

**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 (1.76)

**Project Ratings:**

<b>Project</b>	<b>Title</b>	<b>Average Score</b>
0863101	Triggers of Inflammation in Scleroderma	Favorable (1.67)
0863102	Developing Therapies for Treating Hereditary Spastic Paraplegia	Outstanding (1.33)
0863103	Role of CTF18 in Female Germ Cell Development and Fertility	Favorable (1.67)
0863104	A Microfluidic Model of Drug-induced Liver Toxicity	Favorable (2.33)
0863105	Identification of Biomarkers and Therapeutic Targets in 3D Hypoxic Breast Cancer Mode	Favorable (2.33)
0863106	Role of O-GlcNac Transferase as a Biomarker and Therapeutic Target for Prostate Cancer	Favorable (1.67)
0863107	Piezoelectric Microcantilever Sensors (PEMS) to Detect Methicillin-Resistant Staphylococcus aureus (MRSA)	Favorable (2.00)
0863108	RNA Interference-based Therapy for HIV-1 Associated Neurologic Disease	Favorable (2.00)
0863109	Somatostatin Signaling in Alzheimer's Disease	Favorable (1.67)
0863110	Characterization and Application of a Novel Drosophila Model for CHARGE Syndrome	Outstanding (1.33)
0863111	Multidimensional Shape/Color Distributions as a Computational Biomarker for Cancer Pathology	Favorable (1.67)

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**Project Number:** 0863101  
**Project Title:** Triggers of Inflammation in Scleroderma  
**Investigator:** Artlett, Carol

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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 objective (specific aim) of this project was to evaluate the role of the inflammasome in early dermal fibrotic lesions and autoantibody production utilizing various knockout mouse models.

**Strengths:** The investigators successfully evaluated skin fibrosis in a bleomycin model using several knockout mouse models. They studied skin samples as well as cultured fibroblasts from these mice. In addition, they extended their observations to cultured fibroblasts from scleroderma patient lungs and skin. These data are nicely presented in the text of the progress report with appropriate figures and graphs. Research design and methods were excellent, and the data supported the hypothesis of the investigators. One publication has resulted from this work (*Arthritis & Rheumatism*, November 2011).

**Weakness:** There is no mention of autoantibody studies so the objective was not totally met.

#### Reviewer 2:

The objective of this project was to define the role of the inflammasome in systemic sclerosis. The approach was to utilize a variety of knockout mouse models with deficiencies in key inflammatory signaling molecules and to characterize the response of these knockout mice to bleomycin-induced fibrosis. A total of seven different knockout mouse models were to be tested in the original application. In retrospect, this was likely too ambitious given the time frame of the project and the funds available to support it. Nevertheless, the applicant did test three knockout mice, the NALP3<sup>-/-</sup>, ASC<sup>-/-</sup> and MyD88<sup>-/-</sup> strains. Of the knockout strains listed in the original application, these were probably going to be the most informative. The results do in fact suggest a role for NALP3 and MyD88 in bleomycin induced skin fibrosis. Furthermore, the result with the ASC knockout suggests that ASC may not be involved, which is an interesting result that deserves additional investigation. Analysis of autoantibody production was not completed, although serum samples have been collected. A number of other *in vitro* studies were also performed with scleroderma dermal fibroblasts demonstrating that bleomycin activates the NALP3 inflammasome. Overall, although the rationale for changes to the research protocol was not clearly outlined in the progress reports, strong progress was made during the funding period.

Reviewer 3:

On a broad level, the project met its objective of studying the components of the inflammasome and TLR signaling pathway in fibrosis. The project made some progress on the stated specific aim, “To understand the contribution of inflammatory signaling molecules in early dermal fibrotic lesions and autoantibody production using knockout mouse models.” According to the original application, a timecourse of bleomycin-induced fibrosis, which included three timepoints, was to be examined in seven different knockout mouse strains and wild-type mice. Collagen and TGF- $\beta$  levels in the fibrotic tissue were to be examined, as well as the presence of autoantibodies. In addition, a variety of immune cell populations were to be examined, including T cells, B cells, macrophages, and mast cells. According to the progress reports, fibrosis was examined only in three knockout strains (NALP3<sup>-/-</sup>, ASC<sup>-/-</sup>, and MyD88<sup>-/-</sup> mice). The authors should confirm that the correct sample number is provided in Figure 4, since the sample sizes are very small (n=2-6) and do not match what had been reported for previous progress reports. The PI indicated that no differences in the numbers of T cells or macrophages were found, although no data were included. There is no information provided for B cells and mast cells. One caveat here would be the timepoint at which these cells were examined. If some of the innate inflammatory cells were examined at the 28-day timepoint only and no differences were seen, it could be that the number of these cells peaked at an earlier timepoint. The PI states that the autoantibody levels have not yet been examined, but no reasons for the delay are discussed.

Overall, only some of the original objectives were met; however, additional results from *in vitro* studies that were not originally proposed are reported. While it would have been helpful for the PI to include an explanation for these changes, the *in vitro* studies provide important information about the regulation of inflammasome components in SSc fibroblasts. The authors show higher levels of active caspase-1 and IL-1 $\beta$  in SSc fibroblasts compared to normal fibroblasts, suggesting that the inflammasome is ‘turned on’ in SSc fibroblasts. There is also a series of experiments looking at the effects of bleomycin on the expression of various inflammasome-related pro-inflammatory genes (IL-1, IL-18) in normal fibroblasts. The choice to look at these components at only the mRNA level is somewhat perplexing, since the function of the inflammasome is to cleave and activate these pro-inflammatory mediators at the protein level. Measurement of these secreted proteins in the supernatant may have been a more appropriate readout of inflammasome activation. Overall, the experiments were in line with the original aims of the project, and while all of the specific objectives were not met, reasonable progress was made on the project.

***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 beneficial impact of this project is potentially quite high in that agents to specifically target the inflammasome could be developed which would be a significant benefit for this patient population for which there are very limited and only partially successful treatment options.

Hence these research results could significantly improve the lives of scleroderma patients. Considering the basic-science nature of this research, no new drugs would reasonably be expected.

Future plans: the investigators have submitted an application to NIH to continue and further develop this work.

Weakness: None was noted.

Reviewer 2:

The results suggest a function for the inflammasome in scleroderma pathogenesis. In addition, the results suggest some differences in signaling in the skin. The work provides a foundation for additional studies that might lead to advances in therapeutic treatment of scleroderma.

Reviewer 3:

This project focuses on understanding the mechanisms involved in the development of systemic sclerosis (SSc) using a model of bleomycin-induced scleroderma. The fibrosis associated with this disease can be debilitating and lead to death in some cases; therefore, the project addresses a significant health concern. The project revolves around understanding the importance of inflammasome activation in SSc. Activation of the inflammasome and the resulting production of activated pro-inflammatory cytokines such as IL-1 $\beta$  could be an important initial trigger for inflammation, which is a process known to drive fibrosis. Understanding how the inflammasome is involved in SSc could be important for identifying therapeutic targets to treat SSc. Of particular interest, the results reported here suggest that NALP3 (an integral inflammasome component) and MyD88 (a TLR signaling molecule) are critical for the development of bleomycin-induced fibrosis. The exact plans for this project are not clearly described in the final progress report; however, the PI has indicated that grant applications and an additional manuscript will be submitted in the future, suggesting that the project will continue.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

The investigators have applied to NIH for continued funding to further develop and expand this research idea. Otherwise I did not see evidence for leveraging funds.

Reviewer 2:

NIH grant applications were submitted but ultimately not funded. Additional NIH grant applications are planned to continue the work. The applicant appears to be working diligently to seek external funding to support the project.

Reviewer 3:

In February 2009, a grant was submitted to NIH entitled, "Role of inflammasome in systemic sclerosis." It appears that a revised application (with the same title) was submitted in September

2009. Unfortunately, neither grant was funded. The PI plans to generate additional preliminary data based on the reviewer critiques and submit future NIH grants related to 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:

Strengths: There is one peer-reviewed publication that describes this research in *Arthritis & Rheumatism*, November 2011. This is a first-rate journal with an excellent impact factor. The quality of the publication is excellent.

#### Reviewer 2:

One peer-reviewed publication was published in 2011 based on the work funded by this grant. This was in a journal appropriate for the field. There appears to be additional data sufficient for another peer-reviewed publication. The applicant has met (and will likely exceed) this performance measure.

#### Reviewer 3:

The project was expected to generate at least one publication. One peer-reviewed paper reporting *in vitro* studies supported in part from this grant was published in *Arthritis & Rheumatism* in 2011. It is disappointing that the mouse work has not yet been submitted for publication, but the PI states in the final progress report that one additional publication on the *in vivo* mouse bleomycin studies is expected in the future.

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

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

#### Reviewer 1:

Strengths: The work was carried out to a large extent by a graduate student, Judy Rieger, who is also an author of the paper.

#### Reviewer 2:

No improvements to infrastructure were made, but one pre-doctoral trainee was supported.

#### Reviewer 3:

The funds for this particular project did not appear to support infrastructure improvements or recruitment of new investigators to help carry out the research. One pre-doctoral student was supported by the project.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

Strengths: A collaboration was developed with Dr. Carol Feghali-Bostwick from the University of Pittsburgh who provided the human SSc lung samples.

Reviewer 2:

A collaboration was established with another investigator at Drexel University (Dr. Katsikis).

Reviewer 3:

The project did not lead to collaborations with external researchers or community groups. The proposal was a basic science project, and there was no clinical research component.

***Section B. Recommendations***

***SPECIFIC WEAKNESSES AND RECOMMENDATIONS***

Reviewer 1:

Weakness: Part of the objective was not met regarding autoantibodies in the bleomycin mouse model. It is suggested that these autoantibodies be measured in stored serum from the animals or an explanation provided for why this has not been done. If done, the results should be provided.

Reviewer 2:

None

Reviewer 3:

1. An explanation as to why all of the originally proposed work was not performed should be included. For example, why was fibrosis not examined in all seven knockout strains as originally proposed? Why were TGF/collagen levels or B cell/mast cell numbers not determined in fibrotic tissue?
2. The PI should confirm that the sample numbers provided in Figure 4 are correct. The legend states that n=2 for some strains. If this is correct, then the PI should explain why only two mice per group were used and how statistical analysis was performed with n=2.
3. It would be helpful if the PI could provide more information on whether the mouse data will be submitted for publication. As it stands now, only one publication is listed that described data only loosely related to this project.

## **Generic Recommendations for Drexel University**

### Reviewer 1:

This was a worthwhile project that was nicely carried out, and the results were published in an excellent journal. I would recommend that the university continue to support this important work.

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**Project Number:** 0863102  
**Project Title:** Developing Therapies for Treating Hereditary Spastic Paraplegia  
**Investigator:** Baas, Peter

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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:** Overall, the project met most but not all stated objectives. Specific Aim 2 was ambitious, and it seems that the researchers did not have enough time to complete the experiments proposed in this aim. However, the design and methods that were described in the project were adequate and scientifically sound for the objectives of the project.

The research design and objectives were slightly changed compared to the proposed project. In the original proposal, the spastin mutants were supposed to be expressed in the rat cortical neurons and then the axonal growth and the effect on the transport of intracellular organelles would be examined. In the initial experiments, the researchers used rat fibroblasts. The data from these sets of experiments indicate that many spastin mutations have no effect on microtubule-severing activity, but these mutations cause the disease symptoms in patients. Importantly, these results do confirm the clinical findings that there is no cause-effect between the loss of spastin microtubule-severing activity and the severity of symptoms in hereditary spastic paraplegia type 4.

**Weaknesses:** The experiments described in Specific Aim 2 have been initiated during the funding period; however, the results from these experiments are not included in the final progress report. Despite this weakness there are definitely sufficient published data showing the acceptable progress of the proposed project.

#### Reviewer 2:

The authors proposed to elucidate molecular pathogenesis of SPG4 (caused by most commonly mutated gene SPAST encoding spastin protein). Spastin has several functions, including microtubule severing properties. Recently, additional functions of spastin that are important for endosomal sorting and trafficking have been proposed, but they were not explored in this grant. The authors focused on the microtubular function, and their data clearly point to the gain of function mechanism with possible dominant negative effect of spastin mutations. This is the most crucial question because it suggests that the diminishing of toxic effects of these mutations rather than elevation of spastin function (which would be important if indeed a haploinsufficiency was a likely mechanism) will be important for future disease treatments. That is why I think that the authors' stated objectives were met.

These results are in line with the original research protocols and no modifications or deviations from the proposed studies were done.

Reviewer 3:

I think the applicants have performed critical experiments and have shown remarkable progress, but I do not think the objectives are fully completed.

Strengths:

- The applicant proposes to look from a different angle and focuses on the "gain of toxicity" aspect as a potential explanation for the bases of disease biology, and I think this is a strength.
- The applicant has published remarkably well on the subject including the work that is funded with this grant, and the tools that are recently generated (the constructs with different human mutations) are also very valuable for current and future research. These tools are also right on target for understanding the role of different mutations in the function of spastin with respect to disease progression in hereditary spastic paraplegia (HSP).
- The applicant initially used fibroblast and then moved into cortical neuron cultures from rats, and I think this shift is very appropriate for the relevance of findings.

Weaknesses:

- Even though using mouse or rat cortical neurons for these assays is important for translational research, the limitation is the presence of spastin and katanin proteins in cortical neurons. Therefore, one must first downregulate their expression and then introduce the expression of the mutant gene. The applicant actually tried this with siRNA approaches and suggested feasibility. However, those experiments are not easy and may not be reproducible, since results may change from experiment to experiment.
- To test the various potential therapies for alleviating deficits (Goal 2), a dependable assay system is required. This assay system must be dependable and must be relevant to human conditions. This is not an easy task, and this itself may require its own grant application. I think the applicant must think deeply into potential problems and caveats of the experimental design and how it can be improved.
- It would be best if the applicant tried to culture corticospinal projection neurons and performed experiments using these neurons. Recent publications indicate the presence of corticospinal projection neuron culture paradigms, and they would be most valuable for this study.

***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:

There are no immediate benefits from this project for improving the health of patients suffering from hereditary spastic paraplegia type 4 (*SPG4*). However, the data generated from this project clearly show that mutations in spastin gene are not correlated with spastin microtubule-severing

enzymatic activity. These results strongly suggest that the dysfunctions of mutated spastin might be related to other functional activates of spastin.

Reviewer 2:

Hereditary spastic paraplegia is an untreatable condition, and understanding of axonal biology will also be important for other conditions with axonal degeneration. The results from this grant will clearly facilitate additional experimental work likely focused on suppression of toxic properties of misfolded mutant proteins, and again, this will also be important for other neurodegenerative conditions with axonal degeneration. The principal investigator already has a subcontract on an R01 grant awarded to Dr. Morfini focusing on HSP.

The authors plan to apply for additional grants, and these results will undoubtedly strengthen their future applications.

Reviewer 3:

I find this study to be rather important for helping us understand whether there are gain-of-toxicity effects associated with different mutations of spastin genes in the human. However, I do not think the findings will immediately translate into effective treatment strategies for the disease, and we should not be expecting that.

I think this study will improve our thinking about HSP and will tell us whether protein toxicity is also involved in the process, and if so, it will suggest mutations within the spastin gene that induce this toxicity. These are important milestones.

However, I do not think the findings will immediately be available to take into clinic and to be translational in nature.

***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: During the funding period of the CURE grant, Dr. Baas became a collaborator on one NIH R01 grant entitled, "Axonal transport deficits during hereditary spastic paraplegia." Dr. Morfini is a PI on this grant. In addition, Dr. Baas is also planning to submit more grants for which the data generated from the CURE grant will be critical.

Reviewer 2:

Additional grants will be submitted, and the authors plan to continue to pursue this important question. Again, these results will improve their future applications.

Reviewer 3:

Strengths: Additional funds were generated (i.e., collaboration with Dr. Morfini on an R01), and these funds are well used. The applicant is also planning to apply for grants which will expand the well-developed 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:

Strengths: The funding that supported this project resulted in publications of three manuscripts and one book chapter. Importantly, all three of these manuscripts were published in high impact journals. These manuscripts were partially funded by CURE.

Reviewer 2:

The authors published their research in high impact journals. Even though the number of papers is relative low, the amount of published data and the quality of the papers and journals clearly make their publishing output adequate.

Reviewer 3:

Strengths: I am impressed by the manuscripts published on the topic. The applicant has performed critical experiments, obtained results and shared them with the scientific community. In addition, there are multiple manuscripts emerging from these studies, and I am confident that they will also publish well.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

It does not seem that students were paid by the funds from this project.

Reviewer 2:

No students were included in this grant, and no substantial improvements to infrastructure have been achieved. However, this facilitated a grant from NIH and likely will help the PI to obtain further funding.

Reviewer 3:

The research improved the research environment within the institution. However, I do not find major improvements in the infrastructure of the institution; potentially it was not required at this time.

***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: During the funding period of the CURE grant, Dr. Baas has established a successful collaboration with Dr. Morfini, who works at the University of Illinois at Chicago. The collaboration resulted in the recently funded NIH R01 on which Dr. Morfini is a Principal Investigator and Dr. Baas is a collaborator.

Reviewer 2:

The PI has established collaboration with Dr. Morfini from the University of Illinois and has a subcontract on his R01 NIH grant.

Reviewer 3:

The applicant collaborated with Dr. Morfini at the University of Illinois at Chicago. In the future, the applicant is hoping to generate more fruitful collaborations with other scientists at other institutions and is also trying to form a hub within Drexel University.

***Section B. Recommendations***

***SPECIFIC WEAKNESSES AND RECOMMENDATIONS***

Reviewer 1:

Weaknesses: The experiments described in Specific Aim 2 were initiated during the funding period; however, the results from these experiments are not included in the final progress report. Despite this weakness, there are definitely sufficient published data showing the acceptable progress of the proposed project.

In basic or clinical science where it is necessary to conduct research, it is really difficult to expect that all experiments would work as was originally described in the specific aims. The only recommendation I would have is related to the possibility of getting help from within the institution. Another scientist with expertise related to the project could oversee the timeline described in the project and might also provide intellectual input.

Reviewer 2:

None

Reviewer 3:

I recommend, as a next step, to incorporate corticospinal projection neurons in the assays being developed. It would be most interesting to see how these mutations affect microtubule severing in cortical motor neurons, and as a control the applicant can use another neuron population that does not degenerate in HSP.

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**Project Number:** 0863103  
**Project Title:** Role of CTF18 in Female Germ Cell Development and Fertility  
**Investigator:** Berkowitz, Karen

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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 stated objectives were to determine the function of Chtf18 in mouse oogenesis and folliculogenesis and to determine the cause of subfertility in Chtf18 mutant females. The first aim was largely met; the second was more problematic. Histology showed that folliculogenesis was reduced in mutants, resulting in smaller ovaries with fewer follicles and more abnormal follicles. Determination of the cause of subfertility was weakened by the decision to examine only a fairly late timepoint in development. Mated mice could have been sacrificed much earlier to determine whether the fertilized eggs implanted or even cleaved properly. Either of these might be an expected outcome of a mutation that causes aneuploidy. In general, though, the study was preliminary, and a final determination would not necessarily be expected after such a short time.

##### Reviewer 2:

The project met the stated objectives and went quite a bit further with the analysis. The overall goal was to examine phenotypes in mice on a ctf18<sup>-/-</sup> mutant background. The work is highly relevant, and the experimental plan was well laid out. The data analyzed were very carefully collected, controlled and appropriately statistically weighted. No significant changes were made to the study design as outlined. Moreover, the results are very relevant to human health.

##### Reviewer 3:

The first objective was to determine the function of Chtf18 in mouse oogenesis and folliculogenesis *in vivo*. The PI showed that the deficient mice had a decreased number of follicles and decreased competence of the oocytes. In light of these findings, the size of the cohort was too small to obtain hormonal measurements and lacked some power for statistical evaluation. Hence, the proposed number of subjects was small.

The second objective, to determine the cause of subfertility in <sup>-/-</sup> females, resulted in a fairly large amount of data suggesting that there is embryo loss, but the results of genotyping and phenotyping are still pending. Thus, while much data was derived, some studies are not yet completed and others are underpowered. The data was sufficient to answer much of Aim 1. Aim 2 results are only partially answered. The data generated in Aim 2 at best suggests a limited answer to the causes of subfertility.

There were not significant changes in the protocol. The protocol was not completed. Since work went slowly and procedures needed to be developed, the PI stuck to the proposed protocol. However, all the objectives were not met. The significance of the work is not clear, since prevalence of the mutation in women is not known and the effects of the mutation in women are not known. It is very likely that this mutation may be only a minor or insignificant item in human female subfertility. This question should have been asked first.

While the data was not sufficient to meet all objectives, the data provided was applicable to the project objectives.

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

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

#### Reviewer 1:

The potential significance of this project for improving health was as the PI indicated. The issue addressed is infertility/subfertility, both of which are important health considerations for those affected by them. It is unlikely and should not be expected that this project would have a major direct effect on improving human fertility, since the presence of such a rare allele is likely to be very limited in the population. Nevertheless, basic studies of the general mechanisms underlying various medical conditions, such as subfertility/infertility, always have the potential to provide insight into those mechanisms and thus always have the potential to improve human health.

#### Reviewer 2:

The strength of the project was the significance for improving women's reproductive health. Defects in chromosome segregation during meiosis are extremely common in women and are a major cause of miscarriage. The findings outlined here suggest that the gene Ctf18 plays an important role in maintaining the ovarian pool in mammals; future studies may be aimed at understanding its function in women or developing diagnostics based on the findings to allow personalized therapeutic applications to detect and treat different types of infertility.

#### Reviewer 3:

This project, as is, provides little significance to human health. It will only provide useful data if the data is applicable to women. The authors overstate the case. The manuscript talks about mammals, but only rodents are studied, so the data may not be pertinent to humans.

***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:

Such leveraging was not anticipated and did not occur. The PI was planning on applying for an NIH grant last fall. There was no indication of whether or not this was done.

Reviewer 2:

The PI states that additional funds will be applied for in the future via the National Institutes of Health.

Reviewer 3:

No

***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 have materialized. The PI indicates that one is in the works and will be submitted within a year. No patents or other commercial developments are anticipated.

Reviewer 2:

None are listed.

Reviewer 3:

A manuscript is being prepared entitled, "CTF18 plays critical roles in female gametogenesis and ovarian folliculogenesis in mammals." No article has been provided yet. However, the title may be a hyperbole, as stated above.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

Dr. Berkowitz is a young investigator. When this project began, she had been an assistant professor at Drexel for just a few months. Thus, the project supported the research of a new investigator. It was not clear from the strategic research plan or reports if she was recruited specifically for this project or not. The only other person supported was to be a research technician. Also, a pre--doctoral student was trained. There were no proposed improvements to infrastructure.

Reviewer 2:

The project enhanced the quality of research; it should set the stage for further recruitment of personnel when federal funds are obtained to continue the project. Moreover, the project allowed support of a clinician scientist in research-related activities and mentorship of additional personnel.

Reviewer 3:

Some new protocols have been developed which may be useful to other scientists at Drexel University. A pre-doctoral student was trained.

***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 outside collaborations were indicated.

Reviewer 2:

The researchers will continue the work with federal funding that should be awarded given the quality of the results. Overall the studies were well done and should spark enthusiasm for this system beyond the university that was awarded the funds, allowing for outside collaborations as well.

Reviewer 3:

No

***Section B. Recommendations***

***SPECIFIC WEAKNESSES AND RECOMMENDATIONS***

Reviewer 1:

I found the major weakness to be with Aim 2. The PI could have carried this part of the study farther and potentially given a clearer answer to the question of the cause(s) of subfertility in the mutant mice. Determination of the cause of subfertility was weakened by the decision to examine only a fairly late timepoint in development. Mated mice could have been sacrificed much earlier to determine whether the fertilized eggs implanted or even cleaved properly. Either of these might be an expected outcome of a mutation that causes aneuploidy.

Reviewer 2:

None were noted.

Reviewer 3:

1. The projects that should be completed must be addressed and finished. Additional publications will be necessary to advance Dr. Berkowitz's career.
2. Next steps should be considered. An evaluation as to the likelihood that this mutation would be a significant human problem should be started. From an evaluation of what is known in human subfertility, this may not be a major human issue.

3. The problems seen in mice may not be the only fertility defects. Data should be confirmed by other techniques as well.
4. With what was learned, future studies should have greater numbers of animals in each group for appropriate power.

### **Generic Recommendations for Drexel University**

#### Reviewer 1:

It would have been nice to see what work has been done in the interim, since this project ended in December 2010. One hopes that Dr. Berkowitz has continued and further developed the project. In general, the work done under this grant has largely been of the "effect" variety and has not yet addressed mechanism, and this would certainly be required for her to obtain significant federal research funding to continue the project.

#### Reviewer 3:

Junior investigators would generally benefit from a senior mentor. Work should be discussed in research groups to improve protocols.

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**Project Number:** 0863104  
**Project Title:** A Microfluidic Model of Drug-induced Liver Toxicity  
**Investigator:** Bouchard, Michael

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

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

### **STRENGTHS AND WEAKNESSES**

#### Reviewer 1:

The project had two specific aims: 1) to create layered co-cultures of primary hepatocytes and endothelial cells in a microfluidic device with recirculation; and, 2) to assess the impact of drug exposure on hepatocytes to model drug-induced liver injury.

Goals in Aim 1 were favorably met. Culture conditions were identified that allowed the layered attachment and persistent co-culture of both cell types under continuous flow for 30 days, as well as extension to human hepatocytes for 15 days. In order to accomplish this, surface coating, sterile conditions, media formulation, oxygenation conditions, flow rate and microfluidic configuration and interconnects were optimized. Microchannel leakage was a problem that delayed the project but was solved towards the end of the project period, and therefore Aim 1 is now almost complete. Goals for Aim 2 were not met as a result of this delay; nonetheless, a reasonable explanation was given, and there would have been no way to progress this aim in parallel.

#### Reviewer 2:

Two specific aims were posed for this project. The investigators described in the final report studies towards the construction of a microfluidic system to culture hepatocytes. While the investigators showed good development in building the microfluidic devices, no data was shown to demonstrate co-culture of hepatocytes and sinusoidal cells.

Material issues associated with the device building, compatibility with cell culture, ponding and leak blockers have been addressed in the literature.

The investigators have not addressed Aim 2. This aim is the essence of the whole project.

#### Reviewer 3:

This project did not meet all of the stated objectives, specifically Aim 2. However, the investigators provided sufficient explanations to indicate the problems they encountered with fluid leakage in their microfluidic device and strategies they attempted to troubleshoot the device.

The research design and methods were adequate in light of the project objectives; however, no functional assessments of the liver cells, either primary or cell lines, were conducted. Only gene expression was utilized here, but that does not always correlate with liver functions. Furthermore, longevity data for the liver cells over time was not presented in the figures of the final progress report.

Changes were indeed made from plastic to glass surfaces during the course of the project, but sufficient explanation was provided to justify the change.

Sufficient data and information was provided to indicate that the project met part of the objectives, specifically progress towards completing Aim 1. Very little progress was made using primary human hepatocytes, which is required for modeling and predicting drug-induced liver injury given severe differences in liver metabolism between humans and animals.

The data and information provided were applicable to the project aims/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:

A better *in vitro* model of the human liver that reflects the complexity of multicellular architecture is sorely needed for studies of drug-induced liver injury and hepatic viral infections. The impacts of this design are: that it would require few of the precious resource of human cells; that it captures the key cell-cell interactions of importance in the liver (hepatocyte/endothelial cell); that it incorporates the regional specialization of the liver conferred by nutrient gradients under flow; and, that it has an exit route for toxic biliary products.

In spite of the importance of this proposal area and the significant progress made toward modeling the rat liver (with some inroads to human), the choice of drug and the need for evaluating it in this microfluidic model are not obvious. Is the drug reaction dependent on zonal liver features, on interactions between hepatocytes and endothelial cells, or even observed in a majority of hepatic donors? The PI indicates that 10% of patients suffer from hepatotoxicity, raising the question of whether a typical hepatocyte donor will accurately model the drug response of interest. The use of pathway reporters to explore mechanistic aspects of hepatocyte toxicity and the use of viral transduction of such reporters is novel and has broad potential significance.

### Reviewer 2:

Having an *in vitro* system that possibly mimics liver for potential evaluation of drugs against induced health concerns is highly significant; however the investigators seem to have lost the focus by concentrating on building a microfluidic device. The low number of cells that could be seeded in the microdevice may or may not provide the perfect model for the overall sought

impact. Though a microfluidic device could be a great asset, a meso scale device probably would have been built by now and tested for Aim 2. Smaller may not be good in this case.

Reviewer 3:

This project is one of many in the field to create more physiologically accurate models of the human liver for *in vitro* drug testing and other applications (i.e., modeling of infectious diseases). Given the results of this project as stated in the final progress report, it remains unclear whether the device being built by the investigators will provide any further benefit over systems that are currently being utilized in the field (academia and the pharmaceutical industry) for the aforementioned applications. The results presented by the investigators are unimpressive to date, but considering the dollar amount budgeted for this project, the investigators made sufficient progress in realizing their ultimate objective of creating a microfluidic device with human liver cells, oxygen control and fluid delivery.

No major discoveries, drugs or new approaches for prevention, diagnosis and treatment of disease were created as a result of this project. This project was mostly about technology development (i.e., microfluidic device) for culturing of liver cells in a two-layer configuration (i.e., endothelia layered on top of hepatocytes with an extracellular matrix layer in between the cells).

The future plans for this research project are to culture human liver cells long-term in the non-leaking microfluidic device, control oxygen and fluid delivery to the cells and then use it for drug toxicity predictions, all specifically for humans. The investigators have a long way to go before their system can be commercialized or will be utilized by pharmaceutical companies.

***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:

This project led to an NIH application that is pending.

Reviewer 2:

The investigators secured an R21 from NIH.

Reviewer 3:

Per the investigators, there was a three-month overlap between an R21 grant and this project. It is not clear if the additional funds from the R21 aided the progress of this particular project. The investigators are planning to apply for an R01 and NSF funds using the results of this 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:

Two publications are planned: one on the microfluidic device and the second on the impact of oxygen transport on tissue response.

Reviewer 2:

No

Reviewer 3:

So far, it appears that no publications, licenses, patents or commercial development opportunities have been submitted/explored as a result of this project.

The investigators are indeed planning to pursue one or more of the above. However, commercial development will require culturing of human liver cells, long-term functionality of the cells at levels and longevity better than conventional culture models, and thorough validation using compound sets greater than 100.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

Graduate students were supported by and benefited from this project. It appears that the microfluidic infrastructure has been improved in the PI's laboratory.

Reviewer 2:

The funding fostered collaboration between mechanical engineering faculty and the department of biochemistry and biology. This collaboration is a strength for securing funding from NIH and the National Science Foundation.

Reviewer 3:

Per the investigators, the project did not enhance the quality and capacity for research at Drexel University.

Funds were used to support pre-doctoral students, an assistant research professor (12.5%) and the PI (5%).

***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:

Collaborative efforts between a liver biologist and a microfabrication expert were promoted. This kind of interaction will be fruitful in the future as interdisciplinary research pushes the boundaries of science and technology.

Reviewer 2:

No

Reviewer 3:

It does not appear that this project led to collaboration with research partners outside of the institution or new involvement with the community. This was a collaboration between two professors at Drexel University, Drs. Noh and Bouchard. Interactions with local pharmaceutical companies are in progress.

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

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

Reviewer 1:

1. Incomplete Aim 2. Now that the system is almost working for rat hepatocytes, shifting to human hepatocytes and studying drug interactions (as proposed) is recommended.
2. Incomplete Aim 2. Rather than a sole focus on retonavir, consultation with Merck colleagues to select compounds that exhibit dose-dependent, species-specific, zonally-dependent hepatotoxicity would be ideal, since this would most fully exploit the benefits of the device.
3. Incomplete Aim 1. Characterization of the biliary axis is recommended, since this is a highly novel aspect of the device.

Reviewer 2:

1. The co-culture has not been demonstrated in this report. Data to show a mimic of liver are essential.
2. Progress has been made in building a microdevice. However, Specific Aim 2, the essence of the grant, was not investigated. The investigators are encouraged to focus on Specific Aim 2, even in a meso scale device.

Reviewer 3:

1. Weakness: Lack of attachment and functionality of hepatocytes on glass surfaces coated with collagen.

Recommendation: Attempt covalent linking of collagen and matrix to glass as other groups in the field have tried.

2. Weakness: No data on functionality of liver cells in devices.

Recommendation: Standard methods for measuring albumin secretion, urea synthesis, CYP450 activities and transporter functionalities (i.e. bile canaliculi and transporters) exist in the field and have been used for several years now. These should be coupled with gene expression data to determine long-term functionality of human liver co-cultures in the microfluidic device.

3. Weakness: No data on drug exposure of cells in the device.

Recommendation: Even though the investigators were not successful in getting primary human liver hepatocyte:LSEC co-cultures to survive reproducibly in their leaking devices, they can initially use HepG2 cells and drug exposure to show proof-of-concept of being able to conduct drug toxicity studies in their microfluidic devices. HepG2 cells respond accurately to several classes of liver toxins (especially ones that do not require liver metabolism), and there are several papers on HepG2 cells being used for drug toxicity studies in conventional culture models.

4. Weakness: Only one drug was proposed in Aim 2.

Recommendation: If this device is to garner support from the academic and pharmaceutical communities and has any chance of ever being commercialized, it must be more thoroughly validated for drug metabolism and toxicity studies using compound sets greater than 100.

5. Weakness: Very limited review of other systems in the field in the original proposal.

Recommendation: There are several groups now working on microfluidic liver systems, rodent and human, both in 2D and 3D formats, and perfused and static formats. The investigators should review those publications/systems and determine functional and validation criteria that will set their system apart from others, not just technologically, but with respect to better prediction of human-relevant drug metabolism and toxicity. If the system does not significantly improve the latter, then it becomes one of a plethora of interesting devices that will ultimately not impact human health, at least in the setting of drug development.

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**Project Number:** 0863105  
**Project Title:** Identification of Biomarkers and Therapeutic Targets  
in 3D Hypoxic Breast Cancer Mode  
**Investigator:** Johannes, Gregg

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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 very interesting data generated by the PI. While there were some significant changes in the specific aims, the general context and theme remained centered around the HIF pathway and its role in breast cancer. The progress has been significant in general, although my opinion is that the investigators have given up on the proposed targets too easily, and shifted most of the effort onto the dynamic of HIF induction and degradation of its RNA. This in itself is a very interesting topic, with translational implications.

##### Strengths:

- 3D models.
- The data generated on HIF dynamic should be very important for a future grant.
- The MNK2 data are particularly interesting; it is hard to believe that this target is not important, just based on Bim impact. Additional cell types should be tried.

##### Weaknesses:

- As stated above, the proposed targets were investigated mainly with respect to their ability to regulate Bim in hypoxia. Their inability to affect Bim downregulation by hypoxia led to abandoning them completely.
- This is the first report that proposes ET2 as an HIF2 specific target. However, adding HIF1 or 2  $-/-$  cells would be stronger. In general, adding some genetic models would increase the strength of the data.

The use of MCF10A cells has merits, but I would argue that primary patient cancer cells would significantly strengthen the investigation. These are becoming routinely available at most cancer centers. They are also available from various outside sources, such as Memorial Sloan-Kettering Cancer Center.

##### Reviewer 2:

##### Strengths:

- The project was conceived on the basis of well rationalized hypotheses.

- The feasibility of the project was predicated on the PI having all necessary assays already in use.

Weaknesses:

- The project did not meet its stated objectives.
- The PI essentially abandoned the stated goals on the basis of the proteins not regulating Bim expression.
- Although Bim plays a role in 3D acini formation, other high impact papers have shown that the model can serve to assess multiple transforming and mitogenic events.
- The goal of Specific Aim 1 was to assess target effects on "tissue architecture" not Bim expression, and this was never done.
- The stated rationale for abandoning Specific Aim 2 and Specific Aim 3 are inadequately justified. They are not entirely dependent on Specific Aim 1, and so even if Specific Aim 1 results were negative, this is not justification for not continuing (or even starting) the last two specific aims.

Reviewer 3:

This project utilized the MCF10A-derived 3D acinus culture as a model to investigate the impact of hypoxia on ductal carcinoma in situ (DCIS). Previously, Dr. Johannes' group had identified five hypoxia-induced genes in MCF10A acini using microarrays. The current proposal aimed to: 1) validate the expression and function of five key hypoxia-regulated genes in 3D acini; 2) verify whether the genes identified in hypoxic 3D acini are also altered in hypoxic DCIS tumors; and 3) determine whether altering hypoxic gene expression changes the sensitivity to chemotherapeutic agents in normal and oncogene expressing 3D acini. At the time of this report, Dr. Johannes has completed Aim 1. The focus of Aim 2 was changed as a result of Aim 1's findings. Dr. Johannes has made some interesting findings regarding the differential regulation of HIF1 $\alpha$  and HIF2 $\alpha$  mRNAs in MCF10A cells by hypoxia. Aim 3 was not pursued.

Key strengths:

- Validation that the hypoxic induction of five key genes previously identified in hypoxia-treated 3D acini using microarrays.
- The finding that LOX and ANGPL4 are potential HIF1-specific targets, ET2 is an HIF2-specific target, and MNK2 is a target of both HIF1 and HIF2.
- The finding that HIF1 $\alpha$  mRNA has reduced stability under hypoxia, whereas HIF2 $\alpha$  mRNA stability is not affected.

Weaknesses:

- Some of the figures are not properly labeled and/or have missing data panels.
- Hypoxia-responsible element(s) in gene promoters were not identified. There was no determination of HIF binding directly to promoters using ChIP.
- Although very interesting, the data were not convincing enough to conclude that MNK2 was responsible for hypoxia-induced eLF4E phosphorylation.
- No mechanisms are investigated as to the differential regulation of HIF $\alpha$  mRNA stabilities by hypoxia.

***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:

- A more detailed understanding of HIF induction and general dynamic in hypoxia.
- Predictions with respect to HIF1 versus HIF2 specific inhibitors, as far as their antitumor effects are concerned.
- More complex *in vitro* models than the 2D cultures.

Weaknesses: There was a lack of animal models, in particular genetic models, and almost complete reliance on MCF10A cells.

Reviewer 2:

Weaknesses:

- There was very little productivity from this study.
- No publications have resulted from this project.
- No additional funding has resulted from this study.
- This study will have little or no impact on health.

Reviewer 3:

Strengths: The applicants have identified a panel of hypoxia-induced genes in MCF10A acini, which may provide insights into the role of hypoxia on mammary epithelial differentiation and potentially development or progression of breast cancers.

Weakness: The biological relevance of MCF10A acini to DCIS is not very clear. There was a lack of mechanism-driven investigations.

***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 investigator states that he is planning on applying for NIH grants. No major applications were submitted during the course of this grant (weakness).

Reviewer 2:

Weaknesses: This project is unlikely to garner additional support. A tangential finding was submitted for NIH funding but was not successful.

Reviewer 3:

An application was submitted to NIH but was not funded. Resubmission has been planned.

***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:

Hypoxia suppression of Bim and Bmf blocks anoikis and luminal clearing during mammary morphogenesis.

There was a good quality paper. Additionally, there was collaboration between Drs. Reginato and Johannes.

Reviewer 2:

Weaknesses:

No publications will result from work on the stated goals. A tangential finding is being pursued for publication, but is unlikely to be in even a mid-tier publication.

Reviewer 3:

No peer-reviewed article has been published or submitted. The PI plans to submit a manuscript on hypoxic regulation of HIF $\alpha$  mRNA and HIF2-dependent regulation of ET2.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

Salaries were paid for the investigators and collaborators. It does not seem that the infrastructure was improved as part of this grant.

Reviewer 2:

Weaknesses: This grant made no objective improvement to the grantee's institution.

Reviewer 3:

- This grant has supported the collaboration between Dr. Johannes and Reginato.
- The 3D acinus culture model is likely to be useful to investigators of breast cancer biology.
- No additional investigators were brought in to assist in this application.
- One pre-doctoral student was funded by this application.

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

### **STRENGTHS AND WEAKNESSES**

Reviewer 1:

It looks as if the collaboration was intramural and did not change from the beginning to the end.

Reviewer 2:

Weaknesses: This project did not impact anyone outside of the institution.

Reviewer 3:

None

### **Section B. Recommendations**

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

Reviewer 1:

1. The targets were abandoned purely based on the effect on Bim (or lack thereof). The effect of MNK2 on ERK phosphorylation in hypoxia is very compelling and may well have effects on the biology of 3D structures. The ramifications of MNK2 induction need to be explored more deeply.
2. As proof of principle, test the effects of two targets (rather than one) on Bim repression and on 3D structure viability in hypoxia.
3. For a competitive grant, try to include a mouse model of breast carcinogenesis (MMTV) and test the expression of these targets in the early lesions. Also, test orthotopic xenografts with or without shRNA for the proposed targets.
4. The effects of hypoxia on HIF dynamic (RNA stability) are interesting. The investigators need to try several cell types. Is this a general effect? What are the possible players? miRs? (17-92)? RNA binding proteins?
5. Is the effect of hypoxia on ET2 HIF specific? (HIF1 and HIF2 mouse KO-derived cells would be powerful to test.) The lack of a canonic HIF site is interesting. How many prediction programs have been tested? Also, how many candidate hypoxia-responsive factors are to be considered (nf-kb, AP1)? Is it possible that there are two components? Maybe the canonic HIF sites are outside the region tested, while another regulator (not HIF) is relevant for the luciferase assays using the constructs described.
6. The investigator needs to go beyond MCF10A system, for competitive grants. The effects of hypoxia on both sense and antisense HIF (aHIF) are both interesting and novel. These, if investigated in detail, can lead to a very competitive proposal in the future.

Reviewer 2:

1. Poorly justified abandonment of entire project was a weakness. The grant presented three well-justified specific aims. In the first year of research, some progress was made on Specific Aim 1, and none was made on Specific Aims 2-3. In the second year, again, some progress was made on Specific Aim 1, but not on the final two specific aims. Suddenly in the third year, the PI entirely abandons all of the stated specific aims for poorly justified reasons. Although the five proteins were found not to regulate Bim, this is inadequate justification as to why effects on acinar formation were not studied (Specific Aim 1), why examination of patient samples was not performed (Specific Aim 2), and why drug testing was not even initiated (Specific Aim 3). This was not a grant examining regulation of Bim. It was a grant focused on hypoxia and the five protein targets, all of which may play important roles in tissue architecture (Specific Aim 1), patient tumors (Specific Aim 2) and drug sensitivity (Specific Aim 3). It is certainly not the case that Bim is the sole and central mediator of all of these questions. The lack of scientific perseverance and lack of deliverables from the funding is troubling.

Recommendation: Specific aims for a grant cannot be entirely dependent on each other, in order to prevent a single unanticipated finding from sinking the whole project. In this case, the specific aims were in fact not entirely dependent, but the PI chose to use an unanticipated finding in Specific Aim 1 to justify lack of progress across the entire project.

2. Lack of productivity with no papers and no additional funding resulting from the funded project were weaknesses.

Recommendation: Better scientific perseverance is needed.

Reviewer 3:

1. The biological relevance of the MCF10A acini model to DCIS should be clearly articulated. The potential connection between hypoxia and DCIS should be justified.
2. More mechanistic approaches should be employed.

**Generic Recommendations for Drexel University**

Reviewer 2:

The PI may benefit from more rigorous scientific mentorship.

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**Project Number:** 0863106  
**Project Title:** Role of O-GlcNac Transferase as a Biomarker and  
Therapeutic Target for Prostate Cancer  
**Investigator:** Reginato, Mauricio

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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:

In this project, the applicant proposed to characterize protein post-translational modification by O-linked N-acetylglucosamine (O-GlcNac) as a global process that regulates prostate cancer cell growth and death. The applicant hypothesizes that enzymes regulating protein O-GlcNac, such as OGT, can be used as drug targets to develop therapeutics effective for the treatment of prostate cancer. Specifically, the applicant proposed to determine frequency of OGT over-expression and O-GlcNac modification in human prostate cancer tissue, to characterize role of OGT in prostate cancer cell proliferation *in vitro*, and to determine mechanisms involved in the OGT-mediated regulation of FOXM1, a cell cycle regulator. The experimental approaches are standard and routinely used in the applicant's laboratory. The applicant proposed to repeat the experiments sufficient times in order to obtain statistically significant results. There are several weaknesses in the proposal and they include: 1) use of reagents that have not been validated (i.e., anti-OGT antibody for use in IHC experiments); 2) lack of rationale to carry out proposed experiments (e.g., determine the role of FOXM1 that does not undergo the O-GlcNac modification); and, 3) unfocused experimental plans to directly implicate the possible role of protein O-GlcNac in prostate carcinogenesis. Other weaknesses include the absence of proper literature citation.

##### Reviewer 2:

The goal of the project was to determine the role of O-GlcNac transferase as a marker and therapeutic target for prostate cancer. The PI showed convincing evidence to demonstrate the role of O-GlcNac transferase in regulating prostate cancer cell proliferation and angiogenesis *in vitro* using cell line models (Aims 2 and 3). However, the data from an actual human prostate cancer sample is lacking largely due to the failure of the antibody to detect the target (Aim 1). The demonstration of over-expression of this molecule in human prostate cancer is crucial in order to develop it as either a marker or therapeutic target. Therefore, the overall goal has at the best only partially been met.

##### Reviewer 3:

This project met the goals laid out by the investigators, and their previously published manuscripts are of high technical quality and significant. This is viewed as a clear strength of the proposal.

***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:

Prostate cancer is the most diagnosed cancer type in men, and each year tens of thousands of American men lose their lives because of it. Hence, identification and characterization of mechanisms involved in the prostate cancer progression is highly significant for the development of targeted drugs to improve disease outcome. The proposed project of targeting protein O-GlcNAc may be important in improving the overall health of patients diagnosed with prostate cancer, which affects the lives of too many American men.

Reviewer 2:

The potential impact of this research is the understanding of the role of O-GlcNAc in regulating tumor cell growth *in vitro*. However, whether this is true in actual human prostate and prostate cancer progression remains to be determined. The budget seems to be appropriate for the work accomplished, even though a portion of the critical work has not been done due to lack of the specific reagent.

Reviewer 3:

This project addressed the hypothesis that O-GlcNAc post-translational modification was intricately involved in prostate cancer progression. Their data not only identified O-GlcNAc as a novel therapeutic target for future research aimed at improving health but also delineated at least one specific mechanism (the regulation of a forkhead transcription factor). The researchers, having published previously in a couple of high impact papers, are attempting to get funding for future research from both federal and private sources. Novel OGT inhibitors as possible therapeutic agents are very intriguing.

***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 applicant states that he did not obtain grant funding from NIH.

Reviewer 2:

No additional funding has been obtained, but there is a pending grant from Prostate Cancer Foundation.

Reviewer 3:

They have attempted to secure DOD funding and are reapplying. They also are going for funding from the Prostate Cancer Foundation. Failure to obtain funding to date in this current ultra-competitive environment is seen as only a very minor weakness.

***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 applicant does not list any published manuscripts, posters or meeting presentations. He states that one manuscript will be submitted for publication.

Reviewer 2:

There is one pending publication and no patent or other commercial products.

Reviewer 3:

A peripherally related manuscript was published in 2010 in *Oncogene* and 2008 in *Science*.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

There is no clear indication of whether the project had a positive impact on the quality of research at Drexel University College of Medicine.

Reviewer 2:

There was no impact on overall infrastructure, but it helped to maintain the researchers and pre-doctoral students.

Reviewer 3:

Funds were used for pre-doctoral support.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

The applicant lists Dr. Fernando Garcia and Dr. Keith Vosseller from Drexel University as collaborators. He also states that collaboration with GlaxoSmithKline has begun.

Reviewer 2:

No commercial development.

Reviewer 3:

I expect they are currently seeking appropriate clinical aid for their promising OGT inhibitors in the treatment of prostate cancer.

***Section B. Recommendations***

***SPECIFIC WEAKNESSES AND RECOMMENDATIONS***

Reviewer 1:

The applicant indicates that he created a prostate cancer tissue microarray. No diagnostic tools for prostate cancer resulted from the current studies.

Reviewer 2:

1. Work with companies to develop a more specific antibody against the target.
2. Try to evaluate up/down stream events that may have reliable antibodies (e.g., Fox1) in cancer tissue.
3. Analyze the expression using other techniques (PCR, etc) at RNA level.

Reviewer 3:

None

**Generic Recommendations for Drexel University**

Reviewer 3:

This research looks extremely promising and should be supported as long as external funding is being actively sought.

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**Project Number:** 0863107  
**Project Title:** Piezoelectric Microcantilever Sensors (PEMS) to  
Detect Methicillin-Resistant Staphylococcus aureus (MRSA)  
**Investigator:** Rest, Richard

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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: Given the funding level provided, the project made a reasonable start on the broad objective stated in the project title, "Piezoelectric Microcantilever Sensors (PEMS) to Detect Methicillin-Resistant Staphylococcus aureus (MRSA)."

A device to detect MRSA was produced, as promised in the proposal, and experimental results were briefly described. The goal of building a PEMS for MRSA is a worthy goal. The interdisciplinary approach taken by the researchers appeared to work well and could be developed further in future collaborations.

The general protocol described was followed, and the experimental design appeared to be good.

##### Weaknesses:

In reading the strategic plan one sees the purpose of the grant (IB) is "to develop a real time, highly-sensitive, highly specific, portable, cost effective sensor that can be used to detect MRSA.....leading to improved treatment outcome." It is fair to say that the PEMS devices developed under this grant, thus far, were not evaluated or tested at the level mentioned in the purpose section (IB). Later in the strategic plan (Research Project Overview II) the objective stated in IB is restated as a three-year objective, and it is further stated that three MRSA strains will be investigated. The goals of developing a practical sensor and investigating three strains were not achieved.

Promising results were obtained; however, the quantity of data presented is not large, and it is not clear to what extent the data can be reproduced. The quantity of data is not anywhere close to determining the usefulness of the sensor system in a practical device. Again, the data and information provided were applicable to the project objectives as stated in the title.

In the reports provided there are no discussions of possible cost, size, durability, stability and practicality of a possible "field deployable" PEMS for MRSA. A brief preliminary discussion of these topics should have been provided.

### Reviewer 2:

The major strength of this application is the problem being addressed by the investigators. The ability of clinical laboratories to rapidly diagnose MRSA infections is critical for appropriate antibiotic therapy and to decrease cost for the institution. In addition, the PI and colleagues have previously developed a PEMS array that has successfully detected multiple organisms of medical interest. However, there are multiple weaknesses to this application. First, the PI and co-investigators have already developed the PEMS array, and the addition of new antibodies to an already developed array lacks novelty. Second, there is no discussion regarding how clinical laboratories currently detect MRSA (there are multiple methods, both molecular and phenotypic). What advantage would the PEMS array have regarding implementation of this assay in the clinical laboratory? Third, there was little discussion regarding how the antibody targets will be picked; and, in fact, the targets for the antibodies that were picked were never discussed. There are few antigens that would differentiate MRSA from *S. aureus*; one being MecA (an acquired penicillin binding protein that mediates oxacillin resistance). Experiments using whole cell *S. aureus* vs. MRSA to find rabbit polyclonal antibodies that would differentiate the two strains will most likely be unsuccessful. Therefore, as predicted, the PI used purchased MRSA/*S. aureus* antibodies, but the targets for these antibodies were not discussed in the final progress report. Lastly, it appears that the PI used very few strains to validate these antibodies. Once again, unless the PI uses anti-PBB2A antibodies, these antibodies are unlikely to differentiate between MRSA and *S. aureus* when using a large strain set.

### Reviewer 3:

**Strengths:** A significant amount of time was spent successfully fabricating the PEMS system using lead magnesium niobate-lead titanate freestanding films. Less time was spent on testing the system. Figure 4 shows that anti-MRSA does detect MRSA and that anti-SA antibody did not respond to the MRSA which was illustrated in Figure 5, and the PI concludes that the PEMS system is suitable for detection of MRSA in a direct manner.

### **Weaknesses:**

The major determinant of whether the system will work is the type and quality of the antibodies used. Unfortunately the project is only using American Type Culture Collection (ATCC) isolates which may not reflect clinical strains, and adding some clinical strains to the study should be done to validate the system. Basic experiments, such as determining whether the anti-MRSA antibody interacts with *S. aureus*, were not done. This is an important experiment which could have easily been done to complement the work done in Figure 5. The other major experiment that should have been done is doing mixtures of *S. aureus* and MRSA with the anti-MRSA or anti-SA and determining if the clean reaction occurs as illustrated in Figure 5. Another set of experiments where MRSA is mixed with other bacteria needs to be done to determine if the system can work under real conditions. Other questions to be answered before the project can move forward is whether an antibody made against one MRSA can react as well against unrelated MRSA strains, including a range of clinical isolates.

Originally, it was stated that six specific anti-MRSA antibodies would be tested, but from the figures it appears that only one has been tested.

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

### **STRENGTHS AND WEAKNESSES**

#### Reviewer 1:

The significance of this project for improving health is at best unclear. Clarity could be achieved through further experimental work. It would also be useful to have scientific and engineering analysis of the cost, size, durability, stability, etc. of a field deployable system.

The value of the research completed towards improvement in health outcomes is unclear. Note that the work has not been published or presumably discussed at a scientific meeting; thus, there does not appear to be a compelling case, at this time, for a practical PEMS for MRSA. There appear to be no "relevant measures of impact and effectiveness" for the work reported.

There are no major discoveries. The technology behind resonant and static piezoelectric/piezoresistive cantilever detection of biomolecules was described in the literature well before the work funded on this grant began. See, for example, McKendry et al. *Proceedings of the National Academy of Sciences*, 99, 9783 (2002) and Kooser et al. *Biosensors & Bioelectronics*, 19, 503 (2003). In defense of the proposal it should be noted that the proposal contained no "novelty" claims that were unwarranted.

The future plans for this work do not seem very clear. It can be said that as of the date of the final report no manuscripts had been submitted to journals and no proposals had been submitted to funding agencies.

The funding for this project was not large. Results were achieved, and they were reasonable but not spectacular.

#### Reviewer 2:

The potential benefit of the project is a new method to detect and diagnose MRSA infections, which are pervasive in the United States. This would allow for appropriate antibiotic therapy to be prescribed to patients. However, it is unclear whether a particular market exists for this technology due to the glut of MRSA diagnostics on the market, including genotypic and phenotypic assays.

#### Reviewer 3:

**Strengths:** This is a novel detection system which has been used for the detection of microbes such as BA spores, and thus if the antibodies can be developed that will identify all strains of MRSA specifically, then the potential usefulness is high; and this does have the potential to more quickly identify MRSA infections, which can lead to quicker treatment and the potential for improved outcomes.

**Weaknesses:** The major concern is the quality, specificity and the ability to detect all MRSA strains using specific antibodies, and the amount of information provided on the ability to generate these types of antibodies has not been rigorously demonstrated. Without these reagents

the work cannot progress. Thus, more time must be spent on generating and testing the antibodies that will be needed to make the system work. The project should include clinical isolates, including USA300, not just the three ATCC strains to produce appropriate antibodies. No characteristics are listed for the three MRSA isolates that will be used in the project. Thus, we do not know how related they are to each other and whether they represent the spectrum of genetic diversity found in MRSA strains today. There are large numbers of MRSA isolates that have been completely sequenced, and using some of these to produce the required antibodies, especially for the virulence factors, would be an asset to the project. This would allow one to determine what the antibodies are recognizing and allow for optimization of the antibodies selected for use in the system.

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

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

#### Reviewer 1:

No leveraging of funds appeared to be reported. Plans for future grant activity seemed vague.

#### Reviewer 2:

The PI mentions that the team may apply for future funding, but there are no immediate plans.

#### Reviewer 3:

Drexel University internal Synergy Funds supported the development of the sensor. The report states that the PI planned to submit a grant or contract, but no title was provided to indicate that this was done.

***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 publication was initially expected but has not materialized. No patent or other IP related activity was reported

#### Reviewer 2:

No publications or patents were submitted or filed.

#### Reviewer 3:

No articles or peer-reviewed publications are listed nor were licenses or patents listed. The current project has not reached the stage for commercial development opportunities for detection of MRSA. It is not clear if the system is being commercialized for the detection of *B. anthracis* spores, *E. coli* O157:H7, *Salmonella typhimurium* or Cryptosporidium; although four publications for the detection of these other microbes were published in 2007-2008 (for which the Co-PI Dr. Shih is an author) and are listed in the reference section of the proposal.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

There were no infrastructure improvements. No new investigators or researchers were brought to the institution. Two pre-doctoral students received 25% support. No information on the effects of the project on their training was presented.

Reviewer 2:

There were no improvements made to infrastructure, and no new investigators were added to the institution. Funds were used to support pre-doctoral trainees.

Reviewer 3:

No improvement was made to infrastructure, though the funds allowed the researchers to test a new hypothesis and thus broadened the research activities for team based research spanning several different disciplines at Drexel University. The grant lists two pre-doctoral students supported. No recruitment of out-of-state researchers occurred.

***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 current or future collaborations were reported.

Reviewer 2:

Based on the final report, it is unclear what the plans are for future collaborations or grant applications.

Reviewer 3:

The project did not lead to collaboration with research partners outside the institution or with the community. No new collaborations as result of the research are listed.

***Section B. Recommendations***

***SPECIFIC WEAKNESSES AND RECOMMENDATIONS***

Reviewer 1:

1. Weakness: Lack of focus on a practical device. The initial proposal focused on "practicality," which was in the end not delivered.

Recommendation: Do additional experimental work plus an analysis of the cost, size, durability, stability, deployability, etc. of a practical device.

2. Weakness: Lack of publications, presentations, new proposals or new collaborations. The work completed seems not to have produced many results that will impact the future productivity of the researchers or the institution.

Recommendation: Additional focus on publications, grants, collaborations would be useful. In particular, the students working on projects like this need the intellectual stimulation/professional experience that such activities provide.

3. Weakness: The funding level for this project appeared to fall short of what would be needed to achieve the objectives and goals described in the strategic plan. It can be said that given the funding levels, this project produced reasonable if not spectacular results.

Recommendation: Important questions need to be asked. Should the funding level for this proposal have been higher? Should the proposal not have been funded? Would fewer projects overall, with more funding and better developed work plans, have produced more practical results?

4. Weakness: The periodic (yearly) reports do not seem to have generated any feedback that might have highlighted deficiencies in the project at an early stage when corrections could have been made.

Recommendation: Use periodic reports to provide better oversight.

#### Reviewer 2:

1. The most relevant antibody to differentiate between MRSA and MSSA (methicillin susceptible *S. aureus*), based on years of research, is anti-PBP2A. This is because the only difference between MRSA and MSSA is the presence of a genomic island containing *mecA* (encoding PBP2A) and a variety of other proteins that are not necessarily specific for all strains of MRSA. This aspect of the research needs to be confirmed; it was unclear what antibodies were used in the study. Studies to use rabbits to find unique antibodies that would differentiate between MRSA and MSSA are not relevant.
2. If there is to be some economic benefit regarding this instrument, the PI needs to identify a specific niche and detail the benefits of this instrument against other MRSA diagnostics.
3. The authors need to validate the instrument using a variety of MRSA/MSSA strain backgrounds to determine sensitivity and specificity. Currently, there have been very few strains tested.

#### Reviewer 3:

1. The project should include clinical isolates including USA300, not just the three ATCC strains, to produce appropriate antibodies. The strains used should be well characterized, and using some that have been completely sequenced and are clinical isolates to make the antibodies would be extremely helpful and vital.

2. Determining whether the anti-MRSA antibody interacts with *S. aureus* was not done, and this is an important experiment which must be done to complement the work done in Figure 5.
3. Whether antibodies made against one MRSA work against many other MRSA strains must be determined quickly for the project to be viable.
4. Perform mixtures of *S. aureus* and MRSA with the anti-MRSA or anti-SA and determine if the clean reaction occurs as illustrated in Figure 5.

## **Generic Recommendations for Drexel University**

### Reviewer 1:

I would try to determine if the weaknesses identified for this project are a result of the relatively low funding level or if they are the result of other factors. I would also look to see if there are other projects that fall in the same category as this proposal (i.e., some good initial results coupled with a lack of publications, new IP and a prototype practical device). Should the number/size of individual grants be adjusted so the projects funded have better funding? I recommend that these issues be examined.

### Reviewer 3:

From the papers listed in the references, the PEMS has been in development for a long time, and the amount of effort spent refining the system was most likely valuable for the overall goal of using this for detection of multiple pathogens. However, the critical reagents needed for the MRSA project have not been adequately demonstrated, and thus the project needs to focus on producing, characterizing and testing specific anti-MRSA antibodies in the next phase. Without well characterized MRSA-specific antibodies, the project cannot progress. Work on the system should also be published.

## **ADDITIONAL COMMENTS**

### Reviewer 3:

The major concern is the quality, specificity and the ability to detect all MRSA strains using specific antibodies, and the amount of information provided on the ability to generate these types of antibodies has not been rigorously demonstrated. Without these reagents the work cannot progress. More time must be spent on generating and testing the antibodies that will be needed to make the system work. The project should include clinical isolates including USA300, not just the three ATCC strains, to produce appropriate antibodies. No characteristics are listed for the three MRSA the project is proposing to use. Thus, we do not know how related they are to each other and whether they represent the spectrum of genetic diversity found in MRSA strains today. There are a large number of MRSA isolates that have been completely sequenced, and using some of these to produce the required antibodies would be an asset to the project. This would allow one to determine what the antibodies are recognizing and allow for optimization of the antibodies selected for use in the system. Basic experiments, such as determining whether the anti-MRSA antibody interacts with *S. aureus*, were not done; and this is an important experiment which could have easily been done to complement the work done in Figure 5. The other major experiment that should have been done is doing mixtures of *S. aureus* and MRSA

with the anti-MRSA or anti-SA and determining if the clean reaction occurs as illustrated in Figure 5. Another set of experiments where MRSA is mixed with other bacteria needs to be done to determine if the system can work under real conditions. Another question to be answered before the project can move forward is whether an antibody made against one MRSA can react as well against unrelated MRSA strains, including a range of clinical isolates.

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**Project Number:** 0863108  
**Project Title:** RNA Interference-based Therapy for HIV-1 Associated Neurologic Disease  
**Investigator:** Steel, Laura

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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 is an excellent project and if successfully completed can bring forward valuable information in controlling the HIV/AIDS pandemic. The investigators appeared to have made good progress by evaluating the Tat protein and its control and regulation by HIV as well as by the host proteins.

Therefore, they have made progress towards: 1) characterization of the activity of the Tat-inducible promoter, including in virus infected cells; 2) construction and evaluation of microRNAs that silence CCR2 mRNA; 3) construction of lentiviral vectors for the expression of Tat-inducible microRNAs; and, 4) development of cell migration assays to test the effects of CCR2 knockdown.

They have one publication and they have submitted a manuscript for publication.

#### Reviewer 2:

The investigators made good progress toward their stated objectives; this is a major strength. The data collected is of reasonable quality. Strengths include the careful development of Tat responsive promoter and development of a construct that can silence a CCR2 reporter. They also met the objective of developing this construct in a lentiviral vector and developing the assays needed to assess MCP-1 induced migration.

One minor weakness is that they have not yet been able to show that they can decrease native CCR2 expression via RT-PCR or FACS, both of which would be important steps to take prior to performing a migration assay. It is difficult to predict what the *in vitro* migration assay might actually reflect in terms of *in vivo* transit from blood to brain. The two may or may not be closely related features. Nevertheless, we know that CCR2 is critical for transit from blood to brain, so it would be worth noting whether you can decrease surface CCR2 levels by 50%. If so, it might be worth pursuing an *in vivo* experiment whether or not the migration assay is successful.

A second minor weakness is that I did not see any mention of employing any clinically relevant target cells in these studies. Since macrophages may have very different responses to both viral

infection and RNA interference, the plan should include validation of efficacy of CCR2 suppression in human monocyte derived macrophages.

Reviewer 3:

The stated objective of research project 8, “RNA Interference-based Therapy for HIV-1 Associated Neurologic Disease,” was to determine if Tat-induced expression of interfering RNA targeting CCR2 could inhibit HIV-infected macrophage migration in response to CCL2. The proposed technique, targeting a host pathway known to contribute to pathogenesis instead of targeting the virus, which is highly variable, is a novel approach. Although progress was made on multiple fronts, the research objective was not met.

Strengths:

- HIV-infected macrophages and their trafficking across the blood brain barrier is an important, yet understudied area, and novel therapeutics are needed.
- Targeting a host pathway to circumvent the high mutation rate of HIV is an interesting approach.

Weaknesses:

- Complete CCR2 knockdown was not achieved, and only four CCR2-miRNAs were tested. The best knockdown achieved was only ~50% which may potentially be enough to show proof-of-principle but is insufficient to advance the concept *in vitro* or into animal experiments. More miRNAs should have been tested. A more effective CCR2-miRNA must be identified if this project is to move forward.
- The researchers spent considerable time and effort on characterizing different promoters for expression of their miRNA. It is not clear why CK-TAR was deemed superior to HIV-1 LTR, which gives similar results. Focus should have been more on generating a potent CCR2 miRNA and transfecting macrophages with the construct.
- The CCR2 miRNA construct was never put into macrophages (HIV-infected or not) to address the central objective stated above. A migration assay was designed, yet this central experiment was not performed. Also, HIV-infected (or Tat treated macrophages) were not tested in this migration assay.
- CCR2 levels on cells were never assessed by flow cytometry as stated in the original proposal. This should have been performed on HIV-infected and uninfected macrophages with and without CCR2 knockdown.

***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 progress made so far is reasonable considering the fact that laboratory research takes time to move forward, and the amount of funds spent on the project is also reasonable.

The weakness of the project is that there is no publicly available research information that can directly benefit the health of the public. But, it takes time for the bench side research to reach the bedside.

Reviewer 2:

A major strength of the project is that if it would work, it could have a major impact on the health of patients infected with HIV. The research has very high potential value to improve cognitive outcomes in HIV infected individuals, since this could lead to a new therapy aimed at preventing HIV infected cells from leaving the blood and entering the brain. Theoretically, it might also have a general effect of preventing HIV infected cells from establishing a foothold in tissues that are protected reservoirs from Highly Active Antiretroviral (anti-HIV) Therapy (HAART).

A weakness is that the project is still at a very risky stage. It is still not clear if the planned approach will be successful at decreasing surface CCR2 expression specifically in HIV infected cells. Thus the beneficial impact is still in question. The size of the budget is appropriate for this level of risk/benefit.

Reviewer 3:

HIV-infected macrophages are an understudied yet important component of HIV pathogenesis. Given the recent impetus to characterize and clear/inactivate the latent reservoir (of which macrophages are a key source), the potential significance of this project is large. This enthusiasm is somewhat tempered by the problem of *in vivo* delivery, but the proposed research is an interesting approach that should be explored further. The PI states that an NIH R21 or R01 grant is planned. There are currently new RFAs (R21-R33, R21, and R01) for which this project would be extremely responsive. It is unclear if the PI submitted an application in response to these RFAs.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

It appears that no grant application was submitted to further the work proposed in the funded research, and no patent application was submitted either. This will be considered a weakness in the project.

Reviewer 2:

The final progress report does not elaborate on what grant applications were submitted, and whether or not any of them might have a future chance at funding. It merely states no additional support was obtained and future applications are planned. However, elsewhere in the proposal, additional funding stemming from the current project is mentioned as support for ongoing efforts. A minor weakness is that the progress report was not clear on this topic.

Reviewer 3:

No additional funds were leveraged.

***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 answer to all of the above questions except publications is no. This is considered a major weakness of the project.

Reviewer 2:

One paper was published and a second is in preparation. This seems like good productivity for the budget and complexity of the project and is considered a strength.

One minor weakness is that no mention was made of any intent to file for IP protection or to identify potential partners for commercialization. Agreed, it seems like early days for identifying partners, but why no thoughts on patents? It seems like a novel therapeutic approach is being pursued and if successful, the investigator would want patent protection.

Reviewer 3:

The final progress report indicated that one publication resulted from this project and others are in process.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

It appears that the project contributed to the enhancement of research and development at the grantee's institution. However, it is difficult to judge.

Reviewer 2:

There were no infrastructure improvements. The project engendered a new collaboration between the PI and an investigator at her institution. The project did support several trainees including one who was able to do a presentation based on work on the project. I see no specific weaknesses.

Reviewer 3:

There were no improvements in infrastructure. No new investigators were brought in. One undergraduate and four graduate students were paid from 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 project resulted in inter-institutional collaborations, and this is an important part of the research in general and more important in the case of HIV/AIDS, since it is a serious pandemic, where currently over 34 million people are infected

#### Reviewer 2:

The project supported a collaboration between the PI and Dr. David Weiner at the University of Pennsylvania. I see no specific weaknesses.

#### Reviewer 3:

The PI collaborated with researchers at the University of Pennsylvania. Perhaps the PI should reach out to an miRNA expert.

### **Section B. Recommendations**

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

#### Reviewer 1:

1. There must be more accountability from the project's PI regarding the annual progress report.
2. There ought to be an accountant who can determine if the funds allocated for the research actually benefited the researcher and not the institutions, and if the funds actually reached the investigators and benefited health.

#### Reviewer 2:

1. One minor weakness is that they have not yet been able to show that they can decrease native CCR2 expression. I would recommend using a variety of approaches in addition to the reporter assay, such as RT-PCR and FACS, to confirm suppression of the native message and protein. It is difficult to predict what the *in vitro* migration assay might actually reflect in terms of *in vivo* transit from blood to brain. Nevertheless, we know that CCR2 is critical for transit from blood to brain, so it would be worth knowing if you can decrease surface CCR2 levels and if so by how much. Since the degree of suppression of surface CCR2 expression needed to inhibit migration *in vitro* may be very different than that needed to inhibit transit across the blood brain barrier, it might be worth pursuing an *in vivo* experiment whether or not the migration assay is successful.
2. A second minor weakness is that I did not see any mention of employing any clinically relevant target cell in these studies. Since primary macrophages may have very different responses to both viral infection and RNA interference from a monocytic cell line, I would recommend including validation of efficacy of CCR2 suppression in human monocyte derived macrophages. Secondly, future plans should include pursuing some sort of animal

model to evaluate blood brain barrier (BBB) transit *in vivo*. It might be possible to use either hematopoetically humanized mice or even severe combined immunodeficiency (SCID) mice without needing to develop a second vector specific to mouse CCR2.

3. One minor weakness is that no mention was made of any intent to file for IP protection or to identify potential partners for commercialization. I would suggest early interaction with institutional technology transfer officials. The investigators are working on developing a therapeutic with a potential large market. In addition, the development of the Tat promoter on its own may be a potential technology that could have IP value and may interest commercial partners. The PI should pursue more advice and involvement from her institution to maximize the development opportunities.

Reviewer 3:

1. Complete CCR2 knockdown was not achieved, and only four CCR2-miRNAs were tested. The best knockdown achieved was only ~50% which may be enough to show proof-of-principle but is insufficient to advance the concept into animal experiments. More miRNAs should have been tested. A more effective CCR2-miRNA must be identified if this project is to move forward.
2. As stated above, the critical experiments are: 1) Can CCR2 expression be knocked down in macrophages? 2) Does this alter their migration in response to CCL2? These two experiments are critical to the future success of this project and likely the ability of the PI to attract extramural funds to support future exploration on this project. Both of these questions should be addressed through further experimentation.

**Generic Recommendations for Drexel University**

Reviewer 1:

There must be an internal audit to ensure that the funds allocated for the research project reach the scientist and not the unrelated individuals.

**ADDITIONAL COMMENTS**

Reviewer 3:

The critical experiment was not performed: the migration of HIV-1 infected macrophages (transfected/transduced with and without CCR2 miRNA) in response to CCL2. No miRNA was found that sufficiently knocked down CCR2 expression.

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**Project Number:** 0863109  
**Project Title:** Somatostatin Signaling in Alzheimer's Disease  
**Investigator:** Tallent, Melanie

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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:

Maybe the project was overly ambitious from the start, though this is how it was funded. They attempted significant work and obtained results on *in vitro* work and on LTP, covering a limited portion of the aims. On the other hand (and this is positive), we have to recognize that it looks as if they worked very hard to find differences in LTP in the 3x-Tg mice (as previously reported) and were not able to show any. Two possibilities should have been considered. One is that they could have consulted with (and maybe visited) other labs who have shown this (though it could be above the proposed budget). The second is that what was previously published was maybe an artifact, and if so, the lab should be able to publish this "negative" data (potentially in an open journal) such that the others would be able to comment and verify their own work, so that the scientific community can know the final resolution of such discrepancies.

During the period, two undergraduates were involved in this project. This was likely money well spent, since they exposed undergraduates to research.

They attempted to secure two NIH grants, and one foundation grant. Although this effort was not successful, they likely did the best they could with the preliminary results they had. (Focusing on innovation and *in vivo* could have increased the significance of these applications and have increased their chances of funding. Although not mentioned, adding well-recognized investigators also could have helped.) Moreover, the concept that signaling in hippocampal cilia shares common mechanisms with olfactory transduction is nice, though it might not overly stimulate the enthusiasm of study section reviewers.

In Experiment 3, they made much progress in characterizing the object recognition memory deficits in 3x-Tg mice. They have demonstrated that a major cognitive impairment in SST3 knockout mice is in object recognition memory and that acute blockade of SST3 via IP injection of the systemically active SST3 antagonist ACQ090 leads to a similar impairment. This is a *significant* and potentially highly relevant finding, and it should be pursued.

They have also been able to discriminate the site of action of SST3, which is the dorsal hippocampus, and have data showing that an SST3 agonist can restore cognitive function in an AD mouse model. The latter point is excellent and quite impressive.

Experiment 4 was somewhat completed as planned, though I am not sure the focus was essentially on AD.

The use of aged animals (18-24 months) is seen as a significant strength of the application, especially considering the limited budget.

With the budget and the challenges they faced, it is great they had a *Journal of Neuroscience* paper. And it is also great that they plan to submit another paper that will be on SST3 in the 3x-Tg mice and will include behavioral data and immunohistochemistry. I would also encourage them to try to publish the negative data they obtained.

Weaknesses:

Although most experiments were originally proposed to be essentially *in vivo* and in AD mice, it appears that the research has been more at a cellular level looking at the mechanism of SST3 signaling.

They also focused on testing three different LTP paradigms before they discovered a consistent deficit. (They said that they did not take the opportunity to explore whether activation of SST3 can restore forskolin LTP deficits in 3x-Tg mice.)

There were no results from Experiment 2.

It seems that in some of the experiments the *n* number would appear to be small and may need to be increased (i.e.,  $n=3-4$ ). (This could potentially be explained by the extremely high cost of aged animals and the limited budget available.)

It is not clear that basal levels of cAMP in various hippocampus lysates from WT vs. SST3 are most informative (since many factors can significantly change the outcomes).

Minor detail: the applicant should be careful to make sure to use the same number of decimals, in numerous instances (e.g.,  $41.5 \pm 13\%$ ,  $48.6 \pm 8\%$ ).

Minor comment: the Western blot is poor quality, especially in regard to the housekeeping protein.

Minor comment: The *n* number should be included in the figure legends, and I did a quick search to find out if the error bars were standard deviation (SD) or standard error of the mean (SEM) and could not find it.

In summary, they have provided additional evidence that SST3 could be a target for treating cognitive deficits in AD, though a lot more work is still necessary. They showed that activation

of SST3 receptor restores cognitive deficits in an AD mouse model. They have also shown potentially novel properties of neuronal cilia and their potential role in learning and memory.

Reviewer 2:

In all four experiments the attention to what was proposed and outcomes was excellent. The major weakness was publications.

Reviewer 3:

The progress reports contain preliminary data that are of interest and demonstrate partial achievement of the stated objectives. The data obtained with wt mice and with a transgenic AD mouse model (the 3x-Tg AD mouse) are of interest and were published in abstract form at the Society for Neuroscience annual meeting in 2009.

The research design and methods were adequate, and the results were in line with the original research protocol.

Strengths of the project are the preliminary results and a high-impact publication. The findings presented in the progress reports reveal a novel strategy for development of therapeutics in an AD mouse model. This strategy is based on study of findings with SST3 agonists in the Tg AD mouse model. Progress was documented in achieving certain aims but not others. As expected, the research plan evolved based on experimental findings.

A strength of the proposal is the repeated attempt to obtain further funding for this line of investigation from the NIH and foundation sources. A weakness, however, is the failure of these grant proposals to secure further research funding.

A strength of the proposal is an abstract publication presented at a national meeting (Society for Neuroscience) and a last (senior) author paper published in a high-impact peer-reviewed journal (*Journal of Neuroscience*, 2010). The publication acknowledges research funding obtained from this source. There are no other publications listed or pending from this research funding.

***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 is quite significant, since they have shown that SST3 (with effective drug treatment) could be a target for treating cognitive deficits in AD. They have one excellent paper published, and they plan to have more.

Reviewer 2:

The findings will have limited impact, since they focus on characterization of highly artificial animal models. The benefit is only in careful analysis.

Reviewer 3:

The impact is small. There is, however, one high-impact publication that resulted from this research (*Journal of Neuroscience* 30, 4306-4314, 2010).

A strength of the proposal is that the data may be useful to the field in development of SST3 agonists as a therapy for individuals with Alzheimer's disease, but much more preclinical research is required.

A strength of the proposal is the addition to basic knowledge of the role of somatostatin signaling in object recognition memory.

Although continued grant funding was not secured, additional grant applications may be submitted to foundations with an interest in funding Alzheimer's disease research, particularly drug discovery for individuals with Alzheimer's disease. Typically, efficacy must be proven in a mouse model of AD before human studies commence.

***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:

They have tried hard to get additional funding, though it is a difficult period. Some suggestions have been laid out to potentially assist them in securing future funding.

Reviewer 2:

There is little evidence of other grants, and I am not confident this data would positively support other grants.

Reviewer 3:

No leveraging of funds was expected, and none materialized. Additional grant funding may be sought in the future to continue this line of 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:

There is one excellent paper.

Reviewer 2:

Only two abstracts and one paper are listed. This number is below what would be expected.

Reviewer 3:

Only two publications resulted – an abstract presented and published at the Society for Neuroscience meeting in 2009, and a peer-reviewed publication in the *Journal of Neuroscience* in 2010.

It is not clear if additional manuscripts will be submitted, but the preliminary data presented are of interest and may be further developed into a manuscript for submission to a peer-reviewed journal.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

This is good science. The project supported the PI, and supported staff half-time, along with two undergraduates.

Reviewer 2:

Care in execution and documentation builds capacity in several areas.

Reviewer 3:

No improvements to infrastructure were planned or mentioned. No equipment was purchased.

The grant provided research funding to support a PI (10% effort), a research assistant (50%), and provided research training for two undergraduate students. No other personnel were listed.

***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 was not any yet, though they are encouraged to do so.

Reviewer 2:

None were highlighted.

Reviewer 3:

No outside collaborators were involved in the research, although the abstract lists additional co-investigators, as does the *Journal of Neuroscience* 2010 publication with the PI as last (senior) author.

No new collaborations were mentioned.

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

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

#### Reviewer 1:

1. Although most experiments were originally proposed to be essentially *in vivo* and in AD mice, it appears that the research has been more at a cellular level looking at the mechanism of SST3 signaling.
2. They also focused on testing three different LTP paradigms before they discovered a consistent deficit. (They said that they did not take the opportunity to explore whether activation of SST3 can restore forskolin LTP deficits in 3x-Tg mice.)
3. There were no results from Experiment 2.
4. It seems that in some of the experiments the *n* number would appear to be small and may need to be increased (i.e.,  $n=3-4$ ). (This could potentially be explained by the extremely high cost of aged animals and the limited budget available.)
5. It is not clear that basal levels of cAMP in various hippocampus lysates from WT vs. SST3 are most informative (since many factors can significantly change the outcomes).
6. There was not any collaborations yet, though they are encouraged to do so.
7. Additional grantsmanship is recommended.

#### Reviewer 2:

Additional preliminary data would be of benefit.

#### Reviewer 3:

1. No grant funding resulted from the research support.

Recommendation: Continue to submit grant applications to seek support to continue this line of research. Foundations may be particularly interested in the drug discovery aspects given the limitations of current treatments for individuals with Alzheimer's disease.

2. No collaborations, either inside or outside Drexel University, were mentioned.

Recommendation: Collaborative projects, involving two or more co-investigators with complementary expertise, have a greater chance of achieving research support by competitive grant funding.

Since the proposal includes new research with a mouse model for AD and the PI has no track record in AD research, collaboration with a suitable expert becomes essential to securing grant funding.

## **Generic Recommendations for Drexel University**

### Reviewer 3:

Scientific projects must be peer-reviewed *before* making funding decisions, in addition to *after* the research project is completed.

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**Project Number:** 0863110  
**Project Title:** Characterization and Application of a  
Novel *Drosophila* Model for CHARGE Syndrome  
**Investigator:** Marena, Daniel

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

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

#### **STRENGTHS AND WEAKNESSES**

##### Reviewer 1:

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

The data developed were sufficient to answer the research questions posed, and the data developed were in line with the original research protocol.

Sufficient data and information were provided to indicate or support the fact that the project met its objectives or made acceptable progress, and the data and information provided were applicable to the project objectives listed in the strategic research plan.

##### Reviewer 2:

This project met the majority of the objectives defined in the original proposal. One exception was that the investigators never used this new fly CHARGE model to investigate the genetic interactions between *kismet* and both *atonal* and *daughterless* as described in the original proposal. They also have not fully explored the possibility of using this sensitized genetic background (i.e., *UAS:kis RNAi.a*, the milder allele) for genetic suppressor/enhancer screens which may lead to the identification of new genes that contribute to the pathogenesis of CHARGE. Nevertheless, this is a well-described and well-executed study which resulted in a *Drosophila* model of CHARGE that has face validity (both locomotor and memory defects). Additionally, it has revealed new aspects of *kismet*'s involvement in axonal pruning and migration and has also delineated, in a particularly elegant set of experiments, the role for *kismet* in muscle (post synaptically) as well as inter-neuronal populations in the brain and nerve cord. The investigators, therefore, met the objectives of the proposal.

##### Reviewer 3:

The work fully and comprehensively met all specific aims and objectives. Research design and methods were excellent, and the project made excellent progress.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

The significance of this project for improving health is unknown at this point, and it is also unknown what the value of the completed research is towards eventual improvement in health outcomes.

The future plans for this research project are to do more basic research.

Reviewer 2:

The construction of a *Drosophila* model for CHARGE with proven face validity has enormous potential for improving human health. Although not fully explored in the application itself, the investigators have the opportunity to identify new genes involved in CHARGE which may be druggable targets. The only possible flaw to the report was a lack of emphasis on future plans for genetic and drug screening experiments utilizing this model system. Regardless, the detailed analysis of this fly CHARGE model is an excellent starting point for future projects which will undoubtedly contribute to a better understanding of the pathogenesis and treatments for CHARGE syndrome.

Reviewer 3:

For human health, the project has increased the understanding of CHARGE syndrome and particularly the possible role of atonal and daughterless in the fly has provided new candidate genes for the diagnosis of CHARGE syndrome causative mutations in the one-third of CHARGE cases without CHD7 mutations.

An additional and unexpected result was the dissection of cognitive defects to show that the initiation of short-term memory was disturbed in the authors' CHARGE model flies. This suggests that it may be possible to subdivide mental retardation in CHARGE patients more finely, perhaps allowing possible treatments in some cases.

***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:

Leveraging of funds did materialize. The PI obtained an R21 NIH grant based on the preliminary data obtained with this funding. This is excellent.

The idea is to use the preliminary data obtained via R21 support to apply for an R01.

Reviewer 2:

The applicant proposed to submit an R21 application, and this application was funded: "Characterization of a novel Drosophila disease model for CHARGE Syndrome."

The applicant also states that they will apply for an NIH R01 when the R21 ends. It appears that this grant 5R21RR026074-02 ends in February of 2013. There is time still for an R01 submission.

Reviewer 3:

The research funds given to the researchers were leveraged into a successful NIH grant to continue the studies and further explore the CHARGE syndrome in Drosophila. Given the highly competitive nature of today's NIH funding, this is an excellent result.

***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:

They have quite a bit of preliminary data and are planning further publications.

Reviewer 2:

The applicant published the majority of the data generated during the funding period in a single article in a significant journal (IF>8) in the field of human genetics: Melicharek DJ, Ramirez LC, Singh S, Thompson R, Marends DR. Kismet/CHD7 regulates axon morphology, memory and locomotion in a Drosophila model of CHARGE syndrome. *Human Molecular Genetics* 2010 Nov 1;19(21):4253-64. Epub 2010 Aug 17. PubMed PMID: 20716578; PubMed Central PMCID: PMC2951870.

Reviewer 3:

A high quality manuscript "Kismet/CHD7 regulates axon morphology, memory and locomotion in a Drosophila model of CHARGE syndrome" was published in *Human Molecular Genetics*, an excellent result given the relatively small budget and time period.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

The funds seem to have been spent well. Students were recruited, and the PI made significant progress. This allowed him to submit an R21, which was funded. This is really commendable.

Reviewer 2:

It is unclear.

Reviewer 3:

The work was expanded to an NIH funded project, which allowed the hiring of a post-doctoral student, thus bring a new researcher to the institution to carry out more research in the future. Perhaps more importantly, this work was expanded into an NIH grant and has likely solidified a young researcher's academic position at Drexel University.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

There were none.

Reviewer 2:

It was not expected.

Reviewer 3:

None were reported.

***Section B. Recommendations***

***SPECIFIC WEAKNESSES AND RECOMMENDATIONS***

Reviewer 1:

None

Reviewer 2:

All suggestions are outlined in section A. I would point out, however, that during this limited time when this new fly model of CHARGE is primarily available to the applicant, a larger story should be constructed to provide preliminary data for the R01 application. Specifically, RNAseq or microarray analysis may help to identify transcripts specifically regulated by this chromodomain helicase DNA-binding protein. This preliminary data (presumably generated using either *Heatshock-GAL4* or *C155-GAL4* driving *UAS-RNAi-kis* in the brain) would be a real strength as preliminary data for an R01 application.

Reviewer 3:

I would like to suggest they propose and screen candidate genes for the one-third of CHARGE syndrome patients without clear CHD7 mutations. Given the low cost of genome sequencing now, a comprehensive approach may be an alternative, assuming it is not already being actively pursued.

## **ADDITIONAL COMMENTS**

### Reviewer 1:

The PI did a lot of experiments with his assistants, and they have a lot of nice data. They have a model of how the gene is acting in the brain, and they have some solid phenotypes. They should probe deeper into the mechanism and submit their work.

### Reviewer 3:

The work was outstanding, and the relatively small seed money was fully transformed into a successful larger research program, providing insights into CHARGE syndrome and additionally short term memory acquisition. Overall it is very impressive.

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**Project Number:** 0863111  
**Project Title:** Multidimensional Shape/Color Distributions as a  
Computational Biomarker for Cancer Pathology  
**Investigator:** Breen, David

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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 investigators have done an outstanding job meeting their stated objectives in their entirety. Their goal was to use multidimensional shape/color distributions as a biomarker to predict axillary lymph node status. Their hypothesis was that the structure of the nuclear pleomorphisms found in breast cancers can be transformed into high-dimensional shape distributions using geometric measures, and then the resulting distributions of N pathologic staging categories can be mapped into well-defined regions of high-dimensional distribution space. This would then allow an unknown breast cancer sample to be mapped into this D-space and can then predict, using machine learning, if its lymph node would be positive.

The investigators used 55 breast cancer cases that were node negative and 45 cases that were node positive. Slides were stained into H&E, ER/PR/Her2neu and Ki67 marker. After image processing/segmentation, shape distributions were generated and processed followed by classification via supervised machine learning techniques using a leave-one-out classifier. The investigators were able to get a sensitivity of 77.8% and specificity of 85.5%.

Specific strengths include investigators finishing all their stated aims and developing a machine learning classifier that is able to predict risk of lymph node involvement in breast cancer. They were able to troubleshoot and allow processing of multidimensional characteristics and test 100 primary breast cancer samples.

#### Reviewer 2:

The project has met most of the stated objectives. The strength of the proposal primarily lies in the approach listed. The algorithms proposed to create color distributions from the histologic and prognostic marker images are innovative. The programming methodology is described well. The weakness of the project lies in weak statistics. A discussion on the control slides was not included. The quality of the histological slides should be a major concern. More background information is needed on the standardization of histology slides nationally (and probably internationally). How many immunohistological slides (markers) are necessary/mandatory? Authors mentioned that more histological stains will improve sensitivity and specificity. How much is the error with fewer immunostained markers/slides? Sometimes staining is light due to

reagent concentrations. Are those errors considered? How many  $n$  per patient would be examined? The number of samples studied is low, and more heterogeneity would give a better distribution.

#### Reviewer 3:

The objective of this project is to develop computational techniques for analyzing histology images of breast cancer tumors. Especially, the auxiliary lymph node status of breast cancer tumors will be automatically and objectively predicted through multiresolution image analysis of the primary tumor. This objective is divided into four specific aims.

One major strength of this project is the PI's expertise in image processing and machine learning algorithms. In addition, the project team also includes a senior faculty member, a graduate student assistant, and a research engineer. Therefore, this team has the technical capability to deliver what they promised in the original proposal. Within the one and a half year project period, this team has made reasonable progress and has demonstrated the technical feasibility of the computational pipeline for automated lymph node metastasis status prediction based on analysis of primary breast tumor histology.

Weaknesses: First of all, the PI proposed to validate their approach by testing numerical cases from their breast cancer databank. However, only 100 samples were tested in this project, despite the fact that they had access to 2200 paraffin-embedded breast cancer specimens. This could be caused by the limited efforts the PI and the senior faculty member could put into this project (5% and 1.6% respectively).

Second, the measure of the validation test has not been well-defined. The PI stated that their SPSS BLR classifier with the default threshold value produced the classifications with a sensitivity of 77.8% and specificity of 85.5% over all 100 samples. However, the final report does not describe the gold standard used for the classification test. It is also not clear whether the ROIs used for their gold standard classification of lymph node metastasis status are the same as those for their imaging analysis. Therefore, the conclusion derived from this study is not convincing. Finally, the outcome of this project is a little bit disappointing. There is no major publication from this project, except one poster at a national conference, one poster at a local conference, and one manuscript in preparation. No progress (or future plan) has been made in patent filing, technology licensing, or clinical translational research.

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

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

#### Reviewer 1:

This proposal is promising but still very early in translational application. However, it is intriguing that they were able to use computational data processing to predict risk of lymph node metastasis using a small dataset. This will clearly have to be tested in larger datasets. Moreover, the current sensitivity and specificity are still not appropriate for clinical application, but further

system learning on larger datasets should improve this risk prediction capacity. The investigators have applied for additional funding and are planning to continue their research in larger datasets.

Reviewer 2:

The project's impact is high from an investigative basic science point of view. In terms of actual practical application, there is a long way to go. The major weakness would be the low numbers of samples analyzed, which accounts for the future plans.

The strength lies in the algorithm protocol described.

The project would greatly benefit from incorporating clinical collaborators, such as a breast cancer oncologist.

Reviewer 3:

This project aims at developing computational techniques for analyzing histology images of breast cancer tumors in order to ascertain the metastasis status of the tumor. The strength of this project is its clinical significance. If successful, the technique can be used to determine if a patient's breast cancer has spread to nearby lymph nodes by examining a primary tumor that has been removed from the patient. This image analysis capability will eliminate the need for exploratory surgical removal of lymph nodes; thus eliminating the associated side effects (e.g., pain, swelling and morbidity) and costs.

The weakness of this project is associated with the method. In this project, the PI hypothesized that mapping an unknown breast cancer sample into the high-D space and determining, via machine learning, to which region it belongs will allow them to automatically predict its auxiliary lymph node status. However, the pre-condition of this hypothesis is that the detection methods and the disease-specific biomarkers have sufficient accuracy to determine lymph node metastasis status. Otherwise, any advancement in the imaging analysis algorithm may not help very much because of the "garbage in and garbage out" effect. As the future plan of this project, the PI should pursue close collaborations with clinical and biological researchers to define the imaging algorithms based on more sensitive and specific detection methods and biomarkers.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

The investigators have applied for additional grant funding from the U.S. Army Breast Cancer Research fund in May 2010 based on this preliminary data. In addition, they hope to apply for an R21 as an additional funding source.

Reviewer 2:

The researchers have applied to external grants. None have materialized yet. The researchers have plans in place for applying additional funding to expand the research.

Reviewer 3:

The Department of Defense Breast Cancer Research Program should be a good place to apply for the extramural funding support for this project. However, this program is very competitive and requires extremely innovative research ideas. Considering the current status of the project and the low funding rate, it may be challenging to get extramural funding support at this point. The PI submitted a relevant proposal to the Department of Defense Breast Cancer Research Program on May 2010. No follow-up about this proposal is available from the final report. I suggest adding innovative ideas in the project and collecting preliminary data to demonstrate the technical feasibility before submitting to a federal grant.

***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 one manuscript in preparation with planned submission to the *Journal of Pathology Informatics*. There were no patents filed.

Reviewer 2:

The project did not result in any peer-reviewed publications, licenses, patents, or commercial development opportunities. None of these were submitted/filed.

The researchers plan on submitting a (one) manuscript to the *Journal of Pathology Informatics*. The time line of submission date was not mentioned.

Reviewer 3:

- This project has not yielded major publications yet. However, according to the PI, a paper is currently in preparation and will be submitted to the *Journal of Pathology Informatics*. In addition, the relevant work has been presented as a poster at several conferences.

No invention disclosure, patent, or other commercialization efforts have been reported.

Although it is a little bit disappointing to see no major publication from this project, it is understandable that the PI may need more time to summarize the work for publication. Further follow-up after the completion of the project may be a good option to consider.

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

## ***STRENGTHS AND WEAKNESSES***

### Reviewer 1:

The grant enhanced the research at the institution. The investigators were able to recruit a graduate trained biomedical engineer from the Boston area (Tufts University) for this project. In addition, the proposal provided support for another master's level student.

### Reviewer 2:

There were improvements made to infrastructure.

There were new researchers brought into the institution to help carry out this research.

Funds were used to pay for research performed by a master's student.

### Reviewer 3:

It is hard to evaluate this, since the project has not reached a conclusive result and since the synergy between the PI and other researchers at the PI's institution has not been discussed in the report.

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

## ***STRENGTHS AND WEAKNESSES***

### Reviewer 1:

None

### Reviewer 2:

New collaborations were not initiated.

### Reviewer 3:

It seems that the PI involved Professor Polikar at Rowan University in this project. However, Dr. Polikar's contribution to this project is not clear from the report.

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

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

### Reviewer 1:

The investigator has provided intriguing proof of principle of computational biology. The data is quite preliminary and will need significant testing for any clinical relevance. One caveat for the investigators is that it is currently unclear how many slides/DNA are needed for this; this is relevant, since patients often may have small lesions.

### Reviewer 2:

1. The weakness of the project lies in weak statistics. A discussion on the control slides was not included. The quality of the histological slides should be appropriately addressed. More background information is needed on the standardization of histology slides nationally (and

probably internationally). How many immunohistological slides (markers) are necessary/mandatory? Authors mentioned that more histological stains will improve sensitivity and specificity. How much is the error with fewer immunostained markers/slides? Sometimes staining is light due to reagent concentrations. Are those errors considered? How many  $n$  per patient would be examined?

The number of samples studied is low, and more heterogeneity would give a better statistical distribution.

2. Including a pathologist and/or medical oncologist as a collaborator is encouraged.

Reviewer 3:

1. The measure of the project is not clear. Since the proposal has defined the hypothesis, the project should design the experiment to verify the hypothesis. However, the report did not show a clear measure that can be used to verify the hypothesis. Also, the gold standard for sensitivity and specificity definition is not available. It is suggested that the experiment can be better designed so that the results can be publishable.
2. In this project, the imaging analysis works were carried out based on the available samples and pathology methods. If the available methods do not have sufficient sensitivity and specificity in detecting lymph node metastasis, the algorithm will be “garbage in and garbage out.” I suggest working closely with clinical and biological researchers to explore imaging tools based on novel detection techniques and biomarkers for accurate detection of lymph node metastasis.
3. There are a limited number of publications from this project. I suggest to pursuing publication, patent filing, and technology licensing more proactively.

**Generic Recommendations for Drexel University**

Reviewer 2:

The project is promising and can have a favorable impact on health care. More collaborations and infrastructure/resources would expand the impact.