

# **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 (1.84)

#### **Project Rating:**

<b>Project</b>	<b>Title</b>	<b>Average Score</b>
1085901	Analysis of Small RNAs in the Fetal Placental Maternal Interface	Favorable (1.67)
1085902	miR-210 Regulation of Mitochondria Function	Outstanding (1.33)
1085903	Functional Analysis of the C19MC MicroRNAs in Trophoblasts	Favorable (1.67)
1085904	Microtubule Post-Translational Modifications and Centrosome Dynamics During Mitosis in Normal and Cancerous Cells	Unfavorable (2.67)

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

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## ***Section A. Project Evaluation Criteria***

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

### **STRENGTHS AND WEAKNESSES**

#### Reviewer 1:

- The project met the stated objectives.
- The research design and methods were adequate in light of the project objectives.
- The data generated is in line with the original proposal. However, the data generated cannot address the questions fully due to difficulties in some analyses.
- The explanation given for changes made to research protocol is reasonable.
- Sufficient data and information were provided to indicate or support the fact that the project met its objectives or made acceptable progress.
- The data and information provided were partially applicable to the project objectives listed in the strategic research plan.

#### Reviewer 2:

The project met most, but not all, of its stated objectives. Several technical problems unfortunately prevented the PI from obtaining as many reliable libraries as anticipated using a small RNA sequencing approach. Consequently the PI switched to a different commercial laboratory that successfully constructed and sequenced several libraries but still less than originally anticipated. Nevertheless some interesting results were obtained that nicely support the overall hypothesis of the project.

#### Reviewer 3:

In general, the project met some of the objectives proposed in the original application. They developed several platforms to analyze miRNA expression and transport in the human placenta (at term) and maternal and fetal blood. The group also developed statistical models to evaluate their data.

The initial recruiting efforts to obtain blood and tissue specimens were impressive. However, many samples were lost due to follow-up and technical difficulties.

In many respects, however, the final report is disappointing in that several methods had to be used to isolate RNA and measure the miRNAs in the various compartments; and then they had challenges in finding statistical significance using modeling protocols.

The final sample sizes were very small and made statistical evaluation difficult.

It would have been helpful in the final report to emphasize the initial objectives of the project. In the current form, the project appears to be strictly technical and pilot in nature, though the original proposal outlined fairly well the overarching goals of the work.

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

## ***STRENGTHS AND WEAKNESSES***

### Reviewer 1:

In addition to the biological significance of miRNA in regulating placental development and function, the significance of the study is the potential of identifying miRNA markers in maternal blood for non-invasive diagnosis of pregnancy complications. If this goal can be achieved, the dollar amount budgeted is very much reasonable.

This project has potential and will need the results of the next phase studies (which were in the investigators' plan) to determine the significance.

### Reviewer 2:

The central hypotheses of the project are that small RNAs are transported across the maternal placental fetal interface that are critical for the regulation of gene expression and that abnormal transport of these small RNAs may be important in the pathogenesis of several conditions of pregnancies, such as preeclampsia. These are important, novel and innovative hypotheses that are likely to provide exciting new information that may lead to new therapies for these pathologic conditions. The PI indicates that he recently received a new R21 grant from NIH that will permit him to continue these studies. It is highly unlikely that he would have been successful with his NIH application without the supporting data generated by this project.

### Reviewer 3:

In large measure, this was a feasibility study, though the initial intent was to evaluate miRNA transport among compartments. In this sense, some important data were gleaned. For example, the team has evaluated several methods of miRNA quantification. They have eliminated some methods, especially for plasma-derived miRNAs, but some of the methods have been used successfully by other groups.

These sorts of experiments, even in the pilot stages, can be very costly. So, in general, it was not an unreasonable budget.

However, given the relative paucity of evaluable data and the impossibility of establishing strong statistical modeling validation, the return on investment was not substantial.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

The investigators have obtained an NIH R21 grant based on the data generated and are currently working on this project.

Reviewer 2:

As mentioned above, the PI recently received an R21 grant from NIH.

Reviewer 3:

Some additional funds appear to have been used to support some of the team members. An R21 grant from NIH was applied for and received during the course of the project to continue the studies.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

Two papers are planned, but none has been submitted for publication yet. Also, no licenses, patents, or commercial development opportunities have materialized to date.

Reviewer 2:

The investigator indicated that no publications have resulted from this project to date. The progress report indicates that one or more publications are in process. A check of PubMed indicates that the project is acknowledged in one recent paper.

Reviewer 3:

No publications or abstracts are specifically listed. But, within the biosketch of the PI, there do appear to be some related creative works generated during the grant period.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

The strength of the biostatisticians (PI and his associates) seems to have helped other investigators.

Reviewer 2:

The project enhanced the quality and capacity for research at Magee-Women's Health Corporation. The research is a logical extension of Dr. Sadovsky's work on placenta miRNAs. Students did not participate in the project, but a new investigator was recruited from the University of Southern California (USC). Since the project relies on considerable expertise in bioinformatics, the additional data analysis generated by this project led, at least in part, to the recruitment of two new individuals trained in bioinformatics.

Reviewer 3:

No infrastructure improvements were made. Some new researchers were brought to the project. No trainees were listed on the project.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

This is not clear.

Reviewer 2:

The PI indicated that Dr. Sadovsky is exploring the possibility of a collaboration with the Institute for System Biology in Seattle, WA for new methods to analyze data generated from small RNA studies.

Reviewer 3:

No specific extramural collaborations were forged during the project.

## ***Section B. Recommendations***

### **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

#### 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.

#### 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.

#### 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.
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.

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

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## *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 has certainly met most of the stated objectives, and the investigator has applied a diverse panel of methods that were very well suited to address the goals. He provided enough information for me to understand the progress, and I can clearly see the road to a high-caliber paper in the near future (in line with what the investigator states). Dr. Huang used a large number of ovarian cancer cells, therefore his findings are of a general nature, at least in ovarian cancer.

#### Reviewer 2:

Strengths: The major strengths of this project were that the objectives were clearly stated from the start and the research designed to test the central hypothesis followed a logical stepwise manner. Consequently, the project met its stated objectives as follows. First, the project indicated that despite losses in miR-201 copy number, miR-210 expression levels were generally elevated in established ovarian cancer cell lines so that copy number was not related to miR-201 expression levels. Secondly, NDUFA4, a subunit of the mitochondrial electron transport chain (ETC) complex I that is repressed by hypoxia, was identified as a target of miR-210 such that increased expression of miR-210 was associated with decreased NDUFA4 expression. A miR-210 ‘mutant’ failed to affect NDUFA4 expression, and knockdown of miR-210 could partially rescue NDUFA4 expression, indicating the specificity for miR-210 to regulate NDUFA4 expression.

Consequently, increased miR-210/decreased NDUFA4 expression contributed to cellular glycolysis as noted by decreased mitochondrial respiration, oxygen consumption and ATP production. Lastly, histologic and immunohistochemical studies using 60 primary ovarian cancer specimens localized NDUFA4 expression outside of hypoxic tissue regions as would be expected. However, since there was insufficient tissue available to measure miR-210 expression levels by laser capture microdissection within the same samples as used for NDUFA4 immunohistologic studies, the PI used recently published ovarian cancer TCGA (The Cancer Genome Atlas) data to examine miR-210 expression levels in tissue specimens. In agreement with the cell culture studies, elevated miR-210 expression levels were found in ovarian cancer specimens. Therefore, the project successfully employed a number of techniques to measure the relationship between miR-210 expression levels with hypoxia such that the techniques were

supportive and complementary. The data collected were in line with the proposed research and supported a role of NDUFA4-dependent miR-210-mediated ovarian cancer hypoxia. These data are in agreement with previous studies suggesting a role for hypoxia and drug resistance in ovarian cancer and support, then, the possibility of targeting miR-210 in ovarian cancer. In summary, the data collected were developed sufficiently and were applicable to address the objectives.

Reviewer 3:

Generally speaking, this project has met its stated objectives.

In Specific Aim 1, they found that miR-210 is induced in most ovarian cancer cell lines under 0.5% oxygen for 24 hours treatment. However, from this report, it is not known how many biological replicates have been done for each cell line. More detailed methods and data analysis may help to understand the results. Biostatistics tests may need to be performed before making any conclusion.

In Specific Aim 2, they found NDUFA4 is a novel target for miR-210. Is this the only target they identified for miR-210? There is a potential for several miRNA targets for each miRNA. Biostatistics tests may be needed for this specific aim.

In Specific Aim 3, they found NDUFA4 is localized outside of hypoxic regions. They may adopt one single cell-based RT-PCR for analysis gene expression.

***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 is not envisaged to impact health in the near future. However, it expands our knowledge of a highly biologically relevant player in many tumor types, including ovarian cancer. The pair miR-210- NDUFA4, based on the work performed by the grantee, is emerging as a viable metabolism modifying pathway that may conceivably impact tumor prognosis and response to therapy.

The candidate intends to apply for a National Cancer Institute grant based on his data, which is a highly realistic goal.

Reviewer 2:

Strengths: Given the poor therapeutic options currently available for ovarian cancer patients, especially those with drug-resistant disease, there is a need to develop novel therapeutic targets as well as understand the molecular mechanisms by which such targets mediate poor outcome. While such previous studies have focused mostly on identifying molecular signatures different among drug-sensitive and drug-resistant specimens, this project examined and expanded the possibility of identifying novel therapeutic candidates by studying the expression levels of and consequences of miR-210 for hypoxia in ovarian cancer. Therefore, the results of this project

could eventually have significant clinical impact for improved therapeutic efficacy with a foundation in personalized medicine. Not surprisingly, future plans include further validation and investigations into the molecular mechanism(s) by which miR-210 regulates glycolysis.

Weaknesses: The only minor weakness found is that the overall small sample size used will need to be enlarged for future validation and mechanistic study.

Reviewer 3:

This project generated some preliminary useful data for future study. Investigating miR-210 function in ovarian cancer and mitochondria under hypoxia condition will help to understand the related disease. miR-210 may also serve as a novel biomarker for related health issues.

***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 candidate has obtained a highly competitive American Cancer Society grant. This is a great achievement, since the American Cancer Society payline is extremely low, especially over the past few years. Dr. Huang will be in a great position to apply for an NIH /National Cancer Institute type of grant using these data.

Reviewer 2:

Strengths: With this current support, the PI was able to apply for and garner a significant American Cancer Society grant which started on 07/01/2012 to expand studies on miR-210 regulation of glycolysis. The PI is also planning to apply for additional NIH funding following publication of the current data on miR-210.

Reviewer 3:

The PI already sought \$720K in funding from the American Cancer Society. They also plan to submit an NIH grant application.

***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 manuscript(s) should come in the near future, since the data are solid and novel. The investigator states his intention to submit soon.

Reviewer 2:

Strengths: The researchers are planning on preparing the data obtained from this project as a manuscript for publication.

Weaknesses: A weakness noted is that there is no evidence of public disclosure of the data to date, even as a poster presentation at a scientific conference; although, this should be tempered by the relatively short project time frame. Also, there is no indication that any commercial development opportunities have been initiated.

Reviewer 3:

No paper has been published from this project; however, the PI is planning to submit a manuscript soon.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

The funds were critical for the grantee to maintain his personnel. Without these funds, the American Cancer Society grant likely would not have materialized.

Reviewer 2:

Strengths: A major strength of this project was setting the stage for this institution to enter the research field of cancer metabolism. This is an area of new and growing interest, so Dr. Huang and colleagues are poised to contribute novel findings regarding the role of miR-210 for cancer glycolysis with the potential for development of new therapeutic targets for ovarian cancer. Likewise, this current study supported a post-doctoral fellow, Dr. Zuo, thereby contributing to the training of our next generation of researchers.

Weaknesses: A minor weakness is that no funds associated with this project were used to support pre-doctoral students, thereby failing to contribute to the early training of the next generation of researchers.

Reviewer 3:

This project has expanded the PI's research scope to include cancer metabolism research. One post-doctoral fellow (100%) and one technician (50%) were involved in this project.

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

### **STRENGTHS AND WEAKNESSES**

#### Reviewer 1:

Dr. Huang has established new collaboration within his institution. His access to oxygen consumption rate measurements (via Seahorse XF instrument) is critical for his future success.

#### Reviewer 2:

Strengths: A significant benefit of this project was the development of a new research collaboration between the PI and Dr. Ben Van Houten at the University of Pittsburgh Cancer Institute. This allowed the PI access to expertise in tumor hypoxia biology as well as to that institution's Seahorse system to perform mitochondrial-based analyses. In this way, an additional collaborator and research site were added to the current study, thereby expanding the penetration of the tumor hypoxia studies throughout Pennsylvania.

#### Reviewer 3:

The PI has established collaboration with Dr. Houten at the University of Pittsburgh Cancer Institute to expand their research using the Seahorse system.

### ***Section B. Recommendations***

### **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

#### 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.
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.

#### Reviewer 2:

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

#### Reviewer 3:

1. More function tests may be performed in the future.
2. miR-210 may target multiple gene targets. It may be better to seek other potential targets for this study.

3. One cell-based Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) may perform for a small amount of samples.
4. Biostatistics tests and analysis may be performed.

### **Recommendations for Magee-Womens Research Institute**

#### Reviewer 2:

The institution should continue to support the research efforts of young investigators, since this study has expanded the research focus and potential for this institution.

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**Project Number:** 1085903  
**Project Title:** Functional Analysis of the C19MC MicroRNAs in Trophoblasts  
**Investigator:** Mouillet, Jean-Francois

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### *Section A. Project Evaluation Criteria*

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

#### **STRENGTHS AND WEAKNESSES**

##### Reviewer 1:

- The project has met the stated objectives.
- The design and methods are adequate.
- The data obtained are in line with the original protocol, although some results are not achievable as planned.
- No changes made to the research protocol.
- Sufficient data and information were provided to indicate or support the fact that the project met its objectives or made acceptable progress.
- The data and information provided were applicable to the project objectives listed in the strategic research plan?

##### Reviewer 2:

Dr. Mouillet has made excellent progress in meeting the goals of this project. The research design and methods were adequate, and the data were developed in line with the original protocol. The few changes made in the protocol were well justified.

##### Reviewer 3:

The project met some, but not all, of the stated objectives. Aim 1 sought to inhibit the C19MC miRNA cluster in human trophoblasts and examine the functional consequences. Aim 2 used a complementary approach by exogenously expressing the C19MC miRNA cluster in HTR-8 cell line that does not typically express miRNA genes from this genomic cluster.

Aim 1 showed some interesting yet incomplete results. While the group was able to inhibit several of the 19MC miR genes, they did not detect any overt phenotypic alterations (e.g., hormone secretion or differentiation). It is not clear if any of the miRNAs encoded in the C19MC cluster target hCG or other differentiation genes that would have been presumed functional targets.

Aim 2 was able to show ectopic expression of C19MC miRs in HTR-8 cells using a BAC vector system. They then used microarrays to analyze gene expression in C19MC transfected cells and controls transfected with a C19MC-deleted BAC construct.

Although many genes were either up or down regulated, only a few were authenticated by quantitative reverse transcription polymerase chain reaction (qRT-PCR).

The research design and methods were appropriate.

Changes were made to the methods during the progress of the work. Though a bit disappointing, the changes were appropriate and reflected sound alternative strategies.

***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 proposed studies for determining the role of miRNAs (C19MC family) in regulating trophoblast functions are significant, since this family of miRNAs is expressed with high levels in the placenta. The proposed studies may find pathways for epigenetic regulation of placental development under normal and disease conditions.

This study is still in the early stage of transforming into clinical usage.

There were no 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.

There are no major discoveries, new drugs and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project.

The future plans are: With 46 genes producing highly related miRNAs, the C19MC cluster is a complex genetic cluster. Therefore, multiple approaches will be necessary to capture the extent of its activities. They developed a cellular model that is more amenable to experimentation compared to primary cells. Further analysis of these cells is required to better characterize and buttress conclusions. A mouse transgenic model was also recently established and is being analyzed in detail. These models will be used to carefully dissect the function of the different units from this family of miRNAs.

#### Reviewer 2:

This project is original, novel and innovative and will provide exciting new insights into the molecular biology of the human placenta. Dr. Sadovsky's group at Magee-Women's Health Corporation has made many important contributions relating to placenta miRNAs, and this project is a logical extension of earlier work from the group. The findings may eventually lead to new insights into the pathogenesis of many pathologic conditions of pregnancy characterized by abnormalities in placental morphology and function. Since placenta miRNAs have been a major focus of Dr. Sadovsky's lab, there is no doubt that future studies relating to this project are planned. However, Dr. Mouillet does not provide much information about these plans. He

indicates that additional funding will be sought, but it is unclear whether he will be the principal investigator on new grants relating to the current project.

Reviewer 3:

The PI was a bit over-zealous in stating the important clinical significance of the work.

However, miRNAs are increasingly recognized as being crucial for the ultimate expression of the differentiated genome in nearly all cells. The Pittsburgh group is among the first to appreciate the role of miRNAs in placental functions. The study of the C19MC cluster, given its unique expression among primates and high expression in the placenta, is scientifically logical and interesting.

The budget was reasonable for the proposed work.

Future plans include the further functional analysis of C19MC cluster miRNAs in HTR-8 cells. These studies appear to include study of cell migration and invasion.

***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 leverage additional funds and did not submit any additional grant applications as a result of this project.

Reviewer 2:

The investigator indicates that there are plans to apply for additional funding, but details are not provided.

Reviewer 3:

No additional funds were proposed nor leveraged in a direct way, although other resources from other grants (primarily by Y. Sadovsky, Co-I) may have been used to cost-share technical salaries.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

There are no publications to date, but submission is planned. They also have submitted a patent entitled, "Primary Human Trophoblasts and the Transfer of Viral Resistance," which is currently under review.

Reviewer 2:

No peer reviewed manuscripts are listed. A review of publications by Dr. Mouillet in PubMed, however, lists several excellent published manuscripts with Drs. Sadovsky and Chu (another recipient of a Pennsylvania grant) as well as a comprehensive review article. There were no patents or licenses indicated. A statement indicates that publications from this project are planned, and at least one published paper acknowledges some support from this grant.

Reviewer 3:

Disappointingly, no publication resulted from the work, although a manuscript is planned. But, this was stated in a previous interim report. No meeting abstracts appear to have resulted directly from the work.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

The project did not enhance the quality and capacity for research at the grantee's institution.

Reviewer 2:

There is no doubt that the project enhanced the quality and capacity for research at Magee-Women's Research Institute.

Reviewer 3:

The project was molecular biology intensive, taking into account the expertise of the PI. This is likely to have enhanced technical capabilities within the host institution.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

The project did not lead to collaboration with research partners outside of the institution or new involvement with the community.

Reviewer 2:

This is not stated. A new post-doctoral fellow was recruited to work on the project.

Reviewer 3:

Collaborations with scientists from the main campus of the funded institution were listed.

## ***Section B. Recommendations***

### **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

#### Reviewer 1:

Weakness: 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.

#### 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.

#### Reviewer 3:

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

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**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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### ***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 generated data for most of the objectives.

In many instances the research design and methods were adequate.

The data were developed to a stage where the PIs could draw soft conclusions about the results that are preliminary but require further rigorous experimentation. As it stands, the work is largely descriptive and correlative. Additional studies are required to show that the observed changes between cancer and normal cells are meaningful. As presented, the work is not publishable and would require significantly more experimental work and in-depth analysis to be acceptable for publication.

In its current form, the data provide marginal support for the objectives. Significant additional work and better assays, imaging and statistics would be needed to show acceptable progress toward the objectives.

The data were applicable to the project objectives.

Weaknesses: The objectives were often not adequately met due to issues associated with lack of appropriate numbers of cell lines, low image quality, lack of quantitation and omission of statistical analysis. Statistical analyses are mandatory for generating high-profile, robust data. The lack of statistical analyses is a major weakness of the study, since high-impact journals require such information for publication. Omission of this essential component of scientific research adversely affects the overall quality of the data and its impact. Another major weakness of the project is that the deficiencies discussed above make it difficult to interpret the data accurately, which limits the conclusions that can be drawn from the study.

In general, there were many weaknesses with the research design, methods and interpretation. For example, the lack of robust imaging and quantitation in the research design affected the quality of the data. In addition, the investigators chose to use only a few cell lines in their research design (one normal, two tumor) for all the assays performed (n=11). This seriously

limits their ability to make strong and robust statements about the work and data interpretation. To truly claim that there are differences between cancer and normal cell lines requires a large cohort of cell lines from different tumor types with matching normal cells for each tumor type (breast, colon, prostate, ovarian, etc.). As presented, the number of cell lines used was woefully inadequate. Thus, robust conclusions cannot be drawn from the limited data that compares cancer versus normal cells. Furthermore, there is no evidence shown or discussed that would have the reviewer believe that the data from the few lines used are representative of numerous other cancer-derived and normal cell types. The problematic use of few cell lines is again demonstrated by the data provided, where the PIs show obvious and significant heterogeneity in some of the assays even between the two different tumor cell lines, diminishing the ability to interpret the data.

Another recurring and serious weakness is that the rationale for the use of the particular cell lines chosen for the study was unclear. This aspect of the research design precludes accurate interpretation of the data and limits the ability of the primary investigators (PIs) to publish their work in medium to high-impact journals.

Another major weakness of the study is that the data were not sufficiently developed to address the aims. This left the reviewer to assess a set of weak data that was difficult to interpret. Although results for the objectives were obtained in the project, there were major issues with data robustness, experimental design, interpretation of results, and data development. This diminished the overall quality and impact of the study. In addition, the data does not enable development of strategies for cancer diagnosis, prognosis or therapy. For example, there is no clear pathway from differences in recovery of, for example, centrin fluorescence in fluorescence recovery after photobleaching (FRAP) experiments (Figure 6) to the next logical step toward cancer. It is unclear how differences in turnover of centrin at centrosomes will be used to understand cancer progression. This is true for changes in centrosome protein levels, mitotic index, centrosome numbers, centrosome splitting and most of the other assays. How do any of these inform cancer treatments or cancer progression (cell viability, cell death, cell proliferation, and metastasis)? Even if this data is not required for this study, it is incumbent upon the investigator to provide a clearly articulated scenario for putting this data to work in strategies designed to focus on human cancer.

There were several weaknesses with the data provided as preliminary results for the study. For example, this reviewer believes that the most obvious take-home message in Table 1 is that protein localization is nearly identical between cancer and normal cells, the opposite of that predicted. In Figure 10, visualization of centrioles stained for acetylated tubulin is not compelling under the conditions used. No quantitative analyses were provided to support the PI's visual inspections in this or other figures. Data development is discussed in more detail in Criterion 2, below.

The data were developed in line with the original research protocol, but they were insufficient and underdeveloped.

The data generated, for the most part, supported the objectives to some level. However, the objectives were not completed in a rigorous scientific fashion. As a result, the outcomes of the

work remain premature and uninterpretable both in terms of research quality and in the larger goal of the study, namely to develop assays, methods, strategies to target cancer versus normal cells. The problems with the data and its interpretation are a major weakness of the study and diminish enthusiasm for and the relevance and impact of the work.

The data fell short of reaching the objectives of the proposal for reasons stated above.

Reviewer 2:

The data collection as described followed the original specific aims. In general the specific aims were met. The data presented are applicable to the strategic research plan. However, there is some weakness in the study design, methods and data analysis.

The study goal was to characterize the vital permanent molecules in the mitotic centrosome and discover those which are temporary, along with the post-translational modifications of microtubules that occur in normal and cancerous cells. The PI compared detyrosinated and acetylated microtubules in lung embryonic fibroblasts, lung and breast cancer cells. He used FRAP to study the movement of proteins within the centrosome. He also identified proteins involved in centriole assembly and maturation, as well as microtubule-associated proteins and scaffolding proteins. Two proteins show a difference between noncancer and cancer cells: CP110 (involved in centriole duplication) and NaP1 (involved in cohesion of parental centrioles). Below are comments on each of the specific aims.

Specific Aim 1: The rationale for this specific aim is unclear. It would help to have citations from the literature that suggest there may be a difference in the centriole microtubules in cancer cells, noncancer cells, interphase cells or mitotic cells.

The rationale for the choice of cell lines is unclear. A cell line representing normal breast epithelium or other “normal” breast cells would be a better comparison for the MCF7 cells, rather than lung fibroblasts.

The PI concludes that the data shows that delta-2 tubulin and acetylated alpha tubulin are “highly useful” markers for centrioles. Some information is needed comparing these markers to other currently used markers to validate this finding.

In general, the absence of citations makes it difficult to appreciate how the research questions and design fit in the context of what is known.

Specific Aim 2: FRAP was used to determine the recovery time for GFP-centrin in centrioles in cancer cells vs noncancer cells. More explanation is needed for the conclusion that centrosomes in cancer cells are more unstable. It seems that if the recovery time is longer in cancer cells then the GFP-centrin in these cells is more stable (less movement to and from centrioles). So this analysis as presented seems incorrect. Some clarification is needed.

Specific Aim 3: The PI identified proteins involved in centriole assembly and maturation as well as microtubule associated proteins and scaffolding proteins; two show a difference between noncancer and cancer cells: CP110 (involved in centriole duplication) and NaP1 (involved in

cohesion of parental centrioles). Again, it would have been informative to have a control for the breast cancer cells for a better comparison.

Specific Aim 4: The design for Specific Aim 4 is problematic. The drug treatments were all done at a single relatively high drug concentration (1 $\mu$ M). However, the potency for the drugs varies considerably, and it is well known that the effects of taxanes and vinca alkaloids are concentration dependent. The experiments should have been done at equitoxic drug concentration and more than a single drug concentration. As presented, the data are not interpretable.

The significance of the HDAC inhibition experiment results is unclear. It is not clear if there is a difference between noncancer and cancer cells. Figure 10 suggests no difference. What is the rationale for the statement that HDAC6 inhibition may provide “a novel mechanistic approach to understanding cancer onset at the cellular level?” The PI suggests that HDAC6 might be a novel approach for cancer treatment; however, HDAC6 inhibition is already being tested in clinics.

Reviewer 3:

The project met its stated objectives to a very reasonable extent and was applicable to the project objectives listed in the strategic plan. The data were developed sufficiently to answer the research questions posed and in line with the original research protocol. No changes from the research protocol were made.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

Strengths: The work provides a small amount of weak data suggesting changes in parameters of microtubules and centrosome proteins that are different in cancer and normal cells and show different responses to cancer/microtubule drugs. However, the potential impact of the work on improvements to health of individuals with cancer is marginal to nil. As presented, there are no new discoveries, drugs or approaches that may benefit aspects of human cancer.

The PIs state that they will apply for funding to continue or expand the project. They suggest development of more specific chemotherapeutic agents. However, they do not know how the agents that they already used disrupt specific functions in the cells. Thus, it is unclear how they will achieve more specificity of drug targeting.

Weaknesses: The low potential impact of the work is due to several major weaknesses in the project design and to data that limit robust interpretation of results, question the overall utility of the study and significantly diminish the innovation of the work. As presented, the data in the project are preliminary and not compelling.

One reason for the lack of impact of the project is the lack of novelty. For example, the main strategy is to use conventional methods that have been used extensively by others for decades.

Differential effects of drugs on cancer and normal cells are one of the most common approaches of cancer biologists. Microtubules and their organization/organizing center are the most common targets of such studies. In addition, these strategies have met with only limited success.

Another reason for the lack of impact of the project is the lack of rationale for aspects of the project. The PI states that he will characterize permanent and temporary centrosome molecules, identify differences in cancer and normal cell centrosomes and centrosome movements, study a single posttranslational change of “tubule” (tubulin) and test drug effects on these parameters. There is little to no rationale or citation of literature that supports a potential role for why any of these parameters may be different in normal versus cancer cells. A substantial literature that has already addressed many of these issues is not adequately discussed or considered. For example, differences in centrosome number, structure, size, integrity, organization, coalescence, motility, overall composition, core composition, transient composition, microtubule nucleation ability, assembly, disassembly and function have been identified in cancer cells versus normal cells under control as well as conditions of microtubule loss and regrowth by a number of investigators. In addition, drug effects on microtubules and their nucleating center have been studied for decades, and there is a replete literature on these topics as well. These studies overlap aspects of the current work and should be part of the rationale and foundation for this proposal. This lack of rationale for aims, cell lines, drugs, posttranslational modifications, etc. diminishes confidence that the outcomes of the work will be accurate and if there will be impact on human disease.

Another reason for the lack of impact of the project is that the study is not hypothesis-based. Instead, the results represent only "soft" observations, descriptions or correlations. There are no mechanistic insights that could be developed into diagnostics/prognostics or therapeutics. Another reason for the lack of impact of the project is that the investigators do not take advantage of the large literature developed around their strategies to develop new drugs or methods. In fact, they do not cite some of the most important literature on the topics they develop. For example, there are hundreds of papers about dynamics of centrosome proteins before and after altering microtubule and associated structures. In addition, the centrosome proteome has identified centrosome proteins in cancer and normal human cells, as well as core versus transient centrosome proteins. Most of this work is not adequately discussed, acknowledged or exploited.

Also diminishing the potential impact of the study is the lack of experience of the PI in cancer research. There is no mention of cancer expertise in the personal statement of the PI. Instead, his overall interests lie in fertilization. The lack of tumor biology expertise may explain deficiencies in the proposal, both in terms of the weak research design, and the descriptive rather than mechanistic results. There is also a concern about the PI's low productivity in terms of having only a few first author and no senior author papers published in the last several years. In addition, most of these papers are published in low-impact journals.

A final issue affecting the clinical impact of the work is the lack of confidence that robust, interpretable and clinically relevant results will be obtained given the lack of rigorous statistical analysis and low quality of data. The study lacks rigorous testing for functional differences that inform therapeutic approaches. Even in the absence of further functional data, the PI did not

adequately discuss in detail how potential new discoveries will be leveraged to address issues of human cancer.

Given the weaknesses in the study, it is unlikely to continue. That being said, the PIs state that they will apply for funding to continue or expand the project. They suggest development of more specific chemotherapeutic agents. However, they do not know how the agents that they already used disrupt specific functions in the cells. Thus, it is unclear how they will achieve more specificity of drug targeting.

The PIs appear to shift focus from microtubules and centrosomes as drug targets, to motors and mitotic spindles if difficulties arise. The rationale of the shift is unclear, but it seems to be dictated by drug availability. The PIs state they will use the same questionable approach to look for molecules that are specific for tumor cells versus normal cells. Given the caveats of this approach outlined in this review, this strategy is unlikely to yield novel and robust candidates for cancer therapeutics. Just as microtubules and centrosomes are among the most common current targets of cancer drugs, so are molecular motors and mitotic spindles. This greatly diminishes the innovation of future work on this project.

Reviewer 2:

This study is descriptive. The PI explores differences between normal and cancer cell centrioles. For the most part no differences were found. That is to be expected in descriptive studies. However, the weaknesses in study design, methods and data analysis limit the potential impact.

Reviewer 3:

This project seeks to provide a beneficial impact by understanding the dynamics and molecular composition of centrosomes in normal and cancer cells. This work would provide new understanding of the basic biology of cells and possibly reveal new therapeutic targets. Indeed, understanding centrosomes is an important basic knowledge. The work so far has revealed interesting data, which is a strength; but no clear new therapeutic targets have been identified. It was not for lack of effort. It is just that sometimes necessary experiments reveal that some aspects are not different in normal and cancer cells.

The principal investigator notes in the final report that there were no changes in outcome, impact or effectiveness attributable to the research project, and there were no major discoveries, new drugs or new approaches for prevention. I concur with those statements.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

The investigators were not able to obtain funding from other sources to continue or expand the research.

The investigators state they are seeking NIH sponsorship for potential new targets of cancer therapies.

The investigators claim the work lays a foundation for future work on chemotherapy. However, there are several problems with the work that limit production of useable outcomes. In addition, there is no discussion of *how* the work will facilitate work toward future goals. Importantly, there is no discussion of how future goals will be attained or how they will be highly unique in order to “stand above” the extensive research in this highly competitive field.

Reviewer 2:

This was not done; however, there are plans for submission of an NIH grant application.

Reviewer 3:

The principal investigator notes in the final report that the project did not leverage additional funds and no additional grant applications have been submitted as a result of this project. The researchers are planning to apply for additional funding.

It is not surprising that within the time frame of this proposal that additional grants were not submitted yet. It is unclear if the data are compelling enough at this stage to lead to a successful application. More data showing a significant impact of the differences observed may be needed.

***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:

It appears that no publications, licenses, patents or commercial developments were realized in the completed project. It appears that none of these were expected.

The PIs state that they plan to submit the work if reproducible; but the work has not been completed, the data is weak, and the outcomes are unclear. Based on these serious deficiencies, it does not seem that the work should be continued.

The PIs state that no inventions were patentable, conceived or first actually reduced to practice in the performance of work under this health research grant. No commercial development

opportunities arose from the work. The PIs state that no licenses or patents, or commercial developments will be filed in the future.

Reviewer 2:

There were no publications. There are plans to submit the data for publication once the analysis is complete.

Reviewer 3:

The principal investigator notes in the final report that the project did not result in any peer-reviewed publications, licenses, patents, or commercial development opportunities. The researchers are planning to submit data for publication.

It is not surprising within the time frame of this proposal that papers have not yet been submitted. At the same time it is a weakness, and the principal investigator is encouraged to get some papers submitted soon.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

Weaknesses: Approaches for microtubule imaging were updated and National Cancer Institute resources brought in. No details were provided for the utility of the National Cancer Institute resources that were used during the course of the study. The approaches were not novel but used routinely by researchers in the cancer field. The low quality of some of the data was a weakness of the proposal.

No statement was made concerning updates of infrastructure. It was stated that no out-of-state researchers were recruited to the project. The PI stated that no new investigators were added to the project. It was stated that no funds led to collaboration with researchers outside the institution. No students participated in the project for a semester or summer or received funds from the project. The research did not lead to commercial products. The research did not lead to new involvement with the community.

There appears to be little impact of the funding beyond a focused research effort. The technology was not innovative and did not open new directions in either cancer research or therapy, but rather it brought the group's research up to date in more conventional live cell. The imaging microscopy methods are routine and used in most cancer laboratories, diminishing the innovation of the work.

Reviewer 2:

The PI developed the capacity to use FRAP in studies at his institution. He indicated also that this funding allowed recruitment of collaboration and he expects future collaborations to develop.

Reviewer 3:

The principal investigator notes in the final report that the project led to improvements in infrastructure by instituting new techniques in his laboratory, which is a strength. The final report further indicates an improvement in infrastructure by recruiting new National Cancer Institute resources and by recruiting new collaborations to the Commonwealth. The specific National Cancer Institute resources and new collaborations are not specified in either the progress reports or the final report, so it is unclear as to what they are.

The principal investigator goes on to state that no new collaborations were started with researchers outside the institution, no new researchers brought into the institution to help carry out this research, and no new involvement with the community, which would seem to contradict the statement about new collaborations being a strength. There was no engagement of students or post-doctoral fellows.

The absence of any details on the new National Cancer Institute resources and new collaborations brought into the Commonwealth is a weakness. Also, the absence of any involvement of students or post-doctoral fellows is a weakness for this criterion.

***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 PIs state that a collaboration was formed during the project period. However, this reviewer feels that in the absence of supporting documentation, it is unclear if this was actually accomplished. No collaborators were listed in the application, and there is no indication that new research investigators from outside the institution became active collaborators during the grant period.

Reviewer 2:

The PI indicates that the project did not lead to new collaborations outside the institution.

Reviewer 3:

The principal investigator states that no new collaborations were started with researchers outside the institution, no new researchers brought into the institution to help carry out this research, and no new involvement with the community or businesses. These absences are a weakness for this criterion.

## ***Section B. Recommendations***

### **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

#### Reviewer 1:

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.

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.

3. The study utilizes only one normal and two cancer 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.

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.

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.

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

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.

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.

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.

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.

## **Recommendations for Magee-Womens Research Institute**

### 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.