

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

1. Name of Grantee: Magee-Womens Research Institute and Foundation
2. Year of Grant: 2010 Formula Grant

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

We are cognizant of, and truly grateful for, the impact that the Formula Research Funds have had upon our research at Magee-Womens Research Institute and Foundation. In 2010F, program funds supported four projects that centered on diverse issues in reproductive biology and women’s health, ranging from embryonic development, the placenta, and cancer. We provide the following general comments regarding our management of the program award to MWRIF, and specific comments regarding the 2010F projects.

Over the past five years, MWRIF has established a system for monitoring and oversight designed to ensure scientific success, efficiency, and responsible conduct of research with the Formula Research Funds. This system begins with careful identification of projects that, while ambitious, challenging, and in their early stages of development, have real potential to launch impactful research pursuits. Each investigator’s proposal is discussed with and reviewed by the Director for scientific merit, and refined as needed. Post award, the Director conducts progress meetings with all investigators prior to the six-month and one-year marks. Special attention is given to junior investigators, who meet regularly with mentorship teams to monitor all aspects of research and professional development. Additionally, we have instituted a routine mechanism for internal review of all NIH-level grant applications, as well as grant-in-progress (GRIP) sessions in which established investigators provide key feedback on proposals in development. Administratively, Ms. Cheryl Richards, MWRIF’s Director of Grants and Contracts, provides oversight of our compliance with the program. She assists investigators with all aspects of budget preparation, monitors regulatory compliance in the responsible conduct of research, and ensures that all reports are completed in a timely and thorough manner. Furthermore, Ms. Cheryl Richards maintains detailed records of all investigators and projects funded during the life of the program, and assists the Director in identifying candidates for funding. The success of these policies is evident that, in the past four years, projects have been enthusiastically received, and hardly any reviewed projects received an “unfavorable” score.

We are grateful to the program reviewers for their thorough evaluation of the 2010F projects. Each investigator has provided a detailed response to the reviewers’ critiques in the attached document, and values the reviewer’s input in the research process. We highlight that, given the

\* Please note that for grants ending on or after July 1, 2007, grantees’ Final Performance Review Reports, Response Forms, and Final Progress Reports ***will be made publicly available on the CURE Program’s Web site.***

“seeding” focus of the one-year awards and the germination time of new lines of biomedical research, reporting measurable outcomes of success at the conclusion of the one-year term is very difficult. We believe that this is one of the reasons for the unfavorable score of project 4, which, admittedly, was novel and pioneering in nature. We are now happy to report, within our responses to the reviewers, that the projects have generated important data that have since been presented in national forums, published in quality, relevant journals and buttressed applications for new funding, with success in at least two new NIH grants (Projects 1 and 4) .

We thank the Pennsylvania Department of Health for its support of the Health Research Award program, which has been integral to MWRIF’s status as the top-funded research institute in the United States in the field of reproductive sciences and women’s health. The program has afforded our investigators the opportunity to creatively address key health issues and promote cutting edge scientific discovery under a mantle of conscientious oversight. We look forward to furthering this research to improve the health of women and their infants, in Pennsylvania and beyond.

Comments on the unfavorable score for Project 4:

Remediation plan for investigator(s) with unfavorable reviews:

We continue to make every effort to improve our science and decrease the likelihood of a suboptimal project, which might lead to an unfavorable review score. Nonetheless, we are cognizant of the possibility that a new area of research or a pioneering pursuit in an existing field might require more than one year of support before its full depth and impact become evident. We fear that this situation might have occurred in project #4 within the 2010F cluster. We believe that this project was quite successful, as it led to the foundation of a very exciting research path by Drs Simerly and Schatten. It also supported funding of an NCI grant on stem cells in cancer by this group. We generally expect that the annual report will reflect the researchers’ achievements, as well as their long-term impact on the field.

If the progress, as documented in the final report, does not meet expectations, and generates an unfavorable review, we conduct a thorough evaluation of the researcher’s performance, including items that are distinct from the proposed research. The researcher will not apply for future Health Research Formula Grants or any other state funds until a detailed remediation plan is provided, and corrective action is in place. This should include the adequate completion of the proposed experiments, which is reported to the Health Research Formula Grants Program.

**Project Number:** 1085901  
**Project Title:** Analysis of Small RNAs in the Fetal Placental Maternal Interface  
**Investigator:** Chu, Tianjiao

***B. Briefly describe your plans to address each specific weakness and recommendation in Section B of the Final Performance Summary Report using the following format.*** As you prepare your response please be aware that the Final Performance Review Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.

Reviewer Comment on Specific Weakness and Recommendation (*Copy and paste from the report the reviewers' comments listed under Section B - Specific Weaknesses and Recommendations*):

Response (*Describe your plan to address each specific weakness and recommendation to ensure the feedback provided is utilized to improve ongoing or future research efforts*):

Reviewer 1:

That a large number of different platforms of miRNA arrays were used can be viewed as a weakness. A more cost-effective method should have been utilized.

Response:

In this study, due to the initial technical difficulty with the sequencing of small RNAs from plasma samples, we assessed multiple platforms, including sequencing, TaqMan PCR cards, and microarray, to measure miRNA expression level in maternal plasma and cord blood samples (placental tissues did not pose a challenge). This negatively affected the number of high quality miRNA sequencing libraries we obtained in the study. Nevertheless, through the study, in collaboration with our sequencing service provider, Ocean Ridge Biosciences, we were able to develop an optimized protocol for small RNA sequencing from plasma samples.

To continue the study of the miRNA transport across the maternal-placental-fetal interface, based on the preliminary data obtained in this study, we applied for and received an NIH R21 grant (R21HD071707). With this new funding, using the protocol developed in this study, we were able to sequence successfully 80 miRNA libraries, including 55 from maternal plasma and cord blood samples. This new information greatly expanded our collection of miRNA sequencing data and will enable us to develop a more conclusive and comprehensive model of the transport of miRNAs across the maternal-placental-fetal interface. These data and research tools will also become instrumental for many other researchers.

Reviewer 2:

This was a very ambitious project. The amount of data collected was clearly less than anticipated. Nevertheless, the findings are interesting and important and have led to a successful NIH grant. The research environment is outstanding with a strong general emphasis on the roles of small RNAs on placenta gene expression and function. Dr. Chu indicates that he now has an interest in studying samples from pathologic pregnancies. Since placenta gene expression varies

tremendously from normal placenta to normal placenta and between areas of the same normal placenta, it may be very difficult to detect statistically significant differences between normal and pathologic placentas. Of course, the only way to find out is to do the experiments.

Response:

We thank the reviewers for highlighting this issue. As mentioned in our response to the recommendations by Reviewer 1, we have developed an optimal protocol for small RNA sequencing from plasma samples and have successfully used the new protocol to generate a large number of miRNA sequencing libraries to supplement the data we collected in this study. We realize the challenges we face in applying information obtained from this project to the study of pathological placenta samples. However, with the optimized miRNA sequencing protocol, combined with laser capture microdissection to isolate specific regions of placenta, we are better positioned to address epigenomic mechanisms underlying feto-placental development.

Reviewer 3:

1. It is recommended that there be more attention to detail about pilot research to streamline many of the technical challenges that led to the loss of critical samples that ultimately prevented the team from achieving all of their objectives.

Response: As mentioned in our response to the recommendations by Reviewer 1, through this study we were able to iron out all the technical difficulties related to small RNA sequencing that we encountered in this study and develop an optimal protocol for small RNA sequencing from plasma samples. Using the protocol developed in this study, we were able to sequence successfully 80 more miRNA libraries, which will enable us to develop a more conclusive and comprehensive model of the transport of miRNAs across the maternal-placental-fetal interface.

2. A more thorough understanding of potential pitfalls prior to initiating the meeting would have been helpful. Given the size of the budget, it is unfortunate that so much of the preliminary groundwork had to be done in the early phases of the current project.

Response: We realize that this study has a somewhat higher risk because it involves the use of the relatively new small RNA sequencing technologies to study plasma miRNA samples, which are known to challenge current quantitative techniques. Nevertheless, we have continued our project and have built on the initial investment to accumulate extensive experience in the measurement of placental miRNAs, using various platforms, including Illumina sequencing, Agilent microarrays, NanoString, and RT-PCR. This experience enabled us to develop an optimal protocol in the face of the technical challenges.

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

Response:

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

Response:

**Project Number:** 1085902  
**Project Title:** miR-210 Regulation of Mitochondria Function  
**Investigator:** Huang, Xin

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Reviewer Comment on Specific Weakness and Recommendation (*Copy and paste from the report the reviewers' comments listed under Section B - Specific Weaknesses and Recommendations*):

Response (*Describe your plan to address each specific weakness and recommendation to ensure the feedback provided is utilized to improve ongoing or future research efforts*):

Reviewer 1:

1. One minor aspect is the involvement of NDUFA4 in the ETC complex 4. The grantee should elaborate on this in the future.

Response:

We have established additional Complex IV functional assays in our lab, such as blue native PAGE (BN-PAGE) and Complex IV functional staining assay. We are currently working to further refine these functional experiments.

2. The Cancer Genome Atlas data should be mined a bit deeper for correlations with other mitochondrial targets, which may open a new direction critical for an upcoming R01 application.

Response:

One problem we had during the initial performance of this project was the lack of bioinformatics expertise in our lab. MWRI has now hired a biostatistician. He is expected to greatly expand our capacity to mine the ovarian cancer TCGA database, allowing us to gain much-needed insights into miR-210 and NDUFA4 function.

Reviewer 2:

Any weaknesses noted will be corrected with further expansion of this project with additional funding and career progression of the PI.

Response:

I plan to submit an NIH R01 grant later this calendar year or early in 2014, based on our results.

Reviewer 3:

1. More function tests may be performed in the future.

Response:

We will use our newly established Complex IV functional assays, mentioned above, to further examine miR-210 and NDUFA4 mechanisms of action in ovarian cancer cells.

2. miR-210 may target multiple gene targets. It may be better to seek other potential targets for this study.

Response:

We are in the process of performing Argonaute protein 2 immunoprecipitation (miRNP-IP) experiments. I expect to identify additional miR-210 targets in the next few weeks. Once these experiments are completed, we will functionally validate any targets that are involved in mitochondrial biology to further our understanding of miR-210's role in regulating mitochondrial function. We may expand our pursuits to other cellular metabolic functions.

3. One cell-based Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) may perform for a small amount of samples.

Response:

We currently do not have sufficient experience in single-cell RT-PCR assays. We thank the reviewer for the suggestion. We will review this technology for primary tumor samples where cell number is limiting.

4. Biostatistics tests and analysis may be performed.

Response:

As stated above, MWRI has just hired a biostatistics expert. I will work with him closely to complete the necessary analysis.

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

Response:

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

Response:

**Project Number:** 1085903

**Project Title:** Functional Analysis of the C19MC MicroRNAs in Trophoblasts

**Investigator:** Mouillet, Jean-Francois

***B. Briefly describe your plans to address each specific weakness and recommendation in Section B of the Final Performance Summary Report using the following format.*** As you prepare your response please be aware that the Final Performance Review Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.

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Response (*Describe your plan to address each specific weakness and recommendation to ensure the feedback provided is utilized to improve ongoing or future research efforts*):

Reviewer 1:

This project has made some very interesting findings and developed a useful model for defining the role of miRNAs in primary trophoblast cells. However, it is unfortunate to see that there is no follow-up plan to continue this line of research. Thus, a future plan to continue this research would be important.

Response:

We remain firmly committed to further development of this line of research. This project has actually branched out into several submodules aimed at addressing specific questions related to the role of this unique family of miRNAs. As one of the reviewers stated, the C19MC cluster is a complex genetic entity, and together with its restricted expression in human placental cells, its study poses significant challenges. One approach chosen to interrogate their function is the generation of genetic models suitable for experimentation. Several cell lines expressing transgenic C19MC miRNAs were produced and led to the finding that these miRNAs attenuate the capacity of these cells to migrate. In collaboration with Dr. Carolyn Coyne (U. Pitt, Dept. of Microbiology and Molecular Genetics), we also discovered that the intracellular transfer of C19MC miRNAs can confer resistance to infection by various types of viruses to recipient cells (Delorme-Axford E, et al. Proc Natl Acad Sci U S A. 2013 Jul 16;110(29):12048-53). In addition to modified cell lines, we also produced a transgenic mouse model that recapitulates cardinal features of C19MC expression in humans. For example, as in pregnant women, the C19MC miRNAs in the transgenic mice are primarily expressed in the placentas of pregnant females and are also detected in the maternal circulation. Several aspects of the C19MC miRNA biology are now being investigated in this mouse model, including the global effects of C19MC miRNA expression in pregnant mice, the trafficking of miRNAs between the placental-fetal-maternal compartments, regulation of expression, and resistance to viral infection.

Reviewer 2:

The major strength of this project is that it addresses critically important and fundamental questions pertaining to human placental biology. The experiments should provide important new insights into the regulation of placenta gene expression and differentiation. Another major strength of the proposal is the academic environment. Dr. Sadovsky and his colleagues at Magee-Women's Health Corporation have been interested for many years in placenta gene expression and placenta miRNAs. They have published some excellent papers in the field and have been successful in obtaining NIH grant support.

The major weakness of the project relates to the difficulties associated with *in vitro* trophoblast models. The use of primary trophoblast cells is limiting, since the cells may be difficult to transfect, and there is considerable variation in the magnitude of gene expression among placentas and among cells derived from different regions of the same placenta. This wide variation makes it difficult to detect difference between normal and pathologic placentas. Other *in vitro* models include transformed first trimester cells derived from "normal" placentas and choriocarcinoma cells. Since these are not "normal" cells, gene regulation in these cells may vary from normal due to absence of critical cofactors and epigenetic factors.

Response:

We agree with the reviewer that studies using trophoblast models are not without risks; however, we think that a careful approach combining different types of models, including transgenic mouse models, will allow us to make significant progress in the comprehension of complex biological mechanisms that are inaccessible to direct investigation for technical or ethical reasons. *In vitro* cellular models, by their reduced complexity, can be very useful in investigation of discrete molecular mechanisms; however, their scope is usually limited. Therefore, it is important to generate a variety of these models to fully describe a biological process. It is interesting to note that, despite its availability, many aspects of human placental physiology remain poorly understood.

Reviewer 3:

1. Finish the functional analyses of C19MC cluster miRNAs in HTR-8 cells using proliferation, migration and invasion assays.

Response:

We agree with the reviewer that these assays are essential in the characterization of our modified cell lines. We have completed a series of experiments, including various proliferation (MTS assay, BrdU incorporation), apoptosis, migration (wound-healing assay), and invasion (gelatin zymography, transwell migration) assays. The result of our investigation is that expression of the C19MC miRNAs does not significantly impact cell proliferation or apoptosis, but does clearly affect the migration/invasion potential of these cells. Results from these functional assays also support the results of our microarray analysis and pathway analysis, which showed that several genes associated with "cellular movement" were significantly altered in the C19MC-expressing cells.

2. Recommendations: More thorough analysis of functional aspects of C19MC genes using in silico and bioinformatics tools; refinement of transduction tools for primary trophoblast cultures; and, analysis of the cellular localization of miRNAs from the C19MC cluster.

Response:

We completely agree with the reviewer and are currently engaged in the active development of these approaches. For example we recently obtained encouraging results in the overexpression and depletion of specific miRNAs in primary trophoblasts using lentiviral vectors. In addition, we have initiated a project based on a PAR-CLIP (photoactivatable-ribonucleoside-enhanced crosslinking and immunoprecipitation) approach, in combination with next-generation sequencing and computational analysis, with the goal of identifying the C19MC miRNA target repertoire in trophoblasts. Regarding their cellular localization, we have not been able to produce clear and unambiguous staining of C19MC miRNAs by *in situ* hybridization despite multiple attempts using various conditions as well as different probes against several species. Because we successfully used the same method for several non-C19MC miRNAs, we do not know whether this failure can be attributed to different intrinsic biochemical properties of these miRNAs or whether they may be present in cells with a specific subcellular localization that make them inaccessible to the probes in the conditions tested.

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

Response:

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

Response:

**Project Number:** 1085904  
**Project Title:** Microtubule Post-Translational Modifications and  
Centrosome Dynamics During Mitosis in Normal and Cancerous Cells  
**Investigator:** Simerly, Calvin

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Reviewer Comment on Specific Weakness and Recommendation (*Copy and paste from the report the reviewers' comments listed under Section B - Specific Weaknesses and Recommendations*):

Response (*Describe your plan to address each specific weakness and recommendation to ensure the feedback provided is utilized to improve ongoing or future research efforts*):

Reviewer 1:

The investigators made some insights into differences in centrosome protein parameters that may be useful and could be publishable outside the tumor field, perhaps in the fields of mitosis or centrosome biology. There are, however, several issues with the proposal and the investigator that could have been identified in a pre-funding review.

Response:

We appreciate the helpful, constructive feedback from the three reviewers, which we are incorporating into our current program. Also, we are proud that this work has been leveraged into a 5-year award from the National Cancer Institute (Stem Cells in Cancer; CA163168 for over \$1.6 million). The detailed response to each comment follows.

1. There is a serious lack of statistical significance in the work. Statistical methods must be used for all assays including the intensity of centrosome fluorescence, the mitotic index, centrosome counts, centrosome segregation, centrosome splitting, centrosome movement, etc. Sufficient numbers were not utilized in the project in such assays as counts of cell number, fluorescence intensity, as above. Recommendation: Statistics are required to obtain significant values, as well as standard deviations, p values, etc., which are essential for mathematically, independently and unambiguously expressing the validity, accuracy, reproducibility and interpretability of the results. A number of different statistical methods can be used for different data outputs.

Response:

We agree that a thorough statistical analysis would have significantly strengthened our findings. A number of observations were described as preliminary findings, to be investigated further with additional time and funding. Nevertheless, we did provide a measure of the number of

observations to reinforce our summarized preliminary findings. As we stated, once these observations are statistically confirmed, the findings will be prepared for publication.

2. Problems in the research design are reflected in weak data and weak readouts of the data. Recommendation: The research design can be strengthened by building into each aim discussion of high-quality images, strong quantification, statistical analyses (including the appropriate type of statistics used for certain applications), cell numbers, numbers of times experiments will be done, discussion of duplicate or triplicate numbers for each experiment, and other organizational features and methods that will be implemented in future studies.

Response:

We agree that the results presented are not yet finalized at the level the reviewer suggests we should have achieved. Our view of this 1-year proposal is that it should serve as a valuable tool to define and investigate interesting scientific questions while trying new methodologies and protocols for testing our hypotheses. The funding has been clearly instrumental in generating new leads. We agree that stronger statistical analysis, numbers of times experiments will be done, and discussion of duplicate or triplicate numbers for each experiment would be critical to a final study (see also comment above).

3. The study utilizes only one normal and two cancers cell lines from which conclusions about cancer are drawn. The cell number is inadequate for this analysis as is the interpretability of the data. Recommendation: In order to obtain robust and significant results on differences between cancer cells and normal cells, cells from many different tumor types must be used. Normal cell types that match the tumor cell type must be tested. For example, prostate cancer cells should be matched with normal prostate epithelial cells. The same is true for other cancers. Alternatively, one could limit the conclusions to only the cell types examined, although this weak data would be difficult to publish.

Response:

We agree, if this study were to be a final report on microtubule post-translational modifications and centrosome dynamics during mitosis in normal and cancerous cells. In a 5-year study, this observation is certainly a relevant recommendation, but performing a pilot study on “many different tumor types” could not possibly be accomplished within the constraints of this funded period. We chose two cancer lines (NCI H292 and MCF7) that are well described in the scientific literature in a variety of cancer studies, and compared these against a relevant non-cancer cell line.

4. There is a lack of rationale for many aspects of the study; and the investigator is an expert in fertilization but lacks experience in cancer research. Recommendation: It is important to discuss why experiments will be performed. Be sure to accurately define and record results of published work that sets up a foundation for the study by citing papers that use similar research strategies, methods, assays, statistics, etc. This not only puts the work in perspective but acknowledges others’ work. Discuss the novelty of the work, as well as its potential significance and impact. One can draw on others’ work to support the

rationale. Without good rationale and a solid overall plan, the study can lose focus and become confusing. In addition, without rationale for the choice of aims, assays, cells types and numbers, etc., the reasoning for the aims can be misconstrued.

Response:

We appreciate the comments and recommendations, and we will work hard to better define experimental rationale, solidify our research plans, and cite the appropriate literature for future reviewers.

5. There are issues with the quality of data in the study. Recommendation: Low image quality of centrosome staining can be rectified using higher resolution microscopy, optimized fixation methods, identification of better antibodies (with lower background), and extracting cytoplasm (and associated background) with mild detergents.

Response:

We are confused by the reviewer's comments on data image quality. As stated in our protocols, images were taken on a laser scanning confocal microscope and presented after working RGB images up in Photoshop software, a standard in the industry utilized throughout the scientific field for data presentation in numerous publications. Perhaps the reviewer received a lower quality read out of the original report. A number of different protocols for immunocytochemistry, including the recommended detergent extraction, were utilized in this study, and the data are presented in the clearest possible resolution. We have expertise in these techniques, as proven in our publication track record.

Reviewer 2:

1. The rationale for the study is unclear. How does this study fit with what is currently known? It would be helpful to cite literature describing what is known about cancer and noncancer centrioles.

Response:

We agree and apologize for not providing a more detailed background and rationale for each question and for not appropriately citing relevant literature in our final report. Some of this literature was cited in the original application but failed to be included in the year-end report. We will clearly provide credit to prior publications when the manuscripts related to this work are out.

2. The interpretation of the FRAP studies seems to be incorrect. More detail about these studies is needed with a clearer explanation of why the PI concludes that the centrioles in cancer cells are more stable.

Response:

We apologize for the lack of clarity. We did not state that centrioles in cancer cells are more stable than controls as stated by the reviewer. Rather, we wrote: "These observations suggest that centrosomes in cancer cells may be more unstable than noncancerous cells, with centrin localization easily altered during interphase or mitosis in the centriole lumen."

3. The drug treatment studies are not interpretable. The studies should be done at equitoxic concentrations and more than one concentration.

Response: We agree that more replicates using the employed drug inhibitors over a range of various concentrations will be needed to strengthen these pilot observations and prior to publication of any final results. The studies possible over the 1-year funding period were not sufficient for a robust and impactful publication.

4. It is unclear whether this work adds anything new to the field. The conclusion that HDAC6 inhibition is a novel approach to cancer treatment does not seem to be supported by the PI's work or the current literature. The PI should explain the rationale for the work and the conclusions in the context of what is known.

Response:

We agree that a more detailed rationale and contextual analysis of what is known as it appears in the published literature would have given more support to our preliminary findings. As we extend and confirm these results, we will incorporate these suggestions to improve the clarity of our findings. With the 1 year of funding for this project, we believe we have made a set of preliminary observations that will become instrumental as we advance our research in this field.

Reviewer 3:

1. It is a weakness that no papers have been submitted or published. Recommendation: The principal investigator is encouraged to get some papers submitted soon.

Response:

We entirely agree, and as soon as our preliminary observations are confirmed in studies that are conducted beyond the initial funding period and can be extended with relevant statistical numbers, we will publish our findings in a peer-reviewed journal.

2. The absence of any details on the new National Cancer Institute resources and new collaborations brought into the Commonwealth is a weakness. Recommendation: The principal investigator should provide those details in a revised report.

Response: We appreciate the reviewer's comments and are delighted to report that these pilot funds helped us compete successfully for NCI sponsorship: We received a 5-year award from the National Cancer Institute (*Stem Cells in Cancer*; CA163168 for over \$1.6 million).

3. The absence of any involvement of students or post-doctoral fellows is a weakness. Recommendation: The principal investigator should engage students or post-doctoral fellows in the future projects.

Response:

We appreciate the reviewer's comment, though funding to engage students and post-doctoral fellows is not easily feasible under the current guidelines and conditions. Nevertheless, we will attempt to meet these recommendations in the future.

4. It is a weakness that no new collaborations were started with researchers outside the institution and no new researchers brought into the institution to help carry out this research. Recommendation: The principal investigator should engage in more collaboration.

Response:

We will endeavor to improve outside collaborations if future opportunities and funding are available. We also hope to leverage our new grant, cited above.

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

Response:

We appreciate the insightful comments of these three reviewers and will strive to meet their constructive criticisms in future proposals. We will clearly indicate our study rationale for all proposed Aims and Questions and will provide statistical analysis and proper citation of relevant literature to provide the contextual foundations for our proposals. We are hopeful that some of our findings can be refined and further tested such that confirmation of the data derived in these pilot projects can lead to impactful publications in peer-reviewed journals. We will also clearly state how these monies are leveraged to provide new outside collaborations and involvement of post-docs and student scientists in our research.

We would like to add that we continue to make every effort to decrease the likelihood of an inadequate research project, which might lead to an unfavorable review. Indeed, in the past 2 years, our reviews have been favorable to outstanding. We are aware that, at the end of a 1 year grant, an investigator might not be able to demonstrate the full impact of the research. This probably reflects the pioneering nature of the project and the intent to have a high-impact manuscript that might not be completed within the 1 year of funding. We therefore expect that the report will reflect the achievements and their long-term impact on the researcher's career. As this project generated an unfavorable review, we conducted a thorough evaluation of the researcher's performance, including items that are distinct from the proposed research. These are outlined in our general grant oversight process.

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

Response:

We appreciate the pilot sponsorship and remain indebted to the Commonwealth. We also are pleased to report that this work has been leveraged into a 5-year award from the National Cancer Institute (Stem Cells in Cancer; CA163168 for over \$1.6 million), which will help to train and mentor our most promising scientists in this emerging field.