question is answered.
2. The weakness of the study was that no additional funds were garnered. The researcher does plan on applying for an R21 grant in the future. If an R21 grant is still planned, I would concentrate on attaining preliminary data that shows you can effectively produce reliable deep sequencing data.
3. A weakness of this study is that no definitive measures of success, such as peer-reviewed publications, licenses, patents or commercial development opportunities, were garnered. This is largely a result of the failure in the experiments. Getting more advise from experts in the field may have prevented this failure.
4. The PI states no enhancement of research at the institution was made. This is a weakness. Enhancement of the skills of the PI in carrying out these experiments could be listed.
5. A weakness of the study is that no collaboration outside the institution was involved. I think enlisting skilled investigators in the techniques would have enhanced the probability of success in this case. A mentor with expertise in genomic-scale analysis would have been a good plan.

Generic Recommendations for Magee Womens Research Institute and Foundation

Reviewer 1:

The authors are commended for tackling a fundamental problem impacting human development and health. With a well-designed set of experiments and an adequate amount of preliminary data, it is expected that the authors would be able to develop hypothesis-driven projects aimed at determining the nature of linkage between DNA methylation and aneuploidy.

ADDITIONAL COMMENTS

Reviewer 3:

The project failed to meet the criteria that are listed in this review. In summary, there was a failure to reach the objective of the grant, failure to advance the field, no definitive measures of success shown, and no increase in research infrastructure at the institution.

Project Number: 0990106
Project Title: Antigen Expression and Adaptive Immunity in Human
Endometriosis and Endometriosis-Related Ovarian Cancers
Investigator: Vlad, Anda

Section A. Project Evaluation Criteria

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

Strengths: The project accomplished most of its measurable objectives through two specific aims, as proposed in the strategic research plan. The design, methods and procedures were reasonably appropriate. Statistical analyses together with brief discussion and implication of the data, as well as information collected over the funding period, appear adequate.

In the report minor changes in the observable molecular profiles were found including the Cdh-11 COX-2, and Prostaglandins examination. These were not mentioned in the strategic research plan. However, these are the established markers of EMT and inflammation.

Overall, the progress and achievements are acceptable with regard to objectives of the project.

Weaknesses: A profiling of humoral immunity by measuring the MUC1-specific antibody responses in endometriosis and ovarian cancer using ELISA was proposed in the strategic plan. The status of this goal was not included in the report. Was it an unintended omission or was the goal revised? This is important because the report suggested that MUC1 is a robust tumor-associated antigen and is a potential target for cancer vaccine. However, in absence of data, this speculation will remain invalid and may be a major weakness of the project.

The number of animals to be used was not included in the Specific Aim 2 of the strategic research plan. In the first report (January 1, 2010, to June 30, 2010), tumors were induced in 7 triple Tg mice while Figure 8 of the same report shows that the project used 16 triple Tg mice and 24 double Tg mice. Again, the second report (July 1, 2010, to December 31, 2010) shows 10 triple Tg and 13 double Tg mice were used in the project. Thus, for a valid statistical inference these discrepancies need to be addressed.

Blood samples were not examined before the induction of tumors in mice. Thus, there remains a minor concern whether some of these animals may have non-tumor related pathologies that might have affected their immune status.

Reviewer 2:

The project met its stated objectives within the time frame specified. A significant amount of work went into the acquisition and evaluation of clinical specimens and development of an animal model. The work developed preliminary data and an experimental model system that could form the basis of new grant applications.

Reviewer 3:

Strengths: The main objective of the grant is to investigate the role played by the immune system in the development of endometriosis and ovarian cancer. Emphasis will be placed on determining the complex interaction between transformed epithelial and immune cells within the disease microenvironment in established animal models and patients. This is a clinically relevant topic that could lead to the development of novel strategies of cancer prevention and/or more efficacious immune therapies. The study is innovative, since it aims to be a comprehensive comparative profiling of immune factors/mechanisms in the disease microenvironment and peripheral blood.

Weaknesses: Endometriosis is a precursor for certain subtypes of ovarian cancer, such as endometrioid and clear cell tumors. However, based on the data presented, it is not clear to this reviewer whether the link between endometriosis and ovarian cancer is mediated by an enhanced inflammatory response. If this is not the case, then this type of research will not play a key role in identifying immune-based methods for cancer prevention. In addition, no data is presented to support the major assumption, which is outlined in the proposal, that endometriotic or cancer lesions should be eliminated by an intact immune system or that they develop because of a dysfunction in immune surveillance mechanisms.

Considerable progress has been made towards the original scope of the application, but it does not appear that the specific aims of the original application have been completed. The findings are preliminary and lack detail. For example, the investigation of the mechanisms of immune surveillance in the disease microenvironment and peripheral blood has not been completed.

It is worth noting that although endometriosis is a precursor for certain subtypes of ovarian cancer, no mention is made of whether the scientists attempted to collect these cases only (i.e., the number and characteristics of endometrioid and clear cell cases is not indicated). In addition, the RNA collected from FFPE was largely degraded and could not be used for a detailed and complete genetic/genomic analysis; no attempt was made to collect RNA from fresh samples. In addition, the significant RNA changes identified have yet to be confirmed at the protein level in either tissue or peripheral blood.

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 report described that the findings will not have any impact on the incidence of the disease, death from the disease, and stage of the disease (in response to item # 21 of the final report). However, the findings will improve our understanding of the immune responses against endometriosis and ovarian cancer. It will also reinforce endometriosis as a risk factor for ovarian cancer.

Reviewer 2:

The hypothesis that altered immune function is involved in the development of endometriosis and endometriosis-related ovarian cancer is at the forefront of knowledge in women's healthcare and currently an under-studied focus area. The information gained regarding cellular and molecular details of how defects in immune surveillance could be targeted for diagnostic, preventive and treatment strategies. While the majority of women have endometriosis, there is no screening assay routinely used to detect endometriosis. It is possible that either the compromised immune function contributes to both endometriosis and ovarian cancer separately or that it allows endometriosis to transition into ovarian cancer. The experimental model systems developed will help answer these questions and provide direction for development of prevention and treatment strategies. The inclusion of both laboratory-based models and evaluation of clinical specimens enhances the translation of this project.

Reviewer 3:

Strengths: The results presented, although preliminary, identify a possible link between innate immune effectors and adaptive immunity in endometriosis and endometriosis-associated ovarian cancer. In addition, a novel biomarker was identified in ovarian cancer and could have a possible role in tumorigenesis and a potential as a prognostic factor. Both findings warrant future studies to investigate this possibility. Most importantly, the preliminary data point to MUC1 as a robust tumor-associated antigen, which could be a good therapeutic target. Furthermore, the animal models that have been established recapitulate the human disease and are great *in vivo* systems for the preclinical testing of MUC1-based vaccines.

Weaknesses: The results from genetically-engineered animal models suggest that immune factors, such as MUC1, are not required for tumorigenesis. The role of MUC1 remains unclear. It is suggested, but not proven, that upregulation of human MUC1 contributes to increased tumor metastasis and EMT via Cdh-11 downregulation. For example, no statistical differences were observed between the number of metastases or the presence of ascites in MUC1-KPs compared to KPs. In addition, it is suggested that MUC-1 expressing ovarian tumors are associated with an increased accumulation of Tregs in the spleen, raising the possibility of a disequilibrium in the immune system. Is it possible that this is the result of an immune reaction due to the presence of a foreign human antigen within the murine microenvironment? This possibility is not addressed or discussed.

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:

Strength: Based on the preliminary data generated by this project, a new proposal with large-scale study has been developed and submitted for further funding to NIH (R01) and private foundations.

Reviewer 2:

One NIH grant was submitted and is pending review.

Reviewer 3:

Strengths: Results from this study will create the basis for one pending grant that was submitted to the National Cancer Institute and future grant applications for both NIH and private sector funding.

Weaknesses: Based on the preliminary data presented, the potential for future funding is unclear.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

Strength: No article based on the results of the project was published or submitted. However, the report described that two manuscripts will be submitted for publication this year (2011). Considering the length of the funding period, this is acceptable. No licenses, patents, or commercial developments based on the results of this project were reported.

Weakness: The report did not mention whether any part of the results from this project was presented (abstract) in any meeting of professional societies.

Reviewer 2:

I believe that the data is publishable and that the significant progress made by this project should be continued. The transgenic animal model may be patentable.

Reviewer 3:

Strengths: None.

Weaknesses: The project did not result in any peer-reviewed publications, patents, or commercial development opportunities. Dr. Vlad mentions that two manuscripts will be submitted in the future.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

Strengths: It provided the cost of experiments to generate the preliminary data for further funding (NIH or private foundation) for a larger-scale study.

It provided part of the salaries for the PI and the research technicians.

It helped to maintain a core facility that was also used by other investigators and thus helped the institutional research capacities and facilities.

An undergraduate received a small stipend from the project.

Reviewer 2:

An undergraduate and a graduate student, along with several post-doctoral fellows, were trained. A novel transgenic animal model was developed and a set of clinical specimens collected, both of which contribute to the infrastructure of the institution, if these resources are to be made available to other researchers. The collection of clinical specimens led to new research collaborations with clinicians within the institution.

Reviewer 3:

Strengths: The grant provided salary support for one flow cytometrist who is also providing technical support for the FACS core at Magee-Women's Research Institute and Foundation. This core facility is used by an increasing number of Magee-Women's Research Institute and Foundation scientists.

Research supported by these funds was performed by a group of scientists that included new investigators and undergraduate students.

Weaknesses: None.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

Strengths: The project yielded two collaborations: 1) collaboration with the oncologists and pathologists (University of Pittsburgh Medical Center); and, 2) collaboration with basic scientists and biostatisticians.

Reviewer 2:

There were no new collaborations made as a result of this project, however it is likely that investigators outside the institution will request collaboration with the transgenic mouse model.

Reviewer 3:

Strengths: A collaboration was initiated with clinicians, including GYN oncologists/surgeons/pathologists in the University of Pittsburgh Medical Center medical system and statisticians and basic scientists in the immunology department and the University of Pittsburgh Cancer Institute.

Weaknesses: None.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

1. Development of an immunoassay to measure the humoral immune response against MUC1 will improve the translational significance of the findings of the project.
2. Findings of the project need to be presented and published in peer-reviewed journal(s) so that investigators working in the field will benefit.

Reviewer 2:

The results have not yet been published. My recommendation is to prioritize what needs to be done in order to publish. The publication of preliminary data in peer-reviewed journals will greatly enhance the competitiveness of this project for funding.

Reviewer 3:

1. Weakness: It is not clear based on the data presented whether the link between endometriosis and ovarian cancer is mediated by an enhanced inflammatory response. If this is not the case, then this type of research will not play a key role in identifying immune-based means of cancer prevention.

Recommendation: Strong preliminary evidence needs to be developed to support the major assumption in the proposal that a dysfunction in immune surveillance mechanisms contributes to disease initiation in endometriosis and its malignant transformation to ovarian cancer. It would be interesting to prove that endometriotic or cancer lesions can in fact be eliminated by an intact immune system or prevented by vaccine-activated immune effectors in animal models recapitulating human disease.

2. Weakness: Although considerable progress has been made towards the original scope of the application, it does not appear that the specific aims of the original application have been completed. The findings are too preliminary and lack detail.

Recommendation: The study needs to include a detailed analysis of all categories of immune effectors in order to provide a comprehensive picture of immune surveillance deficiencies in both affected tissues and peripheral blood in animal models and patients. The type and number of infiltrating lymphocytes also need to be analyzed. Antibodies for T cell and B cell markers can be used to identify the makeup of immune infiltrates in the lesion microenvironment as originally proposed by the investigators.

3. Weakness: The RNA collected from FFPE was largely degraded and could not be used for a detailed and complete genetic/genomic analysis.

Recommendation: RNA needs to be collected from fresh samples to prevent RNA degradation. In addition, the significant RNA changes identified have to be confirmed at the protein level in both affected tissues and peripheral blood.

4. Weakness: It is worth noting that although endometriosis is a precursor for certain subtypes of ovarian cancer, no mention is made whether the scientists attempted to collect only these cases.

Recommendation: The authors need to include the number and characteristics of endometrioid and clear cell ovarian tumors collected, respectively.

5. Weakness: The role of MUC1 in ovarian cancer remains unclear.

Recommendation: The investigators proposed in the original application to study MUC1-specific antibody responses in endometriosis and ovarian cancer and measure the IgM and IgG anti-MUC1 antibodies in the sera of animal models and patients by ELISA. It is important that this study be completed in order to facilitate the development and investigate the efficacy of MUC1-based vaccines.

Generic Recommendations for Magee Womens Research Institute and Foundation

Reviewer 2:

While this project has not yet been published or aquired additional funding, the research has been productive in generating infrastructure needed to answer clinically relevent questions that could lead to new diagnostics, prevention and treatments for a significant health problem. My recommendation is to continue supporting projects such as this, and encourage them to prioritize what needs to be done to possibly patent and to publish their work, and then to share the infrastructure they developed with others at the institution and community.

Project Number: 0990107
Project Title: The Impact of Age on the Nematode Germline
Investigator: Yanowitz, Judith

Section A. Project Evaluation Criteria

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

The project met the stated scientific objectives only partially. The analyses of histone PTM changes in the germline over time was incomplete, and little was done to investigate the basis for the change in H3-Ser10 phosphorylation that was observed. However, the award enabled the acquisition of a confocal microscope and software that should significantly enhance the biological imaging at this institution.

Reviewer 2:

The aim of the project was to examine potential links between changes in histone variants and histone modifications and aging. *C. elegans* was used as a model system to address this question. The approach was to use antibody and immunofluorescent staining to identify histone modification and to use confocal microscopy to quantify the results. The overall result was that, apart from small changes in H3S10p levels, no significant changes in histone modifications were noted. Although the data are well-presented and justified, and were in line with the original proposal, the approach lacked the required sensitivity needed to address the main hypothesis in the proposal.

Some additional approaches were included, such as comparing lines bred from young and old mothers. Using this system, the principal investigator (PI) was able to demonstrate that mutations in certain genes (e.g., *rfs1/xnd1*) led to increased sterility in old but not young mothers. This system has the potential to unravel the role of histone modifications (e.g., H2AK5Ac) using a more robust assay (sterility) than the whole nuclei staining for H2AK5Ax originally proposed. This new approach is fully justified given the lack of sensitivity of the staining protocol.

A weakness of the experimental approach was the over-reliance on immunofluorescent staining methods, which can only resolve histone modifications at the level of chromosomes or large chromosome domains. Any changes occurring on small regions of chromatin (gene promoters, large non-coding domains, etc) will not be detected against the overall background of these individual marks. Approaches such as ChIP-seq would provide a more in-depth analysis at the level of individual nucleosomes, allowing for identification of specific genes or regions which are undergoing alterations in histone modifications during aging.

Reviewer 3:

The studies proposed were logical and justified by the background information provided, with potential pitfalls appropriately addressed. Evaluation of the submitted progress reports indicates that the PI made a conscientious effort to accomplish the experiments proposed, which unfortunately did not produce the results expected. In fact, the majority of outcomes were “negative” (no effect of age on the endpoints measured in germ cells). The PI interpreted this as either an invalidation of her central hypothesis, that chromatin modifications (epigenetic changes) serve as a driving force behind increased meiotic recombination errors in germ cells of reproductively aged females, or technical limitations in the approaches employed to evaluate changes in the epigenetic landscape in the *C. elegans* germline with age. Despite these disappointing findings, the PI did pursue an alternative strategy to generate insights into how aging affects female germline function. The PI ultimately worked with mutations in two different genes previously tied to a loss of fecundity and fertility in *C. elegans* over 30-50 generations: *rfs-1*, which encodes a DNA repair protein, and *xnd-1*, which encodes a meiotic chromatin associated protein that regulates histone acetylation. When these mutations were carried through an aged maternal germline, the fertility defects tied to each mutation were amplified compared to worms with the same mutations carried through the germline of young females. These findings indicate that changes in the epigenetic landscape of germ cells leading to impaired fertility may be cumulative across generations. Although this finding is interesting, it still does not provide insight into how aging negatively affects oocyte competence in a given generation, which was the central thrust of the grantee's work. For this criterion, the strengths include the grantee's perseverance in the face of a lot of negative data (which ultimately proved informative in terms of cross-generational observations) and the potential importance of the subject matter under investigation to human reproductive health. The weaknesses include a near-complete absence of new insights into maternal aging, partial dependence of Specific Aim 2 on successful completion of Specific Aim 1 (which did not happen) and the lack of any peer-reviewed publications based on the findings obtained.

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 has the potential, in the long-term, to allow better understanding of the impact of changes in meiotic chromosome structure on proliferative capacity.

Reviewer 2:

Currently, the project has little potential for impact on human health. Further studies using the newly developed *C. elegans* system (based on young and old mothers) may uncover new epigenetic changes linked to sterility and aging. Such results may then provide key information to inform studies in humans. No major drugs/diagnostics are anticipated from the study.

Future plans involve two areas: 1) using the newly purchased microscope to improve their study of meiotic crossover formation; and, 2) to continue the work on factors which drive germline aging.

Reviewer 3:

As acknowledged by the grantee, and as is clear from the progress reports, there is no current or future beneficial impact of this project. Considering the dollars budgeted, which are very high for the work proposed and accomplished (mainly due to the grantee's request for funds to purchase close to \$600K in equipment for confocal imaging analysis that, given all of the uncertainties of the expected outcomes, appears unjustified since the findings obtained could have arguably been achieved with the grantee's existing image analysis set-up), the likely beneficial impact of this project is not judged as reasonable. In other words, the grantee's institution has obtained a new high-end image analysis system for their core imaging facility for little, if anything, in return. In fact, one of the strengths of using *C. elegans* as a model is that it is relatively inexpensive to work with compared to mammalian model systems; yet, a project that actually costs around \$100K to perform ended up costing 7 times as much due to infrastructure investment of a questionable need for project success. This is judged as a significant weakness, as is the lack of any discoveries made, and the absence of any 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?

STRENGTHS AND WEAKNESSES

Reviewer 1:

No current or future applications that would utilize the enhanced imaging capabilities were noted in the final report. This is disappointing given the expenditure involved. Hopefully the PI or one of the twenty labs that have been trained in the use of the instrument will soon rectify this situation.

Reviewer 2:

No additional funds were leveraged, and no new grant applications are noted in the final report.

Reviewer 3:

No additional funds were leveraged as a result of this project, and no grant applications were submitted. Perhaps more concerning, and judged a major weakness, is that the PI is apparently abandoning this line of work with no intention of submitting future grant applications (see section 11B of Final Progress Report). Thus, the actual commitment of the PI to this line of study can be judged as questionable, which raises concerns over the intent of this award application (to build a program to understand the epigenetic mechanisms involved in female reproductive aging or to obtain a \$600K confocal microscope set-up for the institution's core imaging facility).

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:

According to the final report, no papers have been submitted and future plans in this regard are not described.

Reviewer 2:

No peer-reviewed publications were submitted or are planned for submission.

Reviewer 3:

This project did not result in any peer-reviewed publications, licenses, or patents (either in preparation or submitted/filed). In section 20 of the final progress report, the PI provides unclear information related to articles submitted as peer-reviewed publications – not only is the information provided unclear as to meaning, but none of it appears relevant to this specific project (cognition and MRI, lung cancer?). In any case, in alignment with the concern raised earlier regarding the grantee's commitment level to this project, it is unclear why the PI has not prepared, or indicated intent to prepare, a single publication summarizing the outcomes obtained. This is considered a major deficiency.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

The majority of the funds were used to acquire a Nikon confocal microscope, deconvolution software, and a high throughput histology processor. These represent significant infrastructure enhancements. Funds were also used to pay the PI, a student intern, and a research assistant.

Reviewer 2:

Significant improvement was made to the infrastructure through the purchase of a Nikon Confocal microscope. This was used extensively by the PI, and has been constituted as a core facility used by over 20 research groups. This state-of-the-art system will allow for significant advances in research to be made within the host institute, allowing high resolution analysis of cells. The only weakness noted is the lack of specific examples of how the microscope has been used by other groups. Specifically, publications or grant applications where this system contributed critical data.

No new investigators were brought into the project from outside the institution.

The project did allow for the training of both pre-doc and undergraduates (2), enhancing the research experience of these students.

Reviewer 3:

Clearly, the institution's capacity for research has been enhanced by purchase of the \$600,000.00 confocal microscope system using this project's funding, but the project itself did not enhance the quality or capacity for research at the grantee's institution other than provide salary support for a Research Assistant (it was not indicated if this individual was a pre-/postdoctoral student).

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

No new collaborations are noted in the final report.

Reviewer 2:

No new collaborations were noted.

Reviewer 3:

No, this project did not seed new collaborative investigations either inside or outside the grantee's institution, and did not lead to new involvement with the community.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

A disconnect exists between the rationale originally proposed for project (to investigate factors relevant to germline aging and replicative capacity) and the final report (we acquired the microscope and have performed some preliminary studies that gave inconclusive results). Given the expenditures involved in both equipment and salaries, it would behoove the PI to double efforts on the former.

Reviewer 2:

1. The majority of the funding (some \$600K) went to the purchase of the confocal system. It would be helpful to provide some solid evidence (publications, new collaborations with external labs who wish to use this, letters of collaboration etc) that the system has in fact improved the infrastructure.
2. The approach of using primarily antibody staining to look for age-related changes in histone modifications lacks the sensitivity required to address the hypothesis. Although the PI referred to this problem in the initial proposal, the experimental approach did not provide appropriate alternatives. It is recommended that Chip-on-chip or ChIP-Seq approaches are used to address this type of question.

Reviewer 3:

1. Questionable grantee commitment to the project: this could be minimized if the grantee had made an effort to: 1) prepare the findings for peer-reviewed publication; 2) leverage the project to obtain longer term funding for the work (which would reflect the grantee's commitment to this line of investigation); and 3) give some indication that this line of study will be continued after completion of this project, with details as to next steps (immediate and long term).
2. Questionable need for such a large investment in infrastructure improvement: one of the main advantages of the use of *C. elegans* as a model is related to its cost effectiveness for

performing complex genetic studies (compared with, for example, mice); however, that advantage was completely lost by purchase of a \$600,000.00 confocal image analysis set-up that was not really necessary for this project to be pursued. While the use of this system might facilitate some of the work, none of the work was dependent on its use.

3. Lack of current or future beneficial impact: although the grantee's experiments to did produce the outcomes expected, (and thus there is no clear current beneficial impact), some indication that the grantee will take the information obtained and continue to build a research program around this line of study would lend some degree of confidence that, in the future, the project will have a beneficial impact. As things stand now, the grantee has viewed the "negative" outcomes as a reason to simply abandon the work and not pursue either future grant funding or experiments to build on the results obtained.

Generic Recommendations for Magee Womens Research Institute and Foundation

Reviewer 3:

- 1) Ensure that each project submitted for evaluation represents a line of study that not only meets the guidelines of this program but also represents an area of work that the submitting investigator is committed to well past the project period. These types of opportunities should be maximized, not squandered.
- 2) Ensure that large requests for infrastructure improvement are: a) absolutely essential to project completion; and, b) preferably meet the needs of multiple applicants.
- 3) Encourage all applicants to actively involve pre- and postdoctoral students in the work proposed, which has long term benefits in terms of project outcomes even if the experiments proposed do not turn out as planned.
- 4) Encourage all applicants to prepare additional grant applications to other entities (i.e., NIH) during the project period, so that the funding provided can more effectively serve as a bridge to longer term support.

ADDITIONAL COMMENTS

Reviewer 3:

Since it is impossible to accurately predict the outcomes of proposed experiments, the lack of a significant amount of "positive" findings reported by the grantee is not viewed as a weakness since the grantee appeared to make a conscientious effort to complete the proposed studies. With that said, major deficiencies were identified throughout the project in terms of questionable grantee commitment to the project, a lack of effort to submit peer-reviewed publications reporting the outcomes, no intent by the grantee to continue the work or apply for new grants, and unclear justification for spending approximately 85% of the funds (close to \$600,000.00 of a \$700,000.00 budget) on infrastructure improvement that was probably not needed to complete the experiments proposed. In addition, there is no current or future beneficial impact of the project, now that it has been completed.