

# **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 (1.79)

### **Project Rating:**

<b>Project</b>	<b>Title</b>	<b>Average Score</b>
0865401	Molecular Mechanisms Involved in Reprogramming Cells	Favorable (2.00)
0865402	Nanofabrication Lab for Biosensors and Biomeasurements – Research Infrastructure	Favorable (2.00)
0865403	Effects of Nicotine on Mu Opioid Receptor Binding	Outstanding (1.33)
0865404	Validation of Imaging Markers for Use in Cancer Clinical Trials	Outstanding (1.33)
0865405	Understanding the Biology of Residual Neoplastic Disease	Favorable (1.67)
0865406	Individualizing Breast Cancer Prevention in Primary Care	Favorable (2.33)
0865407	Clinical and Molecular Predictors of Responsiveness to Angiogenesis Inhibition in Advanced NSCLC	Favorable (2.00)
0865408	Reprogramming Cells in Studies of Heart and Lung Development and Repair	Favorable (2.33)
0865409	Genome-based Bio-marker Discovery and Systems Biology Engineering	Favorable (1.67)
0865410	Research Infrastructure: Expansion and Enhancement of Rodent Housing Space	Outstanding (1.33)
0865411	Development and Validation of a Tool to Assess Perceived Nutrition Environments	Favorable (1.67)

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**Project Number:** 0865401  
**Project Title:** Molecular Mechanisms Involved in Reprogramming Cells  
**Investigator:** John D. Gearhart, PhD

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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:

This project has an ambitious goal to understand molecular mechanisms underlying cell reprogramming. Two specific aims are designed to understand: 1) Determine the variables and extent of the reprogramming process at the single cell level; 2) Determine whether there are limits to the reprogramming process. The research team made efforts to reprogram: 1) mouse embryonic stem cells to 2-cell blastomeres; 2) somatic cells such as mouse embryonic fibroblasts (MEFs) to the male germ lineage spermatogonial stem cells (SSCs); 3) human fibroblasts into cardiomyocyte-like cells. Preliminary results suggest that the research team made excellent progress on reprogramming SSCs and cardiomyocyte-like cells. The attempt to convert mESCs into 2-cell blastomere appears to have failed. Overall, the scope of research projects is in line with the original goals as proposed. Because other research teams have advanced to successfully convert somatic fibroblasts into cardiomyocytes, this research team can make comparison of their data with the literature and try to further improve the transdifferentiation protocol. The most novel findings would be in line with the preliminary success in the generation of SSCs, although additional rigorous test in animal models via cell transplantation and molecular characterization via transcriptome analysis is warranted. In short, the team has generated a good amount of data that are in line with their strategic goals for this proposal.

#### Reviewer 2:

The broad objectives of the project were met and the research design and methods were highly innovative and appropriate for the proposed studies. The work described in Specific Aim 1 was unsuccessful and was apparently ended after year 1 of the project. The Specific Aim 2 research, as originally proposed, was also unsuccessful. However, this aim was refocused in keeping with the original broad goal for this portion of the project. In addition, a third study was advanced that was not specifically addressed in the original project objectives but fits well with the overall project goals.

Specific Aim 1: This single-cell phototransfection approach is quite exciting and holds great promise for deciphering the mechanisms of cellular reprogramming. Unfortunately, attempts to reprogram primary MEFs to ES-like cells failed. However, it is not clear to what extent this approach was optimized before this portion of the project was ended. It is important to know if other cell types, mRNA ratios and concentrations, phototransfection frequency, etc., were

explored. In addition, the possible explanations for the lack of reprogramming in this system should be addressed.

Specific Aim 2: The objective of this aim was to convert pluripotent mouse embryonic stem cells to totipotent blastomeres. Considerable effort was expended on the identification of markers for totipotency and on constructing the appropriate tools needed for the study. However, reprogramming to a totipotent state was not achieved. In keeping with the original goal of this aim, effort was then shifted to the reprogramming of mouse embryonic fibroblasts to spermatogonial stem cells. While this seems to be a reasonable change, little rationale is provided for choosing this specific system. Significant progress was made in this revised aim with the future possibility for reprogramming fibroblasts to functional spermatogonial stem cells. Cardiac Reprogramming Study: The ultimate goal of reprogramming mouse and human fibroblasts to cardiomyocytes represents a new avenue for this project that was not specifically addressed in the original strategic research plan. However, it certainly falls within the latter, broadly-stated project milestones. It is a reasonable change and a well-justified addition to the project. The data presented demonstrate that significant progress has been made during the project period with very important implications for physiologically assessing the success of reprogramming to cardiomyocytes.

Reviewer 3:

The progress reported does not reflect achieving the stated objectives of the original project.

The research design and methods proposed for the original project were adequate, however not sufficient to fully elucidate the full goal of the specific aims. Nevertheless, in the case of Specific Aim 1, the project was not concluded and only one initial report was provided. My impression is that Specific Aim 1 was dropped during the grant period. In the case of Specific Aim 2, the research design and methods were adequate and followed during the research period, but the progress does not reflect in achievement the goal of this specific aim.

For Specific Aim 1, the limited data provided reflects an initial road-block that was never overcome, and no further information was provided for this aim, which one could interpret as a failure.

For Specific Aim 2, the progress reports indicated that the authors identified a set of factors that could potentially be involved in the reprogramming of cells into blastomere cells. However, at the end of the grant period, no success was reported toward this original Specific Aim 2. Instead, a new direction was taken to study the reprogramming of somatic cells into spermatogonial stem cells (SSC). The data reported for this new project indicated that the authors identified a set of factors that could induce fibroblasts to express genes related to SSCs and to acquired morphological cell shapes similar to SSCs. They were able to expand this modified cell population for an extended period of time while maintaining the expression of proteins considered as SSC's markers. Nevertheless, the authors fall short in giving a full characterization of these cells.

In an additional project reported only in the final progress report, the authors describe data regarding the reprogramming of somatic cells and cardiac fibroblasts into cardiomyocytes.

Although the data suggests significant progress towards the reprogramming of cardiomyocytes, this project was not planned in the original research protocol or project.

Changes were made to the research protocol, however no explanation or justification was provided for those changes.

The data generated and provided is not sufficient to conclude that the progress made met the objectives proposed.

The two new projects reported and not planned in the original proposal, concern reprogramming of somatic cells into specific cell lineages, in this case SSCs and cardiomyocytes. Thus, they are related to the main topic of reprogramming. However, no data was generated regarding the mechanism involved in the reprogramming mechanisms toward pluripotent stem cells as planned originally or cardiomyocytes and SSCs.

The proposal was strong and with innovative ideas and questions that even now are still not fully resolved. The authors proposed a new mechanism or protocol to reprogram single cells into iPSCs, which if it works will have a significant impact in the reprogramming field, and will help to elucidate the mechanism of reprogramming as proposed. With the limited data provided one can appreciate that the authors faced a problem phototransfecting fibroblasts, and it was argued that the cell shape could have a negative impact on this. They proposed to partially trypsinize cells to round them up and to investigate whether this could solve the problem. Alternatively, they could try to phototransfect other somatic cells that have different morphology, such as blood cells, which have been proved to be effective in the generation of iPSCs. Nevertheless, it was not explained which methods will be used in elucidating the mechanisms of reprogramming once the first objective was achieved.

Similarly, in the second specific aim, the author explored an innovative idea of reprogramming cells into totipotent stem cells, going beyond the current limit of pluripotency. They reported significant progress identifying possible markers for this population of cells, and possible targets of genes that could transform the transduced cell into a blastomere. Without much explanation, the authors decided that the cell to use would be mouse embryonic stem cells (mESC). The results suggest the transduction efficiency of selected genes and even reporter genes into mESCs was inefficient. It is known that human ESCs are difficult to manipulate genetically, and thus it is possible to think that mESCs will behave in a similar way. Nevertheless, it could be possible to pursue the reprogramming into totipotent stem cells by using fibroblasts or other somatic cells that are more easily transfected.

***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:

Understanding the molecular mechanisms of reprogramming would be quite beneficial to the field of stem cell biology and regenerative medicine. In addition, the improvement of current

differentiation protocol and purification methods may pave the way of future use of these protocols in cellular reprogramming and regenerative medicine. The successful transdifferentiation of somatic cells into cardiomyocytes or spermatogonial stem cells (SSCs) would be valuable to human health by providing a new source of therapeutic cells for cell replacement and disease mechanism studies.

Reviewer 2:

The directed restoration of genomic potential in a patient's own cells may have direct therapeutic applications for tissue regeneration and repair and for drug discovery. Full realization of these and other applications requires a greater understanding of the molecular mechanisms of this "cell reprogramming" process, the primary focus of this project.

The most significant and promising health benefit resulting from this project involves the eventual reprogramming of mouse and human fibroblasts to cardiomyocytes for restoration of cardiac function. While cardiovascular disease remains the primary killer worldwide, an increasing number of patients are surviving with chronic cardiovascular dysfunction. These patients could benefit greatly from the transplantation or regeneration of functional cardiac cells. This potential health benefit is currently limited, in part, by our lack of understanding of the specific factors required for cardiomyocyte production and assays for assessing cardiac cell function in reprogrammed cells. The project begins to successfully address both of these limitations with the identification of key reprogramming molecules and the development of a reporter system for assessing ongoing physiological changes in the cells during reprogramming. These are significant advances in the field and hold great promise for long-term health benefits. Future project-based work will utilize the physiological reporter system to improve the cardiac reprogramming process in both mouse and human cells.

The progress made toward reprogramming mouse embryonic fibroblasts to spermatogonial stem cells also represents a potentially significant clinical advance in the field of reproductive biology. These studies will be continued in the future.

Reviewer 3:

The original proposed specific aims could have a significant impact on human health conditions indirectly. Once the mechanism of reprogramming is elucidated, as proposed in the evaluated project, this could translate into more efficient protocols and procedures to increase the effectiveness of patient specific reprogramming. Similarly, the direct reprogramming of somatic cells to other cell types could be a benefit from these studies. In fact, the authors apply this knowledge to reprogram somatic cells into SSCs and cardiomyocytes.

As stated above, the elucidation of the mechanism of reprogramming cells would result in improvement in health outcomes. One example is that it will be possible to derive pluripotent stem cells from patients with specific diseases, then use those cell lines as disease models for future studies that could result in drug development and understanding of the disease.

No major discoveries or drugs were developed from this project.

The PI stated that future plans for this research project included use of the data generated for a R01 application and to further conclude experiments that could be published.

The potential impact of this project is significant overall if concluded satisfactorily, but not at this moment. However, at this point it seems that the data generated would serve as an initial set point to pursue studies in the derivation of SSCs and direct differentiation of cardiomyocytes. Taking into consideration that this project did not result in publications and in very limited achievement of the original specific aims, it seems not reasonable in light of the dollars budgeted and spent.

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

### ***STRENGTHS AND WEAKNESSES***

#### Reviewer 1:

No grant application was submitted; although, the PI plans to submit a R01 grant this fall. This is viewed as a weakness of this research project. Given the amount of preliminary results and the sources invested, the PI shall be able to formulate a few competitive grant applications.

#### Reviewer 2:

No additional funds were applied for and/or obtained, based on the health research funds provided during this research project. However, an NIH R01 grant submission is planned in the near future.

#### Reviewer 3:

No indication is given to conclude that extra funds were requested or obtained during the funding period of this project. It was indicated that the PI plans to apply for R01 grant funding to keep pursuing studies in reprogramming.

The authors generated interesting data regarding factors that could induce SSCs, and were even able to obtain a population of cells that express markers of SSCs and to sustain this population for several passages. These data are encouraging and strong to pursue an R01 application for this specific project. However, the data generated to elucidate mechanisms of reprogramming and limits of reprogramming is limited and might not be sufficient for an R01 grant mechanism.

***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 proposed research led to poster presentation at international meetings, but failed to yield original research articles. The PI plans to wrap up some of the most promising projects and to gear up to publish some of the novel findings.

Reviewer 2:

No publications, licenses, patent applications or commercial development opportunities resulted from this project. The lack of publications is not surprising considering the difficulties encountered with the single-cell reprogramming studies and the work on totipotent blastomere reprogramming. However, the researchers do plan to submit their completed works to a “high visibility journal” in the near term.

Reviewer 3:

No publications resulted from this project. The PI has plans for future publications, and it seems that the SSCs studies are close to ending, however not the mechanism of reprogramming or the reprogramming into blastomere cells, the original objectives of this project.

Both additional studies in this project, the reprogramming of somatic cells into SSCs and reprogramming into cardiomyocytes have good data that can potentially translate in publications. Perhaps if the authors are willing to try the phototransfection of other cell types it will be possible to produce a manuscript using the new methodology to reprogram single cells into iPSCs. Similarly, the preliminary data obtained for identifying target genes to induce the reprogramming into blastomeres could result in a publication with high impact because of its originality and novelty. As previously suggested, maybe other somatic cells could be used as candidates for reprogramming into blastomeres, instead of the difficult to transfect mouse ESCs.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

This research project is beneficial to the host institution, the University of Pennsylvania. The grant funding was used to support technical staff, and post-doctoral students. In addition, the purchasing of several important pieces of equipment including a Flow Cytometer and Bioanalyzer enhances the infrastructure of the molecular and cell biology labs at the University of Pennsylvania. The team also leverages on the expertise of outside investigators via meetings and consulting.

Reviewer 2:

A collaboration was established with investigators in the School of Veterinary Medicine and is anticipated with OB/GYN researchers in the School of Medicine at the University of Pennsylvania. Research funds were used to support the training of two post-doctoral fellows.

Reviewer 3:

Equipment was bought using funding from this project such as a flow cytometer, a microscope, and an electrophoresis system.

No out-of-state researchers were brought in to join the team performing experiments in this project.

Three post-doctoral students participated in this project.

The intrinsic nature of this project which was challenging, innovative and high-risk must enhance the research experience of personnel involved. In addition to the three post-docs, three research specialists participated as well in this project, which suggests that enough brain power was at the disposal of 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:

The PI has solicited advice and consulted with outside investigators in the field of gametogenesis and preimplantation development. Additional communications with other experts in cardiomyocyte transdifferentiation are encouraged.

#### Reviewer 2:

No outside collaborations were established or are expected.

#### Reviewer 3:

There was no indication that any collaborations occurred as a result of the research.

Originally, the project was described as in collaboration or support of a scientist with expertise in phototransfection of single cells, a key component of Specific Aim 1. Nevertheless, this aim was interrupted at the beginning of the funding period and no explanation was provided for this. Although enough brain power was at the disposal of this project, key techniques which are innovative and performed only by specialists can become a bottleneck and pose a high risk for the rest of the project. Thus, alternative methods should have been in place to overcome such difficulties.

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

#### **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

#### Reviewer 1:

1. The research team should focus on completing two of the projects including reprogramming somatic cells into cardiomyocytes and spermatogonial cells. Given the resources available, the PI should make efforts to publish those results in peer-reviewed journals.
2. The preliminary data should be useful to generate additional proposals to federal funding agencies such as NIH and DoD.

#### Reviewer 2:

None.

Reviewer 3:

1. Alternative methods should be requested for proposals. In this particular project, the Specific Aim 1 was based on the ability to reprogram single cells into iPSCs using a novel technique of phototransfection. Once this was achieved, then analysis was to be performed to elucidate mechanisms of reprogramming. However, the reprogramming was never established, causing a roadblock to pursue the rest of the aim. Thus, it would be beneficial to request alternative methods at the proposal to further warrant the viability of the project.
2. Evaluation of progress should be more rigorous. In this particular project, a red flag could be observed at the second progress report, since no activity was reported for the Specific Aim 1. In the previous progress report the difficulties of using phototransfection with fibroblasts were stated; however, it seems as though an alternative cell population for this experiment was never considered, nor was an alternative methodology to phototransfection.
3. Embryonic stem cells are known for their resistance to being genetically manipulated; thus, alternative cell populations could be considered for the Specific Aim 2 in order to obtain induced blastomeres.

**ADDITIONAL COMMENTS**

Reviewer 3:

The data generated for this project did not meet the objectives listed in the strategic research plan. In fact, one of the specific aims was dropped from the study, while the other fell short in achieving the objective for Specific Aim 2. Both aims were innovative, challenging and high-risk; however, the possible outcome if achieved will have a significant reward and impact in the field of biology and regenerative medicine. The other two studies related to reprogramming of cells were pursued and reported with relatively significant progress; nevertheless they were not the original objectives listed in the strategic research plan.

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**Project Number:** 0865402  
**Project Title:** Nanofabrication Lab for Biosensors and  
Biomeasurements – Research Infrastructure  
**Investigator:** George Pappas, PhD

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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 appeared to meet its goal of renovating a laboratory for Biosensors and Biomeasurements. The lab is fully renovated and fully occupied. There are 40 investigators supported by the facility, 78 projects are supported by the facility and 64 external grants are supported by the facility. The renovated space is 3,500 sq ft with 1,950 sq ft being a class 1,000 (ISO 6) clean room.

**Weaknesses:** Frankly, the final progress report was very limited. It provided no indication of "ongoing activities" or even potential improvements that the facility can now support. One would think with 40 investigators and 78 projects with 64 external funded grants that there would be something to report.

#### Reviewer 2:

**Strengths:** The objective - to provide a portion of the funding needed to upgrade the Wolf Nanofabrication Facility at the University of Pennsylvania in order to increase clean room and chemical storage space, upgrade physical facilities and provide additional equipment for facility users – was achieved.

Completion of the project (albeit with additional funding from the University of Pennsylvania) contributed to the University of Pennsylvania's efforts to create an interactive environment that both promotes research initiatives and enables the recruitment of new faculty with expertise in micromedical devices.

**Weaknesses:** While the infrastructure of the Wolf Nanofabrication Facility was successfully upgraded as proposed, there was little documentation of the usage of the facility to demonstrate improved capabilities/productivity and collaborations brought about by this facility upgrade.

#### Reviewer 3:

The project was designed to enhance the capacities of the nanotechnology facility established on the University of Pennsylvania. No direct research was planned under this award, and none was performed.

Here is their summary:

“Improvements to the Wolf Nanofabrication Facility, including a 50% expansion of the clean room space, will enable researchers to do many different types of experiments in the future. One of the first devices that will be built using the Facility will be a prostate cancer detector, built from carbon nanotube transistors. Another device will be a sensor, which will be used to map biological and chemical signals in the brain, using nanowires. This will provide a vital platform to understanding the fundamental origins and causes of neurological diseases, ultimately aimed at effective prevention and long-term treatment. Other nanowire devices will be built to detect individual molecules, which are indicators of disease. These and many other experiments will be conducted at the University of Pennsylvania, leading to research publications and patents for medical devices of the future.”

There are no research program or projects tied specifically to this award. It was used for renovation construction and equipment purchases.

***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 promises of nanomedicine remain high and possibly great for mankind and for the development of new medical technology for the future.

Weaknesses: There is little information to support that this facility will contribute to the "NEW WAVE" of nanomedicine devices. I suggest asking the investigators for a progress report now that the facility has been in use for about a year.

#### Reviewer 2:

Strengths: Upgrading the Wolf Nanofabrication Facility will benefit multiple investigators who can utilize the improved capabilities of this facility to create micro- and nanoscale devices to affect and assess cellular processes that have relevance for prevention, diagnosis and/or treatment of disease.

New biosensing and biomeasurement devices and/or approaches to disease originating in the Facility should eventually translate into improvements in health care and/or outcomes.

Weaknesses: For publicity to strengthen and advertise the Facility and its benefits to users within and potential collaborators outside of the institution, it would be advantageous to keep a summary of the projects utilizing the Facility and their eventual outcomes.

#### Reviewer 3:

It is likely that the upgrade greatly increased competitiveness of the nanotechnology program, and increased grant awards and high-impact publications. But, we are not told of this yet. We are told about completion of renovations.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

Strengths: There were \$1.5M in additional funds that were provided by internal Penn Unrestricted Funds for this project. There are supposedly 64 grants that support projects in this facility with 78 projects and 40 investigators who must be receiving funds as a consequence of this facility.

Weaknesses: No additional funds were leveraged, but there are 64 grants that are supposedly supported by this project.

Reviewer 2:

Strengths: The funds from this research facility construction project were combined with \$1.6 million from the University of Pennsylvania to upgrade the Wolf Nanofabrication Facility. This combination of funding allowed not only the construction of additional clean room and chemical storage space, but resulted in the addition of new equipment that increased the capabilities of the facility.

Weaknesses: As mentioned for other criteria, documentation of the breadth of users and the diversity of projects utilizing this facility could help justify new funding, e.g., shared instrumentation grants to provide additional equipment for the Facility users.

No follow-on funding was projected, but generating summary statistics would presumably be useful for other purposes, for example setting a budget for upkeep and service contracts of the equipment within the facility as well as Facility staff salaries.

Reviewer 3:

The University of Pennsylvania provided matching funds, which is a strength.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

Strengths: There are supposed to be 40 investigators, 78 projects and 64 grants that support the activities of this new space.

Weaknesses: No papers were cited. No attempt was made to explain the impact to the University from this facility. This should be documented. What about future patents as well?

Reviewer 2:

Weaknesses: While multiple users took advantage of the newly expanded and upgraded facility, there was no formal documentation of their projects, publications, patents, etc. Presumably large

numbers of researchers that utilized the facility completed work that depended upon the space and/or equipment within the Facility. Although work done in the Facility does not translate into co-authorship on publications of the work, the Facility could be acknowledged within these publications, to increase awareness in the outside community of the services available and the support provided by the Institution.

One strategic plan (FY 09) mentioned usage by 100 investigators between January and June of 2010. It described some specific projects carried out using tools within the Facility and two provisional patents that were filed, but there was no mention of additional examples in other annual progress reports or in the final summary report.

The strategic plan mentioned specific projects (e.g., a prostate cancer detector built from carbon nanotube transistors, a sensor with nanowires to map biological and chemical signals in the brain, other nanowire devices to be built to detect individual molecules that are indicators of disease), but no follow-up information was provided to document that the Facility assisted in these research efforts. Information in the final summary report was very general.

Reviewer 3:

No publications or patents are cited; this would have been helpful to have.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

Strengths: Yes, this was the major goal of this grant and it was achieved.

Weaknesses: How the infrastructure has helped Penn with its nanofabrication mission is unclear.

Reviewer 2:

Strengths: Improvements in the infrastructure brought about by this project and in conjunction with the University's funding for additional facility upgrades and equipment purchases most assuredly resulted in enhancing the capacity for projects involving micro- and nanofabrication of devices.

Weaknesses: Pre- and post-doctoral students were presumably allowed and did utilize the upgraded facility, but as mentioned before, no documentation was generated to provide this information. These upgrades were also proposed as a means of recruiting faculty (strategic plan), but no follow-up information was provided.

The overall project (i.e., the upgrade to the Facility) was to be evaluated by assessing the usefulness to researchers and the ways in which it facilitated research by measuring (1) numbers of investigators supported, (2) numbers of projects supported, and (3) numbers of external grants supported. Few details of these measures were provided in the annual and or final summary reports.

Reviewer 3:

The research capacity is likely to be greatly increased over the next few years, but, again, we do not yet know how this will play out. We are basically informed only about the Wolf lab changes.

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

**STRENGTHS AND WEAKNESSES**

Reviewer 1:

Strengths: Yes, there are 40 investigators, 78 projects and 64 grants that embody this new facility. There are numerous investigators from Penn Engineering, Penn Arts and Sciences, Penn's Medical School and several investigators from Drexel University.

Weaknesses: With the 64 grants involved as stated, there should be more collaborations that have resulted from this infrastructure development.

Reviewer 2:

Strengths: The enhanced capabilities provided by the upgrades to the Facility had the potential to provide many new opportunities for collaboration with research partners outside of the institution.

Weaknesses: The enhanced capabilities provided by the upgrades to the Facility should have provided new opportunities for collaboration with research partners outside of the institution, but, again, little documentation of these new collaborations were provided (general information was provided in the final summary report).

The Wolf Nanofabrication Facility is advertised as “a user facility serving the nanofabrication needs of the Penn community as well as those of external users.” As mentioned previously, there are no statistics to document usage, including that of external users.

Reviewer 3:

None are described.

***Section B. Recommendations***

**SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

Reviewer 1:

Now that the infrastructure has been in place, the resulting merits of the facility should be clear. There should be papers, grants, collaborations and possible patents that have or are resulting from the facility.

Reviewer 2:

1. Specific Weakness Criterion 1: While the infrastructure of the Wolf Nanofabrication Facility was successfully upgraded, as proposed, there was little documentation of the usage of the

facility to demonstrate improved capabilities/productivity and collaborations brought about by this facility upgrade.

Recommendation: Generate some user statistics to document (1) numbers of users/user groups served, (2) number of projects and external grants supported, (3) number of papers, patents, etc., reporting work carried out in the Facility.

2. Specific Weakness Criterion 2: For publicity to strengthen and advertise the Facility and its benefits to users within and potential collaborators outside of the institution, it would be advantageous to keep a summary of the projects utilizing the Facility and their eventual outcomes.

Recommendation: Maintain a user log and solicit information from users on the projects supported by the Facility.

3. Specific Weakness Criterion 3: As mentioned for other criteria, documentation of the breadth of users and the diversity of projects utilizing this facility could help justify new funding, e.g., shared instrumentation grants to provide additional equipment for the Facility users.

Recommendation: While it is acknowledged that no follow-on funding was projected, generating summary statistics would presumably be useful for other purposes, for example setting a budget for upkeep and service contracts of the equipment within the Facility as well as Facility staff salaries.

4. Specific Weakness Criterion 4: While multiple users took advantage of the newly expanded and upgraded facility, there was no formal documentation of their projects, publications, patents, etc.

Recommendation: Presumably large numbers of researchers that utilized the facility completed work that depended upon the space and/or equipment within the Facility. Although work done in the Facility does not translate into co-authorship on publications of the work, the Facility could be acknowledged within these publications, to increase awareness in the outside community of the services available and the support provided by the Institution.

5. Specific Weakness Criterion 5: Pre- and post-doctoral students were presumably allowed and did utilize the upgraded facility, but as mentioned before, no documentation was generated to provide this information.

Recommendation: Document training and encourage usage of the Facility by trainees.

6. Specific Weakness Criterion 6: The enhanced capabilities provided by the upgrades to the Facility should have provided new opportunities for collaboration with research partners outside of the institution, but only a small amount of general information (i.e., numbers only,

no specific information) about these new collaborations was provided in the final summary report.

Recommendation: The Wolf Nanofabrication Facility is advertised as “a user facility serving the nanofabrication needs of the Penn community as well as those of external users.” Specific examples of collaborative projects supported by the Facility could be included in the Facility website information.

Reviewer 3:

Request follow up report in 24 months to show impact of the funding. We do not know yet if this improvement to the nano laboratory was effective as promised. I don't really doubt that it was helpful, but it would be nice to know by how much.

**Generic Recommendations for the University of Pennsylvania**

Reviewer 2:

Document usage and diversity of investigators/projects supported by the Facility and their outcomes as a means of publicizing the Facility for outside users and the community at large.

Reviewer 3:

Please ask for follow-up in 2 years to determine longer term impact.

**ADDITIONAL COMMENTS**

Reviewer 1:

Insufficient data and information were provided to support that the new infrastructure has now contributed to a much better environment for nanofabrication and biomeasurements at the University of Pennsylvania.

Reviewer 3:

It is difficult to assess the overall impact, because the benefits are proposed to emerge in the future, as the lab is used for new types of research.

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**Project Number:** 0865403  
**Project Title:** Effects of Nicotine on Mu Opioid Receptor Binding  
**Investigator:** Caryn Lerman, PhD

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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:

This project sought to characterize the brain mechanisms that explain the association between the mu opioid receptor (MOR). The investigators propose to test for an interaction between a variant located in the *OPRM1* gene and nicotine using a 2x2 experimental design. Data from 24 smokers was obtained and analyzed under the study protocol. Results derived from these experiments were analyzed and published in 2 separate manuscripts.

The project did not fully meet its stated objectives. Presented results focused on 5 brain regions without overarching conclusions and follow-up analyses to better understand observed results. For example, the graphs presented in this report and in the published PNAS manuscript showed effects in one subset of treatment arm or on one side of the brain without a discussion regarding overall statistical significance and possible biologic mechanisms. The manuscript published in *Psychopharmacology* tried to address some of these questions by focusing on the amygdala, more analyses of this type could have been completed.

As mentioned above, the proposed experiments were completed, and produced the type of data that would be needed to address the research question. Overall, the study design appeared to be underpowered to detect clinically meaningful effects given the large number of tests performed. Efforts to validate/replicate initial findings were particularly lacking. A deviation from the proposed study design led to data collection in an additional 6 individuals. However, not much came out of this data. In fact, efforts to further incorporate the data collected from these non-smokers in the main study were stopped. There were no further discussions regarding this subset of the dataset; therefore, it is not clear why the data were collected in the first place.

Finally, the number of published scientific manuscripts is very low; they published an average of one manuscript over the last 2 years. Considering that this project was funded for 5 years, the small number of published manuscripts suggests relatively low productivity.

In summary, strengths of this project include:

The study design was appropriate. However, the number of replicates per group is likely to be too small to lead to valid inference for all the outcomes considered in this project.

The experiment plan, not accounting for the sample size limitation, was adequate and has led to the collection of a valuable dataset in a small subset of individuals.

The plan for normalizing the image data was accurate and done according to existing norms.

If done right, the proposed study could provide valuable insight into an area of research that still has a long list of unanswered questions.

Weaknesses include:

The sample size was extremely small and lacked racial/ethnic diversity. There will always be good reasons for ignoring the racial minorities. In this case, the frequency of the minor allele of the variant of interest was too small in African-Americans (AA), which means that the risk allele (the one presumably associated with smoking cessation and strong withdrawal effect) is more common in this group than it is in European Americans (EA) in this case. Therefore, the burden is likely a lot higher in AA, but they were not included in these analyses.

The decision to include 20 additional controls is questionable. The rationale for doing so was never provided. As it stands right now, it looks like a waste of resources.

Statistical analyses did not follow the plan discussed in the proposal, which seemed more appropriate given the study design. No attempt to replicate/validate results was made.

There was no mention of the screening process. Therefore, it is not clear how the investigators made sure that individuals recruited in this project had the right genotype. What is the average number of screens they had to perform before identifying one individual with non-risk allele?

#### Reviewer 2:

The broad research objective was to characterize the association of the mu opiate Asn40Asp polymorphism of the OPRM1 gene in potentiating nicotine dependence. This was to be approached by observing the effect of iV nicotine on mu opiate binding potential (MOR BP) in the ventral striatum in wimoes with and without the Asp40 polymorphism with the hypothesis that the Asp40 group would have a higher level of MOR BP than those without the polymorphism and that after nicotine exposure the MOR BP would decline further in the Asp40 group. Finally, it was hypothesized that the Asp40 group would have attenuated subjective liking responses to nicotine.

Several cortical regions (e.g., anterior cingulate, middle temporal gyrus and cingulate gyrus and insula) in smokers were found to have significantly altered BP. The results of the studies conveyed in the published articles (two) and progress reports arising from this project are that MOR BP in the amygdala is associated to the motivation to smoke related to negative affect. However, this relationship was not seen with the actual change in affect after a cigarette is smoked (Falcone et al., 2012). No significant relationships were seen with MOR BP in the ventral striatum and smoking urge or smoking effects. However, the clinical significance of this relationship is not clear. No significant relationships of cigarette smoking on reduction of negative affect or relationship between smoking changes in negative affect and MOR BP were found. The authors readily acknowledged this. In smokers, the genotypic G of the OPRM1 Mu

opioid receptor was significantly associated with higher MOR BP in the amygdala (bilateral) thalamus (left) and anterior cingulate cortex (left) (Ray et al., 2011). Among the A118G carrier smokers the change in subject reward with smoking a nicotine vs. non-nicotine cigarette was significantly associated with MOR BP in the right amygdala, caudate anterior cingulate cortex and thalamus. Ventral striatal MOR BP was not found to be associated with genotype or smoking status.

The project did test its stated goals. As is usually the case, the physiological reality is more complex and differs from the view going into the studies. This is why the experiments are conducted. The initial hypothesis that Mu Opioid receptors in the ventral striatum would be of particular importance was not borne out. Instead, it was the amygdala and cortical areas which appeared more relevant. Acceptable progress has been made. In the future, the interactions of Mu opioid systems with nicotinic and dopaminergic factors should be examined.

### Reviewer 3:

The objective of this study was to examine the extent to which the association between a polymorphism (Asn40Asp) in the mu opioid receptor (MOR) gene and smoking-related behavior might be explained by brain-based mechanisms. In smokers, they examined whether smoking a cigarette with nicotine (compared to a denicotinized cigarette) after overnight nicotine abstinence was associated with differential MOR binding potential (BP) and subjective reward by genotype. They predicted that smokers with the “low risk” genotype ( or G allele carriers) compared to the “high risk” genotype (AA) would show less responsiveness to nicotine as reflected by less change in MOR BP in the ventral striatum and less increase in subjective reward to nicotine. They focused on 5 brain regions of interest (ROIs), the anterior cingulate cortex (ACC), amygdala (AMY), caudate (CAU), the ventral striatum (VST), and the thalamus (THA). The research team did an outstanding job in meeting its stated objectives. The design and methods were clear and used state-of-the-art imaging technology to address the project’s objectives. While the investigation initially proposed to use IV nicotine, after running the initial experiments using cigarettes the imaging agent carfentanil was not available, so the investigators focused on the experiments using cigarettes, which seemed to be sufficiently reasonable, and the data collected were able to address the questions of the study (and included N=24 smokers across 2 sessions and N=20 controls for 1 session). They found that regardless of nicotine condition, smokers with the high risk genotype exhibited higher MOR BP in the AMY, THA and ACC. In addition, they found that although subjective reward did not vary by genotype, in smokers with the low risk genotype, subjective reward was associated with MOR BP in the AMY, CAU, ACC and THA. The investigators published these findings in *PNAS*.

They also continued to work with this data to better understand the mechanism underlying their findings and found that increased MOR availability in the AMY to be associated with self-reported smoking to reduced negative mood, but not with actual changes in affect across sessions. The investigators published these findings in *Psychopharmacology*.

The data presented show that the team clearly met project goals.

It is unclear the extent to which the diagnostic instrument used (the MINI) comprehensively assesses both lifetime and current diagnoses for all relevant psychiatric and substance use

problems. The authors may want to clarify this in future work and/or consider an assessment that fully assesses lifetime psychopathology.

Given that the authors did not detect nicotine vs. denicotinized session effect; to what extent might this be due to a carry-over related problem, given that they always administered nicotine in the first session? In future studies they may want to consider counter-balancing these conditions, in order to rule out that possibility.

***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:

As it stands this project will have limited impact. Knowledge derived from the proposed study will likely be incremental instead of paradigm shifting. It will mostly serve to generate hypotheses that could be tested in much larger studies. However, the impact appears to be reasonable given the available budget, and the infrastructure that had to be put in place. Results from this study could potentially lead to the identification of relevant pharmaceutical targets that could facilitate smoking cessation. However, this should be seen as a long-term goal.

With regard to impact, the strengths of the current project include:

Focus on more refined phenotype, relative to self-reported measures. The direct measure of the MOR BP provides enough information to justify the cost of obtaining the pet scans.

Generation of additional data on the brain and more specific regions of interest, which will allow the investigator to address additional questions beyond their stated specific aims.

The fixed cost associated with the collection of this data appears to be one of the proposal's primary weaknesses. It seems that this project could have collected a much larger dataset without these costs.

### Reviewer 2:

The beneficial impact of this project on clinical practice is a fair distance off. These are pretty basic studies. One way in which they could inform clinical treatment is whether opioid acting drugs would be beneficial for people with the opioid polymorphism and would aid in smoking cessation for them.

### Reviewer 3:

This project is likely to have a beneficial impact on preventing and improving smoking-related health outcomes, by helping to elucidate the mechanisms associated with cigarette smoking. The study found evidence that variation in a functional genetic polymorphism in the MOR gene (previously associated with smoking), was associated with MOR BP differences in relevant brain regions, and that variation in MOR BP was also associated with self-reported motivation to smoke to reduce negative mood. These findings may help to guide the development of more

effective smoking cessation treatment. The investigators are planning to follow-up on these results with a study of MORs and nicotine withdrawal to test for the effect of mu opioid receptor antagonist on the process.

A couple of things that may help to guide their future work include: 1) calculating the effect size of their findings, and 2) examining why they did not find any differences in nicotine vs. denicotinized sessions after overnight nicotine abstinence, essentially satiated vs. nicotine withdrawal conditions.

***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 received funding from an R21 during the same time period when this project was funded. However, they did not secure additional funding by leveraging funds received under this mechanism. A follow-up study to evaluate the role of MOR in nicotine withdrawal and a pharmacogenomic study are still in their planning phase.

#### Reviewer 2:

Strength: Further NIH research funding is planned.

Weakness: No applications have yet been submitted.

#### Reviewer 3:

The investigators received co-funding from NIH to support this project, R21DA027066 “Functional Characterization of OPRM1 A118G in Nicotine Dependence.” The investigators are planning to follow-up on these results by submitting an application for an expanded study of MORs and nicotine withdrawal, and plan to test for the effect of a mu opioid receptor antagonist on the process.

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

### ***STRENGTHS AND WEAKNESSES***

#### Reviewer 1:

The results of the study were reported in 2 publications. A PNAS paper in 2011 and a second manuscript published in 2012 in Psychopharmacology. A total of 2 manuscripts over the 5-year period is quite low. It should be noted that a PNAS paper is a notable achievement, keeping in mind that the number of citations is a more appropriate measure of a manuscript's relevance to its field than the impact factor of the journal where it is published.

#### Reviewer 2:

Strengths: Two peer-reviewed publications have been published in high-quality journals.

Weakness: Significant new breakthroughs which would significantly re-orient future progress in smoking cessation have not yet been consummated.

Reviewer 3:

Based on this project, the investigators published two papers, one in *PNAS*: “Human Mu Opioid Receptor (*OPRM1* A118G) polymorphism is associated with brain mu-opioid receptor binding potential in smokers,” Ray et al (2011); and a second related paper in *Psychopharmacology*,” u-Opioid receptor availability in the amygdala is associated with smoking for negative affect relief,” Falcone et al (2012). While the authors do not have plans to submit future peer-reviewed publications based on this project, it would seem as though they have rich data source to fuel some additional important papers.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

There were improvements made to the institution's infrastructure. This research project was the first to initiate 11C-carfentanil PET imaging in the Department of Radiology/Division of Nuclear Medicine at the University of Pennsylvania. Investigators can now build upon this experience for studying mu opioid receptor imaging *in vivo*. No new investigators were added or brought into the institution to help carry out this research, which is not unexpected for a project with a relatively small budget. However, funds from this project were used to support a pre-doctoral and a post-doctoral student.

Reviewer 2:

Strengths: New investigators at the institution were trained.

Weakness: None noted.

Reviewer 3:

This project supported pre-doctoral and post-doctoral training and initiated 11C-carfentanil PET imaging infrastructure in Nuclear Medicine at the University of Pennsylvania, which is now available for other researchers.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

A new collaboration was initiated with Dr. Jon Kar Zubieta at the University of Michigan who provided input on PET imaging protocols and co-authored manuscripts.

Reviewer 2:

Strength: Researchers from other local institutions were recruited for collaboration.

Weakness: Researchers outside the local area were not recruited very much for collaboration.

Reviewer 3:

This project led to a new collaboration with a research partner outside of the institution. Dr. Zubieta from the University of Michigan provided expertise on PET imaging protocols and collaborated on manuscripts. Three hospitals and/or health care professionals were involved in the research project.

***Section B. Recommendations***

**SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

Reviewer 1:

1. The sample size was too small to allow for unbiased estimation of effect size and ultimate evaluation of clinically meaningful effects.
2. About 98% of African-Americans carry the risk allele. Yet, they were excluded from this study because the minor allele was too rare in this population. This approach, independent of how justifiable it might be, tends to create more health care disparities. In this case, the group that may carry a higher burden because they are more likely to carry the risk variants are simply excluded from the analyses because it was more convenient to exclude them.
3. The effect of the *OPRM1* variant is still not clear. It appears to affect the MOR BP, but it is hard to tell what the overall effect is. Reading the PNAS paper did not help. The investigators' insight into possible mechanism or even their plan to investigate possible mechanisms is not presented.
4. Two published manuscripts at the end of a 5-year grant indicates low productivity unless the data collection lasted longer than expected. There was no evidence of such problems in the progress reports.
5. Perform formal power analyses with appropriate adjustments for the expected number of tests before deciding on the sample size of the study. Realistic assumptions about the range of relevant parameters could be made using existing data and the available literature. It is not clear what the justification was for electing to use 6 subjects per cell.
6. Dealing with rare variants in genetics can be tough. However, careful experiments could be constructed in the African-Americans. For example, homozygotes with the major allele could be compared to heterozygotes. Moreover, variants that reach allele frequencies > 90% suggest a selection effect. Population genetic work could help identify the factors that were selected for, and thus provide insight into mechanisms.

7. Statistical analyses should address the main questions first before moving to more detailed analyses. Therefore, a table that showed the effect of the genetic variant, the treatment on each brain region would have been more informative than the graphs. This is particularly true with such small sample sizes. Differences observed with a subgroup do not necessarily translate to the statistically and clinically meaningful differences when the larger groups are considered.

Reviewer 2:

None.

Reviewer 3:

1. It is unclear the extent to which the diagnostic instrument used (the MINI) comprehensively assesses both lifetime and current diagnoses for all relevant psychiatric and substance use problems. The authors may want to clarify this in future work and/or consider an assessment that fully assesses lifetime psychopathology.
2. Given that the authors did not detect nicotine vs. denicotinized session effect; to what extent might this be due to a carry-over related problem, given that they always administered nicotine in the first session? In future studies, they may want to consider counter-balancing these conditions, in order to rule out that possibility.
3. Calculating the effect size of their findings may help to guide their future work.
4. A greater understanding of why they did not find any differences in nicotine vs. denicotinized sessions after overnight nicotine abstinence, essentially satiated vs. nicotine withdrawal conditions, may help to guide any future withdrawal related work.

### **Generic Recommendations for the University of Pennsylvania**

Reviewer 1:

Applications that propose to test specific scientific hypotheses should provide power estimates. Unless it is a pilot study whose objective is to show the feasibility of a project.

The number of peer-reviewed publications can be a very good indicator and provide valuable insights in terms of an investigative team's productivity and relevance of ideas being tested to their specific fields.

### **ADDITIONAL COMMENTS**

Reviewer 3:

The research team did an outstanding job in meeting their project's objectives. The design and methods were clear and used state-of-the-art imaging technology to address the project's objectives. They thoughtfully maneuvered methodological issues and productively recruited participants, completed the study, and published results in high-quality journals. They have expanded their research collaborations, training opportunities, and research infrastructure at their institution. The project should have an important impact on improving our understanding of the mechanisms underlying smoking behavior and may contribute to the development of new

smoking cessation treatments, and the investigators plan to submit new proposals in the future to expand on their important findings.

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**Project Number:** 0865404  
**Project Title:** Validation of Imaging Markers for Use in Cancer Clinical Trials  
**Investigator:** Mark Rosen, MD, PhD

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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:

There were two stated objectives to this project: 1) to develop an infrastructure to support standardized analysis of images performed on cancer trials at the University of Pennsylvania, 2) to explore novel approaches to image analysis to improve imaging markers for cancer trials. The project met its objective by establishing an imaging core which helped to streamline processes between the cancer center and the radiology department (Aim 1) and utilizing novel imaging techniques or biomarkers to enhance the prediction of cancer outcomes.

The Clinical Imaging Core (CIC) led by Dr. Rosen did an excellent job in creating an avenue for communication between the imaging clinical scientists and cancer researchers by integrating or assigning the former into the research program of the latter. In addition, the CIC served as a liaison to facilitate communication and interaction between the basic imaging and clinical cancer researchers. This communication further enhanced by establishing a tumor response service (RECIST core) to report routine imaging results from radiology reports into a format that can be used by the clinical trial operation group in the cancer center. This is accomplished by using existing software and hardware in the radiology department.

Streamlining the process by using a web-based data entry and tracking system increases the efficiency of the system by reducing the time invested by the radiologist and hence reducing cost. The current volume of request of 40-60 cases per month is a huge accomplishment for a unit that was established only three years ago, granted that some of the infrastructure was available within the institution. In order to keep the sustainability and future expansion of the unit at the time, the CIC is implementing a fee-for-service approach and successfully recruiting new entities while maintaining the original collaboration fee free by using existing institutional funding to cover the cost.

The second objective of this project was to explore novel approaches to image analysis to improve imaging markers for cancer studies. In this regard, the focus has been extending the prior work (DCE-MRI) of the PI to the change in vascular targeted anticancer therapies. In order to apply this method as a routine clinical practice lead to adapt a different imaging technique (dynamic radial imaging).

I think the group achieved what they were hypothesizing to do in both specific aims and use of the funding from this grant to leverage institutional support to sustain the CIC which is made possible by this funding. In addition, they obtained extramural funding from the NIH and V foundation to continue their work stated in Aim 2 of this project.

Reviewer 2:

The key specific objective was to develop an infrastructure to support standardized analysis of images performed on cancer trials at the University of Pennsylvania. The project aimed to develop an operational interface between the Cancer Center and the radiology department. The progression of cancer (growth or shrinking) on present clinical trials is commonly evaluated by linear estimates of tumor size and assessing. This approach does not account for tumor necrosis or changes in proliferation. This project aimed to explore new approaches to image analysis such as adjusting size for percent necrosis (identified by the absence of contrast enhancement) or dynamic MRI perfusion measurements.

These objectives were met to a major degree through the formation of the Tumor Response Core of the existing Cancer Center. The Core is using standardized protocol and a web data entry and retrieval system for the analysis of images performed on cancer trials at the University of Pennsylvania. Investigators report improved efficiency of the Core as well as improved workflow for attending radiologists. The new Core is processing about 50 image analysis requests per month, and 10,000 tumor assessments were performed. In collaboration with Dr. Rubin, the team tested software-based tools to facilitate the analysis of linear tumor measurements from cross-sectional imaging studies.

The project also yielded precise and standardized dynamic contrast-enhanced (DCE) MRI for clinical cancer trials. The project contributed to the growth of the DCE-MRI service at the University of Pennsylvania.

As a weakness, the proposed development of new tumor metrics that adjust for tumor necrosis is not specifically addressed in the final report.

Reviewer 3:

The investigators have done an outstanding job in accomplishing the stated goals of the proposal. To a large extent, they have surpassed the original proposal, especially in the area of using DCE MRI as image biomarkers for assessment of treatment response.

The investigators have taken logical steps in the research design and carried out the studies carefully and extensively. The imaging biomarker analysis framework is well done and will provide a useful tool to the cancer research community.

The proposed work has two parts – establishing a technical infrastructure to support standardized analysis of images performed on cancer trials at the University of Pennsylvania and to explore novel approaches to image analysis to improve imaging markers for cancer trials. Both parts have been accomplished with high marks. The implementation of web-based infrastructure is valuable to Penn's cancer research community. Developing an operational interface between the cancer center and the radiology department is much needed to move clinical research forward as

the extraction of quantitative data from imaging examinations is a critical component of the imaging clinical trial. Approaches to image analysis such as adjusting size for percent necrosis or dynamic MRI perfusion measurements are very novel.

A large number of imaging events were carried out. The group has continued to expand the protocol review services of the Imaging Core. The newest expansion of these services is in conjunction with several clinical trial groups in neurological science, including the Penn Stroke unit and the Penn Memory Center.

Sufficient data and information were provided to indicate that the project met its objectives and made acceptable progress.

The data and information provided were applicable to the project objectives listed in the strategic research plan.

***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 main impact of this project would be in increasing efficiency and communication between basic imaging and clinical scientists by standardizing imaging analysis and reporting protocol. In addition, the utilization of novel and enhanced imaging techniques results in identifying changes in tumor early, which can have significant impact on health outcomes such as progression free survival time.

#### Reviewer 2:

The impact of the work performed in this project is beneficial and reasonable in light of the budget. While the project didn't lead to major discoveries, the funding led to more effective clinical cancer trials at the Cancer Center, standardization of image analysis, reduced variability, and increased power of cancer trials that utilize imaging endpoints.

No weaknesses.

#### Reviewer 3:

The beneficial impact of this project is significant for future improvement of health. It lays a foundation for future development of image biomarkers for disease detection, staging, treatment planning and therapeutic response monitoring.

Consider the value of the research completed towards eventual improvement in health outcomes. The results obtained from this project have strong value towards eventual improvement in health outcomes.

This project sets up an infrastructure to facilitate project development teams, including key members of ACC programs and imaging experts in radiology, medical physics, and biomedical

engineering, which will promote and facilitate comprehensive collaborations between University of Pennsylvania Radiology researchers and members of the translational clinical trial services of the Abramson Cancer Center. The analysis from data of their repeatability trial of DCE-MRI in lung tumor patients indicates an overall improvement in the coefficient of variation from 19% to 11%, which is a significant clinical finding if proved to be true independently by other researchers. They also completed processing for trials in targeted therapy in prostate cancer (17 subjects, 43 exams) and renal cell carcinoma (38 subjects, 63 exams). Another technical development is the methods for determining pixel-wise changes in tumor ADC values from diffusion weighted imaging (DWI) in MRI.

There is ongoing data analysis to assess the vascular effects of a radiosensitizing protease inhibitor in locally advanced lung cancer patients. The investigators also initiated quantitative DCE-CT studies and image analysis. Overall, the funding has allowed the group to start potentially high-impact research.

***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:

There was a successful application for two fundings, one federal (NIH) and another from a foundation to continue applying the new method partly developed during this grant period to a different cancer type.

##### Reviewer 2:

The authors report two grant applications submitted as the result of this project, including one NIH grant submitted 12/2009. It appears that both proposals were funded. No weaknesses.

##### Reviewer 3:

The group has successfully attained NIH support based on the data generated from the project. The NIH support provides an impetus to the success of the research.

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

#### ***STRENGTHS AND WEAKNESSES***

##### Reviewer 1:

Currently, there are no publications resulting from this project.

##### Reviewer 2:

Investigators plan to submit manuscripts in the future.

Weaknesses: So far, the project didn't yield peer-reviewed publications, licenses or patents.

Reviewer 3:

None.

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

**STRENGTHS AND WEAKNESSES**

Reviewer 1:

One undergraduate student and five post-doctoral students participated in this project.

Reviewer 2:

There was a significant improvement in the infrastructure of the grantee's institution through addition of technical personnel to the Clinical Imaging Core. This improvement will increase patient throughput. The project also contributed to the growth and technical development of the oncologic MRI Core Service and it improved access of clinicians to new imaging technologies.

No weaknesses.

Reviewer 3:

This project has provided a valuable technical infrastructure for imaging and imaging markers analysis. It has also fostered collaboration within the institution and strengthened the overall research environment at the University of Pennsylvania. New investigators and post-doctoral students were attracted to the University of Pennsylvania and received outstanding training.

***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 focus of the project has been streamlining the communication and increasing efficiency between the clinical and imaging scientists within the same institution (University of Pennsylvania).

Reviewer 2:

The project resulted in collaborations with Daniel Rubin, M.D., of Stanford University and the American College of Radiology Imaging Network (ACRIN).

No weaknesses.

Reviewer 3:

The study has allowed the PI's group to collaborate both within (e.g. Dr. Song's lab) and outside the University of Pennsylvania (e.g., Dr. D. Rubin's lab at Stanford and clinical trial groups).

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

### **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

#### Reviewer 1:

None.

#### Reviewer 2:

1. The reason for abandoning the development of new tumor metrics that adjust for tumor necrosis is not specifically addressed in the final report. Investigators should explain the reasons for changing the initial track.
2. The manuscript describing standardized and automated analyses and record keeping for tumor metrics remains to be submitted.

#### Reviewer 3:

Publications with the PI as senior or first author.

### **Generic Recommendations for the University of Pennsylvania**

#### Reviewer 3:

Overall, this is an outstanding project and the PI, as a physician scientist, has done a great job in executing the proposed research. The attainment of NIH grants also indicated the success of the project. Collaboration with an MRI physicist was very fruitful. Research in imaging biomarkers is definitely needed.

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**Project Number:** 0865405  
**Project Title:** Understanding the Biology of Residual Neoplastic Disease  
**Investigator:** Chodosh, Lewis A.

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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:

This was a preclinical breast cancer proposal from an established and well known breast cancer researcher. The main goals, as described by the applicant, were to better understand the biology underpinning "residual disease" (i.e., tumor recurrence and tumor cell dormancy) using genetically-engineered mouse models.

While mice and humans differ greatly, it is acceptable to use murine systems for these studies as similar types of experiments are far more difficult using primary human cells. Conditional transgenic mouse models had previously been generated by the PI, and a set of experiments were proposed that would take advantage of the bi-directional regulation of the driver oncogene(s) and the ability to do allogenic transfer of mammary tissue into normal mice.

The stated aims were:

- Aim 1: Identify morphological lesions containing residual neo plastic cells;
- Aim 2: Analyze the cellular components of residual neo plastic lesions;
- Aim 3: Determine if autophagy is a survival mechanism for residual neo plastic cells.

As stated, the proposed models would allow for doxycycline-regulated expression or extinguishing of transgenic c-Myc or Wnt1, and explore the major regulators in and of those pathways.

The data as presented in the final and intervening reports is focused mainly on HER2/Neu with some additional information on Ras, myc and Wnt. This shift to HER2 is not a major concern as it is a well-known human breast oncogene; however, the rationale for the shift in models was not apparent nor was it stated.

While the proposal was difficult to follow at points and many of the figures should have been developed and described more thoroughly, the PI has provided a wealth of new, novel insights into the biology of mammary tumor regression and recurrence.

Reviewer 2:

Strengths: Outstanding progress was made on each aim. The proposed methods were useful and the PI adapted methods as findings evolved. The amount and quality of the data was also outstanding. Several new hypotheses were identified.

Weaknesses: Would be nice to see a publication.

Reviewer 3:

The final report details the multiple experiments that were conducted as a part of this project in support of each of the stated specific aims. Work supporting all aims was conducted and thus the project did meet its stated objectives. The research design and methods were all appropriate and, as described, experiments were conducted in a logical and progressive manner. For the most part, the experiments proposed in the original strategic plan document were completed, and sufficient detail was provided to support that objectives were met. One exception is that in relation to Aim III, there is no mention of the proposed evaluation of CCI-779 treatment. No explanation is provided as to why this set of experiments was not conducted.

***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:

While the direct impact on human health has not been determined, the data emanating from this work should inform translational and clinical researchers into possible biomarkers of cell survival and disease recurrence during and after clinical intervention.

The stated further plans were a bit vague, with the PI stating "follow-up studies are planned under the auspices of future projects."

A more thorough discussion of how the PI intended to translate his findings to new, hopefully more clinically focused studies would have been helpful.

None the less, there are many possible important observations made during the course of this project.

Reviewer 2:

Strengths: An important clinical problem was studied and it was identified that innate immunity and autophagy have important unforeseen mechanisms for dormancy. New directions will be stimulated by this research.

Weaknesses: The work needs to be published.

Reviewer 3:

Per the investigator's own statement, no major discoveries were made by this project and no changes in outcome, impact or effectiveness can be attributed to this project. A significant

weakness of the project that further reduces its impact is that not a single paper has been published based on the data generated. The future plans for this research project are quite vague. There is some mention of efforts to pursue additional funding and to write up manuscripts for publications, but absolutely no specifics are provided.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

The PI has stated that no leveraging of funds was attempted during the granting period. DOD and Komen grants are planned.

Reviewer 2:

The PI has not received additional funding, but plans to apply for several grants.

Reviewer 3:

This project did not appear to leverage any additional funds. Again, there is mention of plans to submit additional grant applications, but no specifics are provided. This is viewed as a major weakness as it does not appear that this project has led to a compelling next set of research questions to pursue that could have demonstrable impact.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

No peer-reviewed publications, licenses or patents were generated by this project to date. It is likely, however, that publications will be forthcoming in the future.

Reviewer 2:

Not yet. This slightly diminishes overall enthusiasm, but there is every indication that this work will be published.

Reviewer 3:

It was certainly expected that this project would yield peer-reviewed publications, but none have materialized. The investigator states that he plans to submit articles for publication in the future, but it appears that follow-up studies that are a part of future projects are needed before this could occur. A clear case for why additional data are needed before the data generated by this project can be published is not made.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

No infrastructural improvements were made, and one post-doc was supported. None were recruited.

Reviewer 2:

A post-doctoral scientist was supported by this award.

Reviewer 3:

This is not specifically addressed, but my assessment is that it is unlikely that significant improvements in infrastructure were made as a result of this project. No new pieces of equipment were purchased as a part of this project, and there is no description of any new investigators that were brought to the institution as a part of this project. There was one post-doctoral fellow who was supported by 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:

There were no collaborations stated or proposed.

Reviewer 2:

The PI has made local connections that will be useful as the work progresses.

Reviewer 3:

There do not appear to be any outside research partners that were involved in this work, nor was there any new involvement with community partners.

***Section B. Recommendations***

**SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

Reviewer 1:

The lack of papers, the lack of recruitment, the lack of comprehensive training and the writing style could all use improvement. Despite this, the project is for the most part outstanding.

Reviewer 2:

Given the amount and quality of the data, it would be important to publish this work.

Reviewer 3:

Failure to publish any study results.

Recommendation: Develop a clear and timely plan for presenting study results at national/international meetings eventually leading to peer-reviewed publications.

**ADDITIONAL COMMENTS**

Reviewer 1:

While the work was generally considered to be outstanding, the lack of publications, the lack of clarity regarding the figures and the lack of clarity in describing some of the experimental approaches reduced enthusiasm slightly.

In addition, it is hopeful that the PI will attempt to translate the current findings into human samples.

Reviewer 3:

While this project did conduct a large number of experiments, as were proposed to meet its original set of specific aims, this is really all for naught if no publications come from this work and people outside of this team's investigators are not able to access the knowledge gained. This is a major weakness that can only be overcome through a clear and timely plan for publishing study findings. This project essentially failed to satisfy criteria 2, 3, 4, 5, and 6 as detailed above.

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**Project Number:** 0865406  
**Project Title:** Individualizing Breast Cancer Prevention in Primary Care  
**Investigator:** Susan M. Domchek, MD

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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:

Project summary: The investigator proposed to develop and test a multi-faceted strategy for optimizing the adoption of individualized breast cancer prevention in primary care. Specifically, she plans to: 1) determine whether the collection, calculation and provision of individualized breast cancer risk and prevention information at the time of mammography screening increases the uptake of targeted breast cancer risk reduction strategies (chemoprevention, MRI screening, BRCA1/2 testing) among women at risk, and 2) determine whether the inclusion of an individualized breast cancer risk assessment and prevention module in the electronic medical record (EMR) increases the uptake of targeted breast cancer risk reduction strategies among women at risk. The study population will consist of women between 40 and 70 who have been seen in their university primary care practice in the last 2 years. The target outcomes are: 1) prescription for tamoxifen or raloxifene in the absence of a breast cancer diagnosis, 2) receipt of Breast MRI for screenings, and 3) visit to high risk breast cancer clinic for counseling +/- BRCA1/2 testing. The investigator estimates that 15% of women will meet criteria for tamoxifen/raloxifene chemoprevention, an additional 1-2% of women will meet criteria for BRCA1/2 testing and an additional 3-5% will meet criteria for MRI screening (i.e., lifetime risk >20% but 5-year risk <1.67%). The investigator asserts that if the interventions lead to an additional 500 women at elevated risk pursuing chemoprevention, MRI screening, or BRCA1/2 testing, that between 30 and 50 fewer women in the overall population would die from breast cancer simply based upon the study itself.

A limitation of this project is that the design itself was unlikely to achieve the stated goals. The investigators asserted that breast cancer mortality would be reduced by 6 – 10% (30 – 50 out of 500) if high-risk women were successfully recruited to chemoprevention, MRI screening and/or BRCA1/2 testing. This assertion itself is not plausible, given that the study population was women ages 40 – 70 of whom over 70% are already having screening mammography. Chemoprevention reduced the number of breast cancer cases diagnosed by 50%, but has not been shown to reduce overall breast cancer mortality in this population. It has not been shown that MRI screening improved mortality beyond screening mammography. BRCA testing does not itself decrease breast cancer mortality in the absence of intervention such as bilateral mastectomy/oophorectomy, but even in that circumstance, the projected breast cancer mortality reduction is only 3- 5%.

The fundamental outcome of this project was to develop an Epic-based risk assessment model. While the investigators were able to report on the findings of their population, it is unclear that any actual benefit to these women occurred. They do not know how many women sought out high-risk evaluation by virtue of the assessment model, nor is it clear that any such evaluation led to improved outcomes. It appears that most of the primary outcomes defined in the project's proposal were abandoned as being too difficult to complete.

Reviewer 2:

Original project aims are in *italics*. The project encounters substantial barriers to implementation. This caused the project to fall short of its perhaps overly ambitious aims. However, the work done is quite valuable and perhaps more in keeping with the size of the grant funds. The resulting infrastructure changes and pilot work for other grants is very valuable.

*Aim 1. To determine whether the collection, calculation and provision of individualized breast cancer risk and prevention information at the time of mammography screening increases the uptake of targeted breast cancer risk reduction strategies (chemoprevention, MRI screening, BRCA1/2 testing) among women at risk.*

Aim 1 was not met as the report included no data on health behaviors (high-risk women's use of chemoprevention, MRI screening, BRCA1/2 testing); the researchers are continuing to gather and analyze behavioral outcomes data. The final report provided data on women's intentions. This seems like a more reasonable outcome for the modest grant funds supplied. The project exceeded the objective stated in the final report of enrolling 500 women by enrolling 821 women, of whom 813 had complete data. Thirty-nine percent of the 813 were African-American, making this a valuable dataset. The goal for enrollment is unclear in the initial proposal, so it is not clear whether the project met its initially-stated goal.

*Aim 2. To determine whether the inclusion of an individualized breast cancer risk assessment and prevention module in the electronic medical record (EMR) increases the uptake of targeted breast cancer risk reduction strategies among women at risk.*

Aim 2 was not met as the researchers did not assess impact on health behaviors. The project incorporated a risk assessment into a standard electronic health portal. This is an important increase in the Pennsylvania research and care infrastructure. 913 women have now used this risk assessment portal. Achieving this outcome is an important result of the grant.

*Aim 3. To explore whether the effects of these interventions are independent or synergistic.*

This aim was not met as it was dependent on Aims 1 and 2 being met.

Reviewer 3:

Strengths: This proposal (Project 6) aimed to conduct a population-based study on the possibility of enhancing breast cancer prevention strategies among high-risk women by providing them, as well as their primary care providers, with specific breast cancer risk assessment. This endeavor was undertaken based on the fact that approved chemopreventive agents like tamoxifen and raloxifene were prescribed at significantly lower frequency than what

was warranted, given the risk profile of the patients. The goals of this study were highly laudable and if it had been conducted in its entirety, it would have made a significant impact on the field.

Overall, this study was proposed to be conducted on a large percentage of the 19,000 breast cancer patients or those who were at high risk who sought treatment at one of the six clinics involved in the study. The study met part of its objectives, especially Aim 1, and completed an analysis of about 800 patients. This initial analysis was conducted inline with what they proposed, but the quasi-experimental study did not cover the large population they had anticipated to cover. It appears that the focus of the study shifted slightly to include a SNP analysis, which was funded by a different grant. Inclusion of this aspect enhanced the quality of the data, but the emphasis was more on the SNP aspect, rather than the chemoprevention aspect as originally proposed.

Weaknesses: These include the fact that only Aim 1 was completed, and that too only partially. As the investigator states in the progress reports, they had difficulty in extracting and interpreting EMR data and were not able to build a breast cancer risk assessment module into the EMR. This greatly hampered the execution of Aims 2 and 3; it appears that the investigators have overcome this hurdle by using a baseline risk assessment tool that was developed by the investigators for PCIPS and recently adapted to the patient portal of the EMR. The investigator believes that the late development of this module due to technical reasons has prevented the successful execution of the current project but has laid the foundation for conducting similar studies in the future. Thus, while Aim 1 has been conducted with at least partial success, Aims 2 and 3 have not been completely executed.

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

### ***STRENGTHS AND WEAKNESSES***

#### Reviewer 1:

The project has created a database that may be of utility to the investigators in terms of tracking women at varying breast cancer risks within their primary care system. It is not clear that these outcomes constitute valuable new information. Most disappointingly, the investigator has not provided information about how to better recruit women for high-risk counseling and intervention, which was their primary stated goal.

#### Reviewer 2:

The impact of the project on human health is unknown. However, the improvement in infrastructure for patient care and for research is promising. The researchers will continue to work with the tools developed here and submit an R21 grant application to the NIH. The researchers will also pursue the current research plans as part of a separate grant that is already funded.

Reviewer 3:

Strengths: The study as proposed would have had a significant beneficial impact on subjects who are at high risk for breast cancer. The studies proposed here would have encouraged them to obtain additional monitoring based on their overall risk assessment; this would have included mammograms and MRIs for those patients who are at highest risk.

Weaknesses: While the study was initiated with the intention of assessing how presentation of breast cancer risk data encouraged women at high risk for breast cancer to obtain additional screening, the study did not achieve its end goal, due to a variety of technical reasons. Thus, the benefit of this study, as executed is rather limited.

***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 successful in obtaining additional funding from other sources.

Reviewer 2:

This is an outstanding aspect of the grant. The grantee reports using project data and infrastructure to obtain two large NIH grants (\$2,375,235) awarded to Katrina Armstrong, who is not listed on the Pennsylvania grant, but who is pre-eminent in this field. This leveraging represents an outstanding leveraging of this small state grant. At the time of the report, the PI planned to apply for an R21 grant from the NIH (these are usually up to \$275,000).

Reviewer 3:

Strengths and weaknesses: It is not clear from the application or the progress reports whether the authors succeeded in obtaining additional funding. They mention the submission of a new R21 grant, but it is not clear whether it was submitted. At the same time, the investigators have obtained an additional grant from the NCI to allow them to assess the correlation with SNPs with breast cancer risk. This aspect of the project has made significant progress. Overall, they have not yet succeeded in obtaining extramural funding.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

The investigators have prepared a manuscript for submission entitled “Incremental impact of breast cancer SNP panel on risk classification in a screening population of white and African American women.” While the study is interesting and was made possible by virtue of creation of their database, the study itself is not specifically responsive to the investigator’s stated aims of this project.

Reviewer 2:

The PI reports one paper published in 2013 on data that appear to be tangentially related to the study and another manuscript in preparation. This paucity of research products is a weakness of the study. The PI should produce at least one (and preferably several) papers as the result of a study like this.

Reviewer 3:

Strengths: The investigators have included one research article in Breast Cancer Research and Treatment that was partially funded by this funding mechanism. The main focus of this manuscript is on snps as predictors of breast cancer risk; this is obliquely connected to the current project. Given the technical challenges they have faced in executing Aims 2 and 3, this publication is a testament to their efforts to accomplish the maximum amount of work that was proposed.

Weaknesses: Despite the one publication partially funded by this project, it appears that the productivity in the context of additional funding or publications was lower than what one would have expected of this research team.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

The investigators were successful in creating a risk assessment model and inserting it into their primary care record system, which is a benefit to institutional infrastructure for patient care.

Reviewer 2:

Improvement in research infrastructure is the main contribution of the study. The project incorporated a risk assessment into a standard electronic health portal. This is an important increase in the Pennsylvania research and care infrastructure. The study report mentions work by a doctoral student though it is not clear whether the grant funds supported that student.

Reviewer 3:

Strengths: While the funds did not support the recruitment of junior faculty or graduate students, they did help support research staff, who have been trained in the use of the software and in using the risk assessment module in the EMR. This can be considered a strength, since it will allow the investigator as well as other scientists at this institution to conduct similar studies in the future.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

No major collaborative relationships appeared to be established by virtue of this project.

Reviewer 2:

The project did not appear to lead to new collaborations outside the institution. This is neither a strength nor a weakness of the work done. The number of health care professionals or hospitals reached was unclear. The implementation of the project into a widely used electronic medical system was an important outcome.

Reviewer 3:

Strengths: The investigators are conducting this study in partnership with various primary care centers as well as imaging facilities affiliated with their institution. Thus, this has been a collaborative effort between various scientists.

***Section B. Recommendations***

**SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

Reviewer 1:

1. The database was originally planned to study their screening population to identify high-risk subgroups. While this might have been an interesting pilot study, the investigators would benefit from finding a different study population for their analysis. Instead of studying a population that is already undergoing mammographic screening, they might consider studying a population that fails to undergo screening to see if increased breast cancer risk is a significant problem.
2. The study was intended to provide new information about risk reduction strategies, but the investigators failed to evaluate the population itself to determine what makes them more or less prone to pursue risk reduction. They seemed to assume that if primary care providers were given more specific information about breast cancer risk, that patients would then comply with recommendations and that this would automatically translate into better outcomes. The database that was created might provide some inroad into future studies about social and cultural issues that might improve patient compliance or resistance.
3. The investigators did not appear to have a mechanism set up to determine when women would have high-risk evaluation and change behavior. Setting up this system, which seemed basic to the original proposal, might have better achieved this outcome.

Reviewer 2:

1. Ask the PI to supply the manuscript being developed on the study data and information on any conference presentations.
2. Ask the PI to report the results of the behavioral outcomes for Aims 1-3 as they become available.

Reviewer 3:

1. The studies in Aim 1 have been conducted partially. The studies proposed under this aim are highly relevant to the patient population; it is recommended that the study be expanded to a larger number of patients. As mentioned earlier, the proposal aimed to focus on a large percentage of 19,000 patients seeking treatment at six affiliated facilities, but only less than 900 were covered in the study.
2. It would be highly beneficial if studies in Aims 2 and 3 could be conducted now as proposed in the original application, using the risk assessment module they developed for PCIPS. These two aims formed the major crux of the original application.
3. It is recommended that additional publications be submitted, if this is feasible, based on the data they generated in Aim 1. It is not clear to what extent these studies overlap with those funded by another grant the PI refers to, or to the R01 grant awarded to Dr. Armstrong.
4. It appears that the investigators were not able to leverage this funding to obtain additional extramural grants. While the PI mentions submitting a new R21, grant, its disposition is not known. Submission of additional grants will greatly facilitate continuation of this important study.

**Generic Recommendations for the University of Pennsylvania**

Reviewer 3:

This is an interesting and relevant population sciences project that was hampered by technical difficulties; the investigators could not incorporate a breast cancer risk assessment module into the EMR. This has resulted in partial execution of the project and low overall productivity. Identifying such potential weaknesses and challenges at the outset of the project will lead to successful execution of the project and better return on investment.

**ADDITIONAL COMMENTS**

Reviewer 1:

The investigator planned to: 1) determine whether the collection, calculation and provision of individualized breast cancer risk and prevention information at the time of mammography screening increases the uptake of targeted breast cancer risk reduction strategies (chemoprevention, MRI screening, BRCA1/2 testing) among women at risk and 2) determine whether the inclusion of an individualized breast cancer risk assessment and prevention module in the electronic medical record (EMR) increases the uptake of targeted breast cancer risk reduction strategies among women at risk. It does not appear that either of these goals was met. Although a database was successfully created and staff were trained to use it, none of the other goals of the project have stated explicit outcomes or findings.

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**Project Number:** 0865407  
**Project Title:** Clinical and Molecular Predictors of Responsiveness to  
Angiogenesis Inhibition in Advanced NSCLC  
**Investigator:** Corey J. Langer, MD, FCAP

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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:

Although the project did not meet the objectives stated in the strategic plan, a strength was noted that while Objective 1 sought to assess serum, tissue and radiological markers of response in advanced NSCLC treated with bevacizumab and erlotinib or naïve NSCLC treated with bevacizumab and a cytotoxic regimen, the investigators ended up proposing to assess single nucleotide polymorphisms (SNP). The project moved to genomic markers early on when it was clear analysis of protein changes were not useful.

Related to the strength described above, a weakness is that the sample/patient analysis isn't finished nor is the SNP analysis. This is primarily due to slow accrual and inability to identify a basic scientist to complete the sequencing and evaluation of the resulting data.

Another weakness is in the final report. The investigator claimed to have exceeded enrollment goals, but I cannot track the numbers (32+). I think this may be due to using samples from patients enrolled in similar trials, but this is not clear.

A final weakness is that the second half of the project that endeavored to use novel imaging approaches compared with conventional measures of response was unable to be completed due to issues with support for the co-investigator charged with those projects.

#### Reviewer 2:

The initial proposal was to study a number of tissue and serum markers in advanced NSCLC who are slated to receive bevacizumab in combination with either erlotinib or other chemotherapy. Due to bevacizumab's toxicity, the authors proposed to study a number of correlative parameters including serum levels of multiple factors, tissue histology and various radiographic imaging technologies to predict who would respond to the chemotherapy drug and spare predicted nonresponders which would minimize cost and morbidity.

Shortly after beginning the protocol, the authors reported most of the planned investigational biomarkers had been discredited in other studies and abandoned the search. In the meantime, they had banked biospecimens from 40 patients half of whom had pemetrexed or pemetrexed in

combo with bev. They plan to examine other undefined markers in order to make use of the collected biospecimens.

The authors state that the University of Pennsylvania contributed about 30% of patients to two IRB approved clinical trial protocols. They also state that the proposal served to fund the research nurse along with establishment of the biospecimen repository.

This represents a major change to the proposal, but the explanation seems reasonable since many labs have been working on biological correlates with targeted chemotherapy. Such correlates have not been surprising. Nonetheless, useful clinical trial data has been collected.

The project on tumor microvessel density was not discussed in the final protocol and was based on the idea of the mechanism of bevacizumab with blood vessel development.

The second project reported, which was based on imaging correlates was similarly shelved, and was not performed.

The main strength of this project was the completion of clinical trial information on bevacizumab, but major weaknesses include the failure to complete two of three proposed projects.

Reviewer 3:

Strengths: The investigators were able to bank biospecimens on about half of their target population, but have not yet conducted the analysis. They plan to combine these samples with about 20 additional samples, which would allow reaching a target of about 30 total patients.

Weaknesses: The biospecimen project has not yielded any data to date (only specimen collection), and the imaging project was never performed.

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

**STRENGTHS AND WEAKNESSES**

Reviewer 1:

It is likely that these trials will find that bevacizumab-containing therapies will improve lung cancer survival, but the data herein are not mature.

The investigators have immature data and analysis of these data will determine the significance.

There were no major discoveries or new therapeutic strategies that have emerged from this project.

Reviewer 2:

There appear to be no obvious future plans directly resulting from this project.

The beneficial impact relates to the findings that bevacizumab has a beneficial role in the treatment of advanced NSCLC, but has failed to contribute much to our understanding of the mechanisms of action or predicting the patients who may benefit.

This beneficial impact was in concert with other trials, thus it is uncertain how much the funding from the present trial contributed to our understanding of bevacizumab. There are no major discoveries of insights from the present trial.

Reviewer 3:

Strengths: The promise of a useful biomarker of response to antiangiogenic therapy remains hopeful, but many larger studies have failed to achieve this.

Weaknesses: This study is likely too small to identify new biomarkers of response, but could help validate existing ones (which are still few in number and far from established). Therefore, this work is unlikely to significantly impact the lung cancer population, and as noted above, has not generated any data, to date.

***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:

There was no leveraging of funds, and the investigator will apply for additional funding to facilitate completion and extension of the clinical studies.

Reviewer 2:

No additional funds are reported as being leveraged from this project. There appear to be no proposals for further work and funding in the future.

Reviewer 3:

Strengths: The project did use resources to assist with another clinical trial recruitment/accrual in a similar theme.

Weaknesses: The leveraging of funds reported by the PI is relatively minimal and no grants have been secured.

***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:

A weakness of this project is that no patents or licenses were applied for nor are there any publications that directly relate to the strategic plan objectives. IRB-approved clinical studies associated with the studies reviewed herein have been presented as a meeting abstract and a

manuscript. Once the data are mature, the investigator intends to submit abstracts for clinical research meetings.

Reviewer 2:

No patents or commercial opportunities arose from this project. In the final report, the authors report presentations at various meetings but do not report any publications acknowledging the Pennsylvania funding. The authors report plans to apply for further funding for projects related to this study, but report no submitted applications or funding obtained.

Reviewer 3:

Strengths: None.

Weaknesses: No publications or other results were reported.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

A strength of the work is that the primary investigator finds that his institution recognized the importance of angiogenesis inhibitors in advanced NSCLC and have made these trials a part of the Genetics and Environmental Interaction initiative.

A weakness of this project is that no new researchers were added to this project, and this was a concern with respect to the inability to complete the second and third objectives of the strategic plan.

Reviewer 2:

There are no reported infrastructure improvements. No new researchers/faculty were added as a result of the project. No students/post-docs were reportedly involved in the project. The author states that the project helped to jump-start the further development of clinical trials of NSCLC at his institution that has led to his becoming the North American PI for a major trial.

Reviewer 3:

Strengths: This project supported the infrastructure by employing a research coordinator and helping with biospecimen collection and storage.

Weaknesses: No new researchers or students appeared to be involved.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

A strength was that two new studies arose from this proposal and resulted in a collaboration with a nearby institution.

A weakness was observed in the lack of engagement of researchers outside of the institution, no commercialization, and no new community involvement.

Reviewer 2:

The authors report significant clinical trial accruals resulting from two additional trials associated with the Pennsylvania funding. No obvious interaction with other Pennsylvania hospitals is reported.

It is unclear how much the present proposal contributed in view of the other two reported IRB-approved clinical trials being responsible for most of the data collection.

Reviewer 3:

Strengths: There was report of collaborations on other clinical trials relating to this project, but not directly leading from this project.

Weaknesses: It is unclear that the other clinical trials, including one jointly with Fox Chase, resulted from this work (more likely were just in the same theme of research).

***Section B. Recommendations***

**SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

Reviewer 1:

1. Accrual into trials aimed to answer Objective 1 (Aim 1) was slow and as a result, data were not evaluated at the time of the final report. It was hard to determine how many separate clinical trials contributed samples to address Aim 1, but the slow accrual and change in plan to evaluate SNPs instead of tumor proteins seemed to delay evaluation of data. Further, because a new treatment protocol was in place in the trials, the measured outcomes (PFS, RR, OS) required waiting many months for a change in patient status. The recommendation is to have viable initial and alternative plans of action. Because SNP analysis was not the primary molecular outcome to be measured, no investigator or contractor was identified to prepare and analyze the samples, thereby delaying reporting.
2. For the 2<sup>nd</sup> and 3<sup>rd</sup> objectives (Aim 2) in the strategic plan that related to radiographic correlatives for outcome and staging of tumors, the project suffered from loss of departmental support for the co-Investigator in charge of this study. The PI had no backup plan for completion. I would expect that each objective would be constructed with an alternative approach in mind. Perhaps, the PI could have sought the same expertise outside of the institution.

Reviewer 2:

1. The author needs to better recognize the contribution of this grant to publications.

2. The author states that two of three proposed projects were abandoned after attempts to jump start it. Mechanisms to perform studies should be in place that such problems should not occur, e.g., assign projects to post-docs or students.
3. It is surprising that no post-docs, graduate or medical students played a role in this study. There should be more training opportunities, e.g., to jump start a project.

Reviewer 3:

1. This project is borderline between favorable and unfavorable because, to date, only half of the target specimens have been collected and no data have been generated. To be consistent with the expectation of a favorable rating, the investigators need to perform the additional work and analysis on the existing biospecimens in the next 6 months and provide clinical correlative survival and response data. Ideally this should result in a report (publication or presentation at a meeting) of their results within the next 12 months.
2. The imaging work was not completed. In the opinion of this reviewer, it would not contribute significantly to this work at this point and does not need to be initiated. The grantees have indicated that they returned this portion of the funding.

**Generic Recommendations for the University of Pennsylvania**

Reviewer 2:

The institution should help encourage involvement of basic lab researchers and students to see that proposals are given a fair chance to produce results. Bevacizumab has nonetheless become part of the chemotherapy regimen offered to patients at least partly due to the funding of this project.

**ADDITIONAL COMMENTS**

Reviewer 2:

It is unfortunate that none of the biological/imaging endpoints were achieved. Nonetheless, it seems that the Pennsylvania money was used as seed money to help open other clinical trials related to bevacizumab that accrued well. The grant also funded a biospecimen repository that the authors state will be used to examine other newer relevant biomarkers. This still remains speculative with no hard research plan. I suspect many of the biomarkers that were reported as being irrelevant were close to being published as such at the time of writing.

It is somewhat puzzling that the authors could not "jump start" the imaging proposal or the blood vessel density study as proposed. No real explanation is given.

It is also surprising that the author reports no publications acknowledging this funding, since the other reported trials were "linked" to the current proposal.

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**Project Number:** 0865408  
**Project Title:** Reprogramming Cells in Studies of Heart and Lung Development and Repair  
**Investigator:** John Gearhart, PhD

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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 objectives were only minimally met, and in Aims 2 and 3 the work did not directly address the stated objectives in the strategic research plan. No publications were reported from the work. In Aim 1, the work primarily recapitulated previous published work, albeit using a different endpoint for monitoring cardiomyocyte differentiation. However, there is no evidence that the described approach yielded a feasible strategy for producing cardiomyocytes or their progenitors that could be used clinically or therapeutically. The results reported represent only a minor incremental advance. In Aim 2, the studies merely collected tissue samples for isolation of cells, which the investigators used to confirm feasibility of generating iPSCs. There is no progress directed at the stated aim of uncovering the molecular basis for the various congenital heart diseases being studied. The final aim showed similar lack of progress toward the stated goal of determining whether non-pulmonary cells could be reprogrammed to the lung epithelial lineage and whether Wnt signaling is important in lung epithelial regeneration. Irrelevant work was described confirming the importance of Wnt in lung development, but results into the feasibility of generating lung epithelial cells from non-lung cells were lacking. The findings on miR02/367 are of potential interest, but were not investigated in the context of non-lung cell differentiation into lung epithelial cells.

#### Reviewer 2:

The research team performed a significant amount of experiments towards the objectives, as originally proposed. Aim 1: They developed a system that will allow them to monitor the success of “direct reprogramming” and began evaluating a number of transcription factors that will be used for direct reprogramming. Aim 2: This aim was designed to generate iPSC from patients with heart disease. The aim was only partially accomplished, as one of the co-investigators that was in charge of the specific experiments left. Aim 3: The experiments in this aim were a combination of in vitro and in vivo studies. Some of the in vitro reprogramming of non-pulmonary cells into lung epithelium was done; however, the research team took a different direction than was originally proposed. The in vivo experiments were not done.

The original design and methods of analysis were appropriate for the proposed study. However, as indicated above, the studies proposed for Aims 2 and 3 were not entirely accomplished. Aim

2 was not achieved due to the absence of the co-investigator in charge. Aim 3, took a different route, and although interesting data was achieved, they did not accomplish the goal.

Significant and innovative data was achieved by the research team. However, for Aims 2 and 3, the totality of the data could not definitively prove or disapprove the research hypotheses. Most of the data was indeed developed according to the original experimental plan.

There were changes to the research plan of Aim 3, and the team has acknowledged these changes in the previous progress reports. The experiments of Aim 2 and 3 were not completed and the team reported it only in the final progress report.

The project resulted in a large amount of experimental data. The data presented was very relevant to the proposed study; however, it failed to achieve all the goals, as originally proposed and expected. It must be noted that the research team had made significant progress in the field of cardiac disease, and thus, the data that was obtained may be considered as acceptable progress.

Strengths: Development of methods to screen for transcription factors that can facilitate direct reprogramming. Establishment of a preliminary list of transcription factors for direct reprogramming. Identification of novel miRNA to promote lung repair and regeneration. Reprogramming of cells from patients with cardiac disease.

Weaknesses: The studies of Aim 2 were only partially accomplished.

#### Reviewer 3:

The project did not meet the stated objectives. The original application has three specific aims. Aim 1 is to reprogram adult cardiac fibroblasts to cardiac progenitor cells. This aim was supposedly to include both human and mouse fibroblasts, but the reported result is on mouse only. There is zero data related to humans. The mouse data is at a descriptive stage and the outcome for the in vivo studies is not certain. Aim 2 was planned to recruit congenital heart disease children patients and to generate iPScells from these patient's fibroblasts. The report results indicate the additional recruitment of 3 more patients is needed. However, with the departure of Dr. Gruber from the University, the project has suddenly stopped. It is not clear if the project will be continued. In addition, it is also not clear if iPS cells/cell lines have been developed from these CHD fibroblasts from these patients. Aim 3 was to look into the potential of microRNA as the tools to generate both heart and lung progenitor cells for injury and repair purposes. Impressive studies were shown in the lung system but no results related to the heart area were reported. However, most of microRNA studies are limited in the mouse model. The application of the study to humans is not sure. Further, there are other funding sources in addition to this program's funding related to this aim seen in Dr. Morrisey's lab, that may raise some concerns on the grantsmanship of the application. At the publication level, Aim 1 has a paper submitted but the outcome is not certain. Aim 2 has zero publications. Aim 3 has 4 publications, but they are related to lung progenitor cells in injury and repair. Lastly, there is also a lack of collaboration between lung and heart studies in the program. There is no collaborative evidence between the PI and Dr. Morrisey.

The original research design is acceptable. However, there is a lack of discussion on the species difference between human and mouse. Thus, the finding in Aim 1 of mouse studies may not be applicable to humans. The same is true for the mouse microRNA approach not being applicable to humans. In addition, the departure of Dr. Gruber of Aim 2 may further damage the overall research design since there is no new scientist with the same skill and commitment identified to replace Dr. Gruber.

As mentioned above, the mouse data may not be applicable to humans, unless there is strong evidence to support the claim. Thus, the data generated in Aims 1 and 3 of mouse cell studies are not in line with the research plan for human cells.

There is no explanation on how difficulties were to be overcome. The departure of Dr. Gruber from the project should be immediately addressed in order to sustain the project.

A preliminary demonstration that both the mouse cell studies in Aims 1 and 3 can be used for human cells would be helpful.

***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 described work has minimal impact on the field of regenerative medicine and is primarily of confirmatory value. The ability to reprogram cells is well documented; hence, the stated objectives are only of incremental value given that they are designed mainly for confirmation using slightly different approaches. The progress was limited and thus the impact is minimal. The future plans for the project are not adequately articulated.

### Reviewer 2:

Heart and lung disease are the number one and three causes of death and illness in the United States. The ability of the heart and lungs to repair themselves is very limited and poorly understood. The proposed research aimed at understanding how these two organ systems respond to injury as well as try to affect repair. As such, successful accomplishment of the proposed goal has a potential to have a dramatic impact on both human and financial costs of these illnesses. The data generated on differentiation of cells for heart and lung repair could improve the outcomes of people with cardiac and lung disease.

At this stage, the research is in its basic stage, so it is hard to predict if it will eventually have a significant impact on human health. The experiments in Aim 2 were supposed to yield some data for development of new drugs and prediction, but this aim was not completed.

With the reprogramming of cells to a cardiac fate, the research team has opened a new and potentially transforming modality for treating heart disease.

Strengths: Preliminary data that may shed new light on generating new cell sources for treatment of heart and lung diseases. Identification of agents that may promote lung regeneration.

Weaknesses: The data did not amount to achieve tangible outcomes that can directly impact human health. No new drugs/treatments were developed.

Reviewer 3:

Based on the progress report and the statement, the impact of the studies is not high. For Aim 1, it is a descriptive study on the generation of mouse iPS cells. Similar approaches with the same findings have been reported before. The novelty of the study is not high. For Aim 2, with the lack of the project leader's input, the impact is non-existent. Aim 3 is on mouse lung cell repair, but the significance of the finding to a human setting is uncertain.

As stated above, the significance of this project for improving health is none since there is no progress report related to human cells and tissues.

There is still a long way to go towards eventual improvement in health outcomes since critical experiments relevant to human cells and tissues have not been done.

There is some progress with mouse fibroblast-derived iPS cells for cardiomyocyte development, but it is uncertain whether such an approach can be implemented for human cells. Without this development, Aim 2 is difficult to carry out. Aim 3 is largely mouse microRNA, and the existence of similar microRNA in humans has not been addressed. Therefore, the current studies are still far from having a significant contribution with regard to producing major discoveries, new drugs and new approaches for prevention, diagnosis and treatment.

The progress report did not specify any future plans for this research project . It is assumed that the future focus is on the human relevance of the study.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

No attempt was made in this regard, and also not stated in the strategic plan.

Reviewer 2:

No leveraging of funds was listed. The researchers plan on submitting grants to the NIH and to the AHA later this year.

Strengths: Generation of a significant amount of data that can be turned into research proposals.

Weaknesses: No additional funds were received and/or applied for.

Reviewer 3:

The PI has no other RO1 grant funding other than this. There is an indication that the PI plans to submit an application based on their limited data in mouse iPS cells derived from fibroblasts. However, the content of the application is not clear. The co-Investigator, Dr. Morrissey does have an excellent funding record. It is not clear if these fundings are new and derived from the funding of this proposal. Despite this, there is no indication if Dr. Morrissey will submit a grant application based on the current findings in this application.

It is not clear at this moment whether leveraging of funds will occur. The researchers plan to apply for additional funding in the future as is indicated in the progress report. However, the content of the application and the relevance of the application toward this proposal are not clearly described.

***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:

None in any of these categories- which reflected the lack of progress. Only one manuscript is being reviewed for submission.

Reviewer 2:

Four manuscripts were published as a result of this research project. One manuscript is still pending review. Manuscripts were published in high-impact journals, which justifies the low number of total manuscripts published/submitted.

Strengths: Significant contribution to advancement of the scientific understanding in the field.

Weaknesses: None.

Reviewer 3:

As stated before, Aim 1 headed by the PI has resulted in one submitted paper this year, but the fate of the submission is not disclosed. It is quite disappointing that there is no other publication relevant to this grant generated from the PI's lab in the past years. For Aim 2, there is no publication listed and the progress report is incomplete. For Aim 3, progress is excellent with 4 relevant publications. However, it is not clear if publication with a human focus is planned for Aim 3.

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

**STRENGTHS AND WEAKNESSES**

Reviewer 1:

The purchase of several pieces of equipment (including a confocal microscope) has been contributory in this regard. Only one post-doctoral fellow worked on the project; otherwise, there is no evidence that new investigators were recruited to the institution to conduct research on the project. Overall, however, the project has not substantially enhanced either the quality or capacity for research at the grantee's institution.

Reviewer 2:

Imaging (microscopes) and chemistry analysis (HPLC) equipment were added. No new investigators or researchers were brought into the institution to help carry out this research. One post-doc participated in the research.

Strengths: Significant improvement in the analytical tools of the institution.

Weaknesses: No new investigators were added.

Reviewer 3:

It is not clear if this funding had any significant impact on the quality and capacity for research at the University of Pennsylvania. Both the PI and co-Investigator, Dr. Morrisey, belong to the Regenerative Medicine group. In some sense, the funding may help the program. However, the departure of the project leader of Aim 2 raises a concern on the impact of this grant to the institution. In addition, the interaction between the PI and Dr. Morrisey is not evidenced. There was some improvement made to infrastructure at the equipment level, such as the purchasing of confocal and Olympic microscopes, etc.

It is not clear whether any new investigators were added or researchers were brought into the institution to help carry out this research. The first person needed is to replace the project leader of Aim 2.

Funds were used to pay for research performed by a post-doctoral student.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

The project did not lead to any extramural collaboration, and no plans were indicated in this regard.

Reviewer 2:

It was not indicated that any collaborations occurred or that any are planned as a result of this research.

Strengths: There is a potential for new collaborations.

Weaknesses: No new collaborations were made so far.

Reviewer 3:

The progress report has not indicated there is a plan to lead to collaboration with research partners outside of the institution or if there is new involvement with the community.

There is no clear indication that the researchers are planning to begin any collaborations as a result of the research.

***Section B. Recommendations***

**SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

Reviewer 1:

1. The lack of progress is a key weakness, and the obvious recommendation is to devote greater effort to address the stated objectives. The lack of progress in Aim 2 was implied as being due to the premature departure of Dr. Gruber, thus prompt identification and recruitment of a replacement should have been a priority.
2. The work is mainly of incremental value since it mainly focuses on repeating or extending previous work without providing significant new insight into development of iPSCs or direct reprogramming of cells into an effective or feasible therapeutic approach. More novel strategies and approaches are recommended.

Reviewer 2:

1. Continue with the proposed studies of Aim 2: generate more iPS lines from patients with CHD in order to accomplish the goal.
2. Develop the finding of Aim 3, miRNA regulation on lung cell differentiation, to a treatment approach.
3. Use the preliminary data and study design to apply to more research grants.
4. Team up with other investigators to complete the studies proposed for Aim 2.

Reviewer 3:

1. The PI's productivity is low.

Recommendation: The PI should establish more research collaboration and recruit a new project leader to replace Dr. Gruber for Aim 2. Aim 1's study is very descriptive and therefore, there is only one submitted paper. The PI should carry out some of these studies with a more mechanistic feature in order to increase the productivity.

2. Lack of a project leader to focus on Aim 2.

Recommendation: With the departure of Dr. Gruber from the University, there is a need to recruit the new project leader to focus on Aim 2. Aim 2 is the most important study, and it is the main purpose of this grant application.

3. Because of the departure of Dr. Gruber, the progress report for Aim2 is incomplete.

Recommendation: To recruit a new project leader with similar research training and commitment to this research to carry out the work. It will be an advantage to have this person to have ongoing research in this related area.

4. Most of studies are mouse cells. There is a lack of research relevant to the proposal's human health relevance study.

Recommendation: Research relevance to human health is important for this project. Investigators have to demonstrate a similar finding from mouse to human in order to sustain the research impact of the study.

5. There is no plan to address the species difference between human and mouse in these studies.

Recommendation: Since it is relatively easy to generate iPS cells from mouse fibroblast with so many publications in the literature, the PI should attempt to extend his study to iPS cells derived from human fibroblasts. For Aim 3, the use of microRNA involved in mouse lung injury and repair is excellent work. However, these microRNA may not be applicable to humans. Therefore, there is a need to look into this deficiency by addressing it and searching for new microRNAs relevant to human cells.

### **Generic Recommendations for the University of Pennsylvania**

#### Reviewer 1:

It may be helpful for the institution to designate an oversight committee to monitor progress and evaluate the needs of the project to achieve the stated objectives.

#### Reviewer 3:

Lack of collaboration.

Recommendation: The institution should encourage research collaboration. There is no clear indication if the PI and co-Investigator of Aim 3 collaborated, or even if they were in the same regenerative medicine center.

The departure of the Project 2 leader.

Recommendation: To have the position filled as soon as possible.

### **ADDITIONAL COMMENTS**

#### Reviewer 3:

The progress report contains some progress for Aim 1 and lung studies for Aim 3. For Aim 2, there is no progress. In addition, there are several weaknesses in this progress report. These

weaknesses are: 1) The PI's productivity is weak; 2) The departure of Dr. Gruber who is responsible for Aim 2; 3) The progress report for Aim 2 is incomplete; 4) Most of studies are mouse cells, there is a lack of research relevant to the proposal human health relevance study, 5) Lack of the indication of research collaboration among the investigators, and 6) There is no plan to address the species difference between human and mouse in these studies.

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**Project Number:** 0865409  
**Project Title:** Genome-based Bio-marker Discovery and Systems Biology Engineering  
**Investigator:** Junhyong Kim, PhD

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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 overall aim of this project by the Penn Genome Frontiers Institute led by PI's, Dr. Junhyong Kim and Dr. James Eberwine, is to develop novel biomarkers using high throughput genomic sequencing as well as computational modeling and analysis and micro and nano engineering. Two aims were proposed including, 1) development of novel biomarkers for normal and diseased states in humans using high throughput sequencing, cell based screening, bio-photonics and single cell genomics and in Aim 2) develop systems model of identified biomarkers and develop single platform diagnosis devices using identified biomarkers. In order to understand system biology and to develop platforms for specific biomarkers, a series of subprojects were proposed including single cell transcriptome, RNA-RNA and RNA protein interaction and single cell imaging as well as microfluidics for lab on a chip assay along with using a system to model circadian rhythm manifestations to drug deliver over a three-year project timeline.

**Strengths:** Overall, the investigators have been highly successful and innovative in this three-year grant funding period in this outstanding grant proposal. They have been able to develop several novel technologies based on cutting-edge genomic technologies and use of computational biology and synergy of the two PIs, including single cell analysis as well as novel biomarkers for system biology such as circadian rhythm.

In this three-year grant, the investigators had several successful applications. For Aim 1 studies, they initially developed a novel assay to monitor double strand breaks, which would be important in monitoring chemotherapeutic response using a FRET-based reporter system but large scale was limited due to loss of sensitivity of the system. They next investigated utility of single cell whole transcriptome profiling in various sources, such as mouse cardiocytes, brown adipose cells, hippocampus neuron or dorsal raphe neurons, and showed strong correlation amongst cells such as those from cardiomyocytes but also heterogeneity amongst cells such as those from dorsal raphe. This single cell variability analysis has now been leveraged into a large U01 grant by the PIs.

Moreover, they have shown interestingly that presence of cytoplasmic intron sequences retaining transcripts, or CIRTs, can indicate novel functional compartment using their single cell RNA analysis.

For Aim 2 studies, they have been similarly successful in using system level modeling to identify signatures of circadian rhythm and identify a novel component, Chrono, which they identified using Bayesian models. Knockout studies were performed of this novel molecule as well as validation assays. This finding has been leveraged to a large grant from NIH for \$3 million and a paper has been submitted. In addition, as their overall goal is to use genomic biology to develop novel biomarkers they used primary samples of normal and retinoblastoma samples to perform next generation sequencing and mutational analysis along with miRNA studies and identified that low level mosaicism is present in 7.2% of patients with unilateral retinoblastoma probands. This discovery will change genetic counseling for risks of second cancer in the proband.

Weakness: None.

Reviewer 2:

The project has two aims (1) to develop novel biomarkers for human normal and disease states using high-throughput sequencing, cell based screening, bio-photonics, and single-cell genomics, and (2) to develop systems models of identified biomarkers and develop single-platform diagnosis devices using identified biomarkers. The project period spans from 2009-2013. The researchers made some progress that resulted in sizable NIH grant.

The progress did not fully meet the stated objectives. The researchers continually changed the model (cell/disease) from year to year without reasonable explanation. In 2009, the team reported the development of an assay to monitor double strand breaks of DNA. Microphages were used as a model for high-throughput cell based screening; however, the model was soon abandoned. In 2010, the team goal was to develop molecular signature of 20 matched normal and Retinoblastoma tumor pairs. However, no data were reported then. The team selected circadian rhythm system for Aim 2. Statistical analysis was carried out. In 2011, the team reported 7 family of miRNAs, which were significantly differentially expressed in RB as compared to normal retina. No progress was reported on Aim 2. In 2013, there was no reported progress on Aim 1. Progress on completing the prediction model for Aim 2 was reported.

Hence, the project did not fully meet the stated objectives. The anticipation of developing biomarkers that distinguish between normal and disease cells was not fully achieved; however, the team developed an assay that can possibly be utilized at least for one cell model. The cell signature variability from patient to patient is a serious pitfall for the project and has not been addressed. The models used for Aim 1 and Aim 2 are not identical. The translation from Aim 1 to 2 is broken.

Reviewer 3:

As stated by the investigators, the overall goal of this project is to develop novel biomarkers using genome-scale data, through the development of novel reagents, computational modeling and analysis, and micro- and nano-engineering.

The project was divided into two specific aims.

Specific Aim 1. Develop novel biomarkers for human and normal disease states using high-throughput sequencing, cell-based screening, bio-photonics, and single-cell genomics.

Specific Aim 2. Develop system models of identified biomarkers and develop single-platform diagnosis devices using identified biomarkers.

Strengths: Overall, the goal of this research has a strong rationale, as biomarker development is a critical translational area to improve patient management and provide tools to practice evidence-based medicine. The potential translational relevance of this proposal is a major strength, and the expected clinical implications of the findings are high.

The investigators plan to identify such clinically useful tools by using genomic, engineering, system-based, and advanced technologies. These are all innovative and cutting-edge technologies and approaches.

The investigators are highly qualified to achieve the goals of this project, as they have a solid track record of accomplishments in the field of computational and system biology and genomics. The group has already established some of these devices, and generated preliminary data. For example, they have developed microfluidic-based systems for cell-level, time-controlled samples, in addition to the PAIR and APRA techniques.

The investigators acknowledge the fact that the technologies that will be applied to the systems are high-risk. However, there are several areas where progress has been made.

One is on the application of genomic screens to retinoblastoma, where patient samples (at the DNA and RNA levels) have been sequenced and important discoveries have been made. For example, the let-7 family of miRNAs has been identified as differentially expressed in retinoblastoma compared to normal retina; moreover, the proportion of normal to variant miRNAs was significantly different between normal retina and retinoblastoma samples. On the basis of this finding, the investigators were able to elucidate the effect of variant miRNAs on novel targets. In addition, mutations in the RB1 gene and in other genes belonging to the apoptosis pathway have been detected. Finally, low-level mosaic mutations have been identified in retinoblastoma samples.

Another area where significant progress has been made is on the identification of biomarkers of the circadian rhythm. The analysis of mouse data (gene expression profiling) led to the development of tools for systems biology modeling of the circadian rhythm system. From these analyses, genes were identified as the potential core circadian rhythm genes. Towards the end of the grant period, a more refined modeling analysis has narrowed down a major clock component of the mammalian cell, and a new gene has been named “Chrono.” This gene has been functionally characterized using knock out mouse models.

The investigators planned to devise new technologies to aid the development of biomarkers. In this area, they have also made progress as, 1) they developed a new photoactivatable molecular reagent to be used in vivo called TIVA and tested this procedure using mouse brain slices and also demonstrated that TIVA-mediated RNA analyses from single cells in tissues can be conducted using this new technology, and 2) they also develop a new assay to detect double-strand breaks of DNA in live organisms by creating a new biosensor of ATM kinase activity using FRET technology.

Another major research progress has been on the single-cell transcriptome analyses using mouse data from different tissues, where computational pipelines of QC of sequencing data have been devised and multi-dimensional transcriptome signatures have been generated.

Additional results included: 1) carrying out a siRNA, high-throughput, cell-based screening in human primary macrophages that required further optimization using a commercial source of cells, and 2) carrying out a high-throughput cDNA overexpression screen to identify TFs that regulate the STAT1 pathway, and more than 30 factors have been identified. Although these approaches are somewhat more traditional and were not based upon the application of innovative technologies and devices, these results will be useful to the identification of new biomarkers. Overall, these results are quite encouraging, and fit well both with the goals of Aims 1 and 2. They clearly show the group has made some progress in some of the planned work.

Weaknesses: This plan is overly ambitious. As a matter of fact, many projects of the planned work have not been accomplished or even initiated. The stated research plan has been only followed in part. The investigators should provide a detailed explanation for why a certain set of planned experiments has not been conducted, and why other sets of experiments have been conducted instead, and how these fit the overall goals of the grant. It is true that the investigators have used an approach aimed at initiating multiple projects at a proof-of-principle stage, but there are many large projects in the original plan that have not been initiated, and many new technologies that were developed by the investigators, but have not been applied.

The applicants have provided very clear criteria for the evaluation of the success of this project, setting the bar quite high. These criteria include publications, generation of intellectual property, additional external funding including even large center grants, dissemination of new tools and databases to the research community. The applicants have also defined standard metrics for each of these criteria (number of publications, size of grants etc.). Many of the milestones have not been met, and the investigators should clearly state whether the results obtained so far might still meet those milestones and why.

The applicants acknowledge the fact the technologies used are high-risk; however, they have not articulated any pitfalls in each of the proposed research projects and ways to overcome them. This section should have been introduced, as, in fact, deviations from the original plan have been made in order to overcome some technical issues. Such deviations have impaired fast progress on the 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?***

### ***STRENGTHS AND WEAKNESSES***

#### Reviewer 1:

Strengths: The investigators were able to accomplish the goal of using system biology and computational methodology to develop novel biomarkers and have gone on to obtain additional extramural funding. Primarily, they have been able to deliver three products including identification of single cell study for transcriptome analysis, a circadian rhythm project for

pharmacogenomic application and a study to identify novel retinoblastoma biomarkers. Funding for two of these projects has already been attained from the NIH and additional funding will be sought.

Weakness: None.

Reviewer 2:

Identifying signature biomarkers for the state of cells is significant for the diagnosis and development of therapies. The team proposed to develop a complete system where, in one hand, they will identify the biomarkers from both normal and diseased cells and develop a system that takes advantage of such identification to predict/diagnose the abnormality. However, the change between the models used between Aim 1 and Aim 2 makes it difficult to judge the success of the proposed approach. The progress for both Aim 1 and 3 is modest, despite the award of NIH grants.

Reviewer 3:

Strengths: The potential significance of this project for improving health is high, as new biomarkers will guide the decision making process on prevention, diagnosis, and treatment. The new tools, technologies and system-wide approaches gathered from this project will also lead to further expand our basic knowledge on the biological meaning of the genome. Several discoveries have been made, and they are described in detail in the above section.

Weaknesses: With regard to the impact on patient health, these results are still very preliminary and basic. Hence, this research did not lead to a direct translation into improvements in human health. The investigators should describe their near term plans to translate this information to the clinic. The results obtained so far by the investigators are still very far from reaching the main goal of this grant, such as generating new biomarkers. First, only a few new candidate genes have been identified. Second, biomarker development is a very lengthy process, and requires multiple steps of demonstrated analytical validity, and clinical validity and utility. The investigators need to outline the plan for bringing the most promising candidate biomarker from the stage of discovery to that of a clinical application.

No investigator with a clinical or medical degree has been part of this team, and such expertise should be brought in, in particular for further clinical development of the most promising biomarkers.

The focus for future plans should be established. It is important to develop tools that can be applied to different systems and therapeutic areas, but the plan needs to move forward in a more focused manner, identifying specific disease areas where all the investments made so far will be capitalized.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

Strengths: The investigators have been highly successful in leveraging data from this proposal for additional funding. They have been able to obtain >\$9million from NIH from a U01 center grant for single cell mRNA analysis. In addition, the investigators were able to use the system level modeling analysis to identify a new novel circadian clock gene, termed Chrono which represses known clock gene BMAL1 and knockout studies were performed in mice showing that Chrono affects circadian rhythm. This work was then used to obtain additional NIH funding for five years totaling over \$3 million.

Moreover, additional NIH R01 grants will be submitted to further characterize the chrono circadian rhythm gene as well as characterize biomarkers from their retinoblastoma project.

Weaknesses: None.

Reviewer 2:

The team reported the acquirement of two NIH grants.

Reviewer 3:

Strengths: The investigators have leveraged the preliminary data obtained on the single-cell transcriptome analysis in the mouse and obtained a U01 for over \$9 M over 5 years to continue developing discoveries based upon the single-cell analysis program.

Additional NIH funding was obtained based upon the preliminary funding on the work conducted on the circadian rhythm.

The success in getting NIH funding has been excellent.

Weaknesses: The role of the PI and Co-PI in the above grants should be indicated, as it is not clear whether they are also PIs/Co-PIs on those grants or just Co-Is.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

No manuscripts have been published so far.

Strengths: This is a highly productive group based on their track record in obtaining high-level funding for future projects. They are planning on submitting three manuscripts based on their work to high-tier journals including Cell and Genome Research and anticipate they will be successful. These manuscripts are complete and focus on mosaicism in retinoblastoma; machine learning approaches used to identify novel circadian rhythm regulators as well as methodologies for single cell sequencing. In addition, another manuscript is in the preparation stage for Science journal submission.

No patents have been developed.

Weakness: None.

Reviewer 2:

The team reported a number of peer-reviewed publications as a result of the project work.

Reviewer 3:

The applicants have drafted two manuscripts, and two others have been submitted (one of them being revised).

Weaknesses: The investigators have not mentioned filing for any licenses or patents, or described any commercial development opportunities in the future. Because the grant supports the development of new technologies, it seems the investigators might miss an opportunity to protect their rights on the IP on these technologies.

No abstracts or presentations at scientific meetings on these results have been reported.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

Strengths: The project was used to fund multiple young investigators including 14 undergraduate students, 5 pre-doctoral, and 4 post-doctoral students. In addition, the funding by this mechanism has allowed Penn Genome Frontiers Institute in improving interdisciplinary research across the University of Pennsylvania system in allowing collaborations between different schools such as Engineering, Arts and Sciences and Medical School. Furthermore, it enhanced computational infrastructure within the institution.

Weakness: None.

Reviewer 2:

A number of students and post-doctoral fellows were hired on the project and collaboration among a number of researchers is clear in the project reports. However, there is discrepancy in the reporting effort for the main researchers.

Reviewer 3:

Strengths: The added value to the institution is implicit, as a result of the development of new models and technologies. The critical mass will be also enhanced, attracting new investigators.

Weaknesses: It appears that no major improvements to the infrastructure resulted from this research. No indication is given whether any pre- and post-doctoral students were funded by this research. Opportunities for mentorship of junior faculty have not been described.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

No collaborations have been planned outside of the institution.

Strengths: None.

Weakness: None.

Reviewer 2:

Some collaboration has been reported. The team purchased/obtained cells from vendors and other collaborative facilities and acquired data from other institutions.

Reviewer 3:

Strength: This group of investigators is highly qualified, the infrastructure is excellent, and the resources available are outstanding.

Weaknesses: No new investigators were added and it seems this proposal did not engage collaborators or institutions outside of the University of Pennsylvania.

It does not appear the applicants plan to begin any new collaboration as a result of this research. Because the group is very strong, those above are minor weaknesses.

***Section B. Recommendations***

**SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

Reviewer 1:

None.

Reviewer 2:

Strengths:  
Development of protocols to identify signature biomarkers.  
Evaluation of prediction model using a known data set.  
NIH grants.

Weakness:

Lack of connect between the work on the two aims.  
Inconsistent models from year to year.  
Lack of solid reporting (during the 4year project) on both aims.

Recommendation: Keep model consistent.

Reviewer 3:

1. Weakness: This plan is overly ambitious. As a matter of fact, many projects of the planned work have not been accomplished or even initiated.

Recommendation: The stated research plan has been only followed in part. The investigators should provide a detailed explanation for why a certain set of planned experiments has not been conducted, and why other sets of experiments have been instead conducted and how these fit the overall goals of the grant.

2. Weakness: Many of the milestones have not been met.

Recommendation. The investigators should clearly state whether the results obtained so far still meet those milestones and why.

3. Weakness: Most of the results are still very preliminary and basic. Hence, this research did not lead to a direct translation into improvements in human health.

Recommendation: The investigators need to outline the plan for bringing the most promising candidate biomarkers from the stage of discovery to that of a clinical application.

4. Weakness: The focus for future plans is not clearly established.

Recommendation: The future plan needs to move forward in a more focused manner, identifying specific disease areas where all the investments made so far will be capitalized. Such a plan should be outlined.

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**Project Number:** 0865410  
**Project Title:** Research Infrastructure: Expansion and  
Enhancement of Rodent Housing Space  
**Investigator:** Glen N. Gaulton, PhD

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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 overall project resulted in a great expansion and improvement in research infrastructure for housing research animals.

The research design and methods were adequate in light of the project objectives.

No changes were made to the research protocol.

The final report describes the successful completion of all proposed components of the expansion of the barrier facilities and improvement in sanitation and working conditions in the animal facility which is located at the core of the medical school campus.

The data and information provided were applicable to the project objectives listed in the strategic research plan.

The project met all of its stated objectives and was completed satisfactorily on time. Further, funds were obtained from NCRP = \$700,000 and Internal University Funds = \$1,111,514 with Total Co-funding = \$1,811,514.

##### Reviewer 2:

The overarching goal of this infrastructure project was to improve disease prevention and control, and enable increased murine housing in the Clinical Research Building (CRB) animal facility vivarium. Three specific aims were proposed to encompass a comprehensive institutional plan to upgrade and expand the CRB vivarium: 1) Enhance CRB housing capacity through conversion/renovation of neighboring laboratories, 2) Improve sanitization by upgrading the CRB ventilation system and expanding the barrier, and 3) Provide working conditions that meet optimal occupational safety standards for facility staff.

The PI indicated that success in achieving these aims could be measured by 1) renovation and occupation of the space (August 2010), 2) expansion of cage capacity behind the barrier from

4,382 to 12,000 (August 2010), and 3) increase in numbers of external grants supported (December 2012).

The PI also provided time frame and milestones for measuring project progress:

Design and approval: 1/1/2009-6/30/2009.

Construction: 7/1/2009-6/30/2010.

Full occupancy: 7/1/2010-6/30/2011.

Follow on reporting: 7/1/2011-6/30/2012.

Follow on reporting: 7/1/2012-12/31/2012.

Major strength: The stated aims of this infrastructure project were fully achieved.

Weaknesses: It is not clear whether the full occupancy of this renovated facility is achieved. It is also not clear whether there was an increase in numbers of external grants supported by this expansion.

#### Reviewer 3:

The project fulfilled the stated goals of building and equipping an expanded and updated rodent facility in the Clinical Research Building (CRB) at the University of Pennsylvania Medical School. The specific aim was to increase the total CRB cage capacity from 7,000 to 12,000, including an increase in barrier capacity from 4,382 cages to 12,000 cages. This is a worthy and important goal for research operations at the University of Pennsylvania School of Medicine.

***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 quality of the animal care facilities at the core of the medical school campus were significantly improved and expanded where total cage capacity increased from 7,063 to 15,000, with barrier capacity tripling, from 4,382 to 15,000.

The improved facilities will contribute significantly to the success of all health related research projects conducted under the improved conditions for housing experimental animals. Furthermore, this project allows the number of such studies to be conducted to double.

The improvements significantly improved sanitary working conditions for animal care workers.

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

This project has met the rare standard of attracting funding from multiple sources, being completed on time, and meeting all objectives which will have a big impact on health-related research. The funding invested in infrastructure improvements will be returned many fold

through successful completion of extramurally funded research projects which will be conducted there.

Reviewer 2:

Weakness: The newly upgraded facility is expected to provide important animal infrastructure support for the current and future needs of the University of Pennsylvania scientific community. However, there is no evidence to indicate whether this newly upgraded facility has provided improved animal infrastructure support to the needs for animal studies at the University of Pennsylvania.

Reviewer 3:

Renovation of the CRB vivarium will improve the school's research programs by providing state-of-the-art animal care/husbandry and facilities for the University of Pennsylvania's present and future programs, including Genetics/Genomics, Metabolism (diabetes/obesity), Immunology/Transplantation and Neurosciences (behavior/neurodegenerative diseases), among others. It will also reduce the potential spread of infectious agents among the animals, and improve working conditions for animal care staff. This facility will meet AAALAC standards, improve the quality and quantity of rodent housing, and more adequately meet the needs of NIH-funded research.

*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:

Matching funds were obtained from two other sources.

Funds from the other sources were obtained and contributed approximately 50% of the total cost of the project.

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

The need for the project was clearly established and the project itself was well-planned, which greatly aided in attracting funding from both the University of Pennsylvania and federal sources in addition to Health Research Grant funds to perform the needed renovations and expansion.

Reviewer 2:

Weakness: The newly upgraded facility is expected to be used by investigators to compete for grant funding for further exploration of genetic and diabetic diseases. However, there is no evidence to indicate whether this newly upgraded facility has enhanced the capacity of competing for grant funding at University of Pennsylvania.

Reviewer 3:

No other funding sources were sought; however, there was \$700,000 provided from the NIH-NCRR to support this total project in addition to institutional funding to complete the project.

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

**STRENGTHS AND WEAKNESSES**

Reviewer 1:

This criterion is not directly applicable to the project as performed.

Reviewer 2:

Weakness: There is no evidence that the project has resulted in any increased peer-reviewed publications, licenses, patents, or commercial development opportunities at the University of Pennsylvania.

Reviewer 3:

No, not applicable.

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

**STRENGTHS AND WEAKNESSES**

Reviewer 1:

The quality of the rodent housing facilities at the core of the medical school campus were significantly improved. Further, the capacity was expanded where total cage capacity doubled and the number of cages within a barrier facility to ensure the health of the rodents housed there tripled.

There were improvements made to infrastructure.

No new investigators were listed as having been added or brought into the institution to help carry out this research, but it is understood that such improved rodent housing facilities will be critical for recruiting new research faculty for many years to come.

No funds were used to pay for research performed by pre- or post-doctoral students.

The completed renovations and expansion of the small animal facility will contribute to an expanded number of research grants and publications from the University of Pennsylvania for decades to come.

Reviewer 2:

Major strength: The project has enhanced the capacity for animal studies at the University of Pennsylvania.

Weakness: It is not clear whether this renovated and upgraded facility has enhanced research capacity, resulting in recruiting new investigators, animal caregivers, or research personnel.

Reviewer 3:

This project of renovating and updating the rodent facility in CRB absolutely enhanced and increased capacity for research at the University of Pennsylvania.

***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 importance of the project is highlighted by the ability to obtain funding from three sources including NCRR to undertake the renovation.

Reviewer 2:

Weakness: There is no evidence to indicate any increased collaboration with research partners outside of the University of Pennsylvania community.

Reviewer 3:

No, not applicable.

**Section B. Recommendations**

**SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

Reviewer 1:

None.

Reviewer 2:

Specific weakness: The PI indicated that success in achieving these aims could be measured by occupation of the newly upgraded animal facility and increased numbers of external grants supported by this facility. However, there is no follow-up measurement performed to evaluate the impact and value of this project on improving the research capacity at the University of Pennsylvania.

Recommendation: The PI may document all the publications, grant applications, funded grants, patents, and commercial development involving animal studies using this newly upgraded facility, thereby generating evidence of this project's impact/value on improving the research capacity at the University of Pennsylvania.

Reviewer 3:

None.

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**Project Number:** 0865411  
**Project Title:** Development and Validation of a Tool to  
Assess Perceived Nutrition Environments  
**Investigator:** Karen Glanz, PhD, MPH

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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:** A very rigorous and systematic process was utilized to develop the new instrument that was derived from established theoretical and conceptual models, published literature, expert review, and input from the target population.

Methods included community-based participatory approaches and quota sampling to insure a diverse sample to test the new instrument. As a result, there is greater confidence in the representativeness of the study sample with the larger population.

Recruitment goals were exceeded for full testing of the instrument (Aim 2).

A very large and diverse sample of food stores and restaurants was sampled, which aids with the potential generalizability of findings.

**Weaknesses:** The final report fails to provide a description of findings related to Aim 3, and thus it is difficult to ascertain whether this objective was completed as written in the original application or whether reasonable progress on that aim was made. Specifically, there is no mention of findings from the path analysis suggested in the original strategic plan to answer the question of the independent and additive mediators of the relationship between self-reported nutrition environment and eating behaviors (stated health outcome).

##### Reviewer 2:

**Strengths:** Very thorough protocol for developing the base list of items to include on the Nutrition Environment Measures-Perceptions (NEMS-P) scale.

The use of cognitive testing to refine the instrument, with specific examples of how the instrument was modified as a result.

The new instrument includes measurement of perceptions of placement and promotion of both healthy and unhealthy foods.

Data for test-retest reliability of the NEMS-P survey composite items were presented.

Weaknesses: The specific aims are listed below (as in the strategic plan). The research team accomplished Aims 1 and 2 successfully. However, Aim 3 was not accomplished.

- 1) To pilot-test an instrument designed to evaluate perceived nutrition environment in a convenience sample of 16 individuals.
- 2) To determine the psychometric properties of the instrument developed to measure perceived nutrition environment in a sample of 200 adults: 100 residing in an area of high socioeconomic status and 100 from an area of low socioeconomic status.
- 3) To explore whether observed nutrition environment and perceived nutrition environment are independent and additive mediators of the relationship between Self-Reported Nutrition Environment and eating behaviors.

The research team has several publications in-progress that both describe the development of the NEMS-P measure as well as the association between the observed and perceived nutrition environments in stores and restaurants. However, none of these results are provided.

Nor are results provided for the proposed analyses to examine the relationship between the perceived nutrition environment and diet/weight status. Although this was an aim, no measure of dietary behaviors or weight status was included in the survey administered to the 221 participants, and there is no explanation provided as to why the analyses examining perceived nutrition environment and observed nutrition environment/dietary behaviors was not conducted.

#### Reviewer 3:

The project proceeded as planned in a timely fashion. The aim of the project was to develop and validate a standardized measure of perceived nutrition environment. This objective was met by achieving the project's first two specific aims of pilot testing the instrument and determining the instrument's psychometric properties. The methods for achieving these aims were rigorous and the statistical approaches were appropriate. Preliminary results reveal that the project has met its objectives.

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

#### ***STRENGTHS AND WEAKNESSES***

#### Reviewer 1:

Strengths: The instrument developed from this project is a very important tool for better understanding the potential disconnect between what people think their food environment is and what it really is.

Applications from this work could include interventions to bridge the gap between perception and reality (if any) and/or leverage healthier environments with behavior change programs. The new tool is likely the first of its kind.

Plans include making the new instrument widely available and future research studies.

Weaknesses: The final report fails to include a description of findings from the path analysis or alternative analysis (if changed) that includes assessment of the stated health outcome (eating behavior). The final annual progress report suggests some differences in perceived availability and eating behaviors by neighborhood characteristic, but these findings were not presented again in the final report, nor do they address the scope of the research question eluded to in Aim 3.

There are no other changes in outcomes, major discoveries, etc., resulting from this work.

Future plans are not described in detail, so it is not as clear what the future impact could be of this work.

#### Reviewer 2:

Significance: The neighborhood food environment is related to food consumption and obesity prevalence. More work is needed to learn about how neighborhood residents perceive their food environment, and how this is related to dietary behaviors and obesity. This research project developed and tested a measure of the perceived food environment.

Changes in risk factors: None.

New approaches: While the final progress report states there were “none,” development of a standardized measure of the perceived food environment is a somewhat new approach, and the inclusion of a measure of placement/ promotion of healthy foods in stores and restaurants is innovative. It would have been nice to see an objective measure of placement/promotion of healthy foods in stores, as well as the perceived measure, since NEMS-S does not measure placement/promotion.

Future plans: The research team plans to publish findings from the survey development and pilot testing and the main results for the store and restaurant nutrition environments. The team also plans to collaborate on projects that would incorporate the NEMS-P tool.

Strengths: Development of a standardized measure of the perceived food environment, which includes measurement of placement/promotion of healthful foods in stores and restaurants is needed.

Weaknesses: There were no risk factors measured.

The future plans section did not include plans to carry out Aim 3 of the proposed project: “To explore whether observed nutrition environment and perceived nutrition environment are independent and additive mediators of the relationship between Self-Reported Nutrition Environment and eating behaviors.”

Reviewer 3:

This project will result in a validated measure of the perceived nutrition environment. The nutrition environment is one factor that influences eating behaviors. Eating behaviors are directly related to obesity which greatly impacts health. Being able to rigorously measure the perceived nutrition environment will have a beneficial impact on the ability to analyze the relationship between the nutrition environment and obesity.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

Strengths: None noted.

Weaknesses: Did not leverage this award for further funding despite significant history of work in this area and stated need/gaps in the literature.

Future plans are for applying for additional funds, but without health outcome data demonstrating impact, it is not as clear how competitive this work would be in the current funding climate.

Reviewer 2:

Strengths: There are plans to apply for funding to continue the research related to the NEMS-P.

There are plans for future research collaborations with other research institutions using the NEMS-P tool to predict eating behaviors and weight.

Weaknesses: Although the strategic plan stated that “an R01 NIH grant proposal will be submitted following the pilot phase of the study (October 2010),” it does not seem that this submission happened.

Reviewer 3:

No other funds have been received, but the PI plans to apply for additional funding to refine and further test the measure and to work with other institutions to use the instrument to predict eating behaviors and weight status.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

Strengths: None noted.

Weaknesses: No published project results.

All publications are still “in progress” despite a 4-year project period.

Future plans for papers suggest analyses that were not part of the final report (i.e., relationship between the perceived nutrition environment and diet/weight status; predictors of the home food environment).

Reviewer 2:

Strengths: Publications are currently in-progress that both describe the development of the NEMS-P measure as well as the association between the observed and perceived nutrition environments in stores and restaurants.

Additional analyses include the relationship between the perceived nutrition environment and diet/weight status as well as predictors of the home food environment (i.e., accessibility and availability of healthy and unhealthy food within the home).

Weaknesses: No publications had been submitted at the time of the final report.

It is unclear how the research team will submit papers regarding analyses between the perceived nutrition environment and diet/weight status, as it seems they did not collect dietary or weight status variables in the survey. Perhaps these data are coming from another source.

Reviewer 3:

No publications had been submitted at the time of the final progress report, but several publications were in progress that describe the development of the instrument and the association between the observed and perceived nutrition environments in stores and restaurants. Additional publications may involve analyses of the relationship between the perceived nutrition environment and weight status and predictors of the home food environment.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

Strengths: Study involved 8 undergraduate and graduate students.

New relationships with community organizations and other researchers on campus were described.

Weaknesses: Well-established researcher with history of development and dissemination of similar instruments, but no discussion of how this full line of research has/will be used to form sustained relationships/collaborations to address the proposed health problems in the community.

As described, it is difficult to ascertain the nature of the reported new collaborations, and how this has/will enhance the existing infrastructure to conduct this line of work.

All publications are still “in progress” despite a 4-year project period. Future plans for papers suggest analyses that were not part of the final report (i.e., relationship between the perceived nutrition environment and diet/weight status; predictors of the home food environment).

Reviewer 2:

Strengths: The project funds were used to pay for training and research conducted by six undergraduate and two master’s-level students.

The research team developed relationships with community organizations that have subsequently helped with recruitment efforts for other projects and researchers.

Through this project, the research team has developed new relationships with other researchers at the University of Pennsylvania.

Weaknesses: There were no new investigators brought into the institution to help conduct the research.

Reviewer 3:

The project involved 6 undergraduate and 2 master's-level students in research opportunities, but did not involve any pre- or post-doctoral students. The project did not fund improvements to infrastructure.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

Strengths: None noted.

Weaknesses: Twelve new investigators were involved in the expert review for the instrument development, but it is not clear if (how) this has led to any ongoing collaborations with this line of research or planned research.

Reviewer 2:

Strengths: Twelve experienced investigators working in obesity prevention and nutrition were invited to assess the face and content validity of the items.

Relationships with community groups were established to assist with recruitment.

While the research team stated that they did not initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects, this work did involve development and testing of a tool to measure the perceived nutrition environment among both low- and higher-income residents.

Weaknesses: None.

Reviewer 3:

The project allowed researchers to develop relationships with community organizations that have been beneficial in their recruitment efforts for other projects and researchers. The project also helped researchers develop new relationships with other researchers at the University of Pennsylvania.

***Section B. Recommendations***

**SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

Reviewer 1:

1. No data related to Aim 3 as described in the original strategic plan. The grantee should provide data analyses/findings from path analysis or alternative statistical analysis that addresses the question of the association between perceived and observed nutrition environment and stated health outcome (i.e., eating behavior).
2. No publications or papers under review. Provide a list of specific papers in progress, estimated submission dates, and journals targeted for submission.
3. Limited evidence of new collaborations. Provide more details about the community organizations and additional researchers collaborating with and briefly describe how the grantee is working together with these organizations/researchers to extend the funded research.

Reviewer 2:

1. As no results are provided for the association between the perceived and objectively-measured nutrition environment, these results should be written up and submitted for publication.
2. Results for the proposed analyses to examine the relationship between the perceived nutrition environment and diet/weight status should also be written up and submitted for publication.

Reviewer 3:

Recommend involving pre- and post-doc students in future projects to help develop these students' ability to conduct this type of research.