Response Form for the Final Performance Review Report*

1. Name of Grantee: Drexel University

2. Year of Grant: 2008 Formula Grant

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

Drexel University conducts a rigorous review process to select the proposals that are funded by the Commonwealth "CURE" funds. A brief description of this review process is highlighted below.

<u>Review Process</u>

- Grants are reviewed by two independent reviewers.
- Grants are assigned to reviewers based on the following criteria:
  - Reviewer has expertise in the topic.
  - Reviewer is neither a collaborator with the applicant nor in the same department as the applicant.
  - Ideally, grants are assigned to a clinical scientist, a basic scientist, population scientist, biomedical or engineer depending on expertise required.
  - Departmental Chairs do not serve on the review panel.
- Phase One: Reviewers evaluate their assigned grants according to the following criteria:
  - Overall scientific merit
  - Innovation
  - Strength of the PI and research team
  - Ability to successfully complete specific aims within time frame and limitation of the funding
  - Likelihood of success in developing a competitive application for extramural support
  - Fit with the program for which it is submitted *and for potential for translational research or therapeutic application*
  - Reviewers assign a score to each application. These scores represent an estimation of their scientific merit as if they were reviewed by an NIH Study Section.
- Second Phase and Study Section
  - Scores are submitted to the Office of the Vice Dean for Research.
  - The top ranked proposals are selected for review at Study Section.
  - Proposals are distributed to the entire study section for review.

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

    - The Study Section review process is conducted in the manner used by NIH panels. For the meeting of the panel, the grants are scored according to standard NIH procedure. In particular, proposals are scored from 1 (best) to 10 (worst).
- Study Section
    - The panel is chaired by Kenny Simansky, Ph.D., Vice Dean for Research, who does not review any individual applications or vote. Noreen Robertson, D.M.D., Associate Vice Dean for Research in the DUCOM Office of Research, and Frank A. Ferrone, Ph.D., Associate Vice Provost for Research at Drexel University, are present, *ex officio*.
- Funding decisions
    - Funding decisions are made on the basis of the review for merit.

<u>Monitoring of Progress and Submission of Reports</u>

The Office of the Vice Dean for Research is responsible for monitoring progress of the individual intramural projects towards their stated aims. This is accomplished with the following procedures and timeline:

1) The Associate Vice Dean reviews the Annual Reports and Final reports prior to submission to the Commonwealth and submits the reports to meet the Commonwealth's deadline. This review evaluates the amount and quality of data collected to that point, the extent to which the data address the aims that were funded and the likelihood of successful completion of the aims during the remaining period of the award.
2) The Associate Vice Dean notifies the Principal Investigator (PI) of each project whether the progress is considered *acceptable* or *questionable*. Note that the first report for new projects are received from the PI's only 6 months after the start of the project. Thus, progress may be limited. The evaluation takes that into account. Nonetheless, if a project is considered questionable, the Associate Vice Dean requires a follow-up report from the PI, with a plan to maximize the opportunity for scientific success. At the end of the first year, PI's who request no-cost extensions are required to provide scientific justifications for that action. The Associate Vice Dean reviews those justifications and requires a further plan intended to meet the scientific goals of the project, as necessary.
3) Patricia Groshon, Senior Director, Research Administration, Office of Research Administration ensures submission of the Audit reports.

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

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

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

<u>Reviewer 1:</u>

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.

<u>Reviewer 2:</u>

None

<u>Reviewer 3:</u>

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

<u>Reviewer 1:</u>

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.

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

Due to personnel turnover we have yet to finish some of these studies. We are waiting for samples from a few more mice before we complete ANA serum analyses part of the study. As we do not have staff that are dedicated to the project as this point, the final data collected will take some time to finish.

Personnel turnover hampered the data collection. We have sections cut from the biopsies for staining and this is currently being undertaken. As we do not have staff that are dedicated to the project as this point, the final data collected will take some time to finish.

The figure legend was incorrect. N=3.

Once we have completed the studies with the mice we wish to publish this final piece of data.

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

Response:

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

Response:

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

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

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

<u>Reviewer 1:</u>
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.

<u>Reviewer 2:</u>
None

<u>Reviewer 3:</u>
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.

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

Since the original funding period, the project has continued to grow, and we have brought into the project additional collaborators, chiefly Dr. Daniel Marenda of the Biology Department and Dr. Patrick Loll of the Biochemistry Department. With ongoing support from the College of Medicine (through a multi-user institutional grant), we are attempting to expand the project so that Hereditary Spastic Paraplegia becomes both a basic research and clinical strategic priority. Dr. Terry Heiman-Patterson, whose role was originally as a consultant, is now actively involved in our efforts to generate a transgenic mouse model for the disease.

Dr. Marenda is working with us to express the spastin mutants in Drosophila neurons, and we are assaying both for degeneration of the axons and for behavioral deficits. As originally planned, we are now moving forward with expressing the mutants in cultured rat cortical neurons, and we are assaying for degeneration, impact on microtubule dynamics, and organelle transport. We are also generating a transgenic mouse that expresses the mutants in motor neurons, in collaboration with Dr. Heiman-Patterson. We will take the advice of the reviewer and express the mutants in different populations of neurons, although we think it is likely that they will produce degeneration in any neuron population where they are expressed. The specificity of the disease to corticospinal tracks, we suspect, is because the specific spastin isoform that is most toxic when mutated is only appreciably expressed in corticospinal neurons.

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

Response:

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

Response: The project is moving forward at many fronts. We are preparing a paper for submission in 2013 that will include the fibroblast work, the primary cultured neuron work, and Drosophila work. We have submitted an NIH R01 application on the project, as well as two small foundation proposals.

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

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

Reviewer Comment on Specific Weakness and Recommendation (*Copy and paste from the report the reviewers' comments listed under Section B - Specific Weaknesses and Recommendations)*:
<u>Reviewer 1:</u>
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.

<u>Reviewer 2:</u>
None were noted.

<u>Reviewer 3:</u>
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

<u>Reviewer 1:</u>
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.

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

PI Response:
The PI agrees and in fact, since the time of the CURE grant, the PI made the decision to investigate embryonic lethality of *Chtf18*-null embryos at an earlier time-point. The project has already been revised to examine one-cell zygotes for aneuploidy. One-cell zygotes contain discrete maternally and paternally derived pronuclei, which are easily distinguishable by the larger male pronucleus. Adult females (mutant and wild-type) are superovulated and mated with wild-type males. Females with a copulatory plug are injected with colcemid to arrest cells at the first-cleavage metaphase, then females euthanized and zygotes flushed from oviducts and fixed. Air-dried chromosomes are prepared and stained with Giemsa, and the number of chromosomes in individual pronuclei are counted. Presence of more than or fewer than 20 chromosomes in one pronucleus indicates aneuploidy.

The proposed project will be completed; the work has continued and advanced. A paper on *Chtf18*-null mice has been published recently (November 2012) in *PLoS Genetics* and another manuscript describing the female mutant phenotype will soon be submitted for publication (see below, "*Additional comments in response to the Final Performance Review Report*").

Our morphometric analysis revealed that *Chtf18*-null ovaries are smaller and contain significantly fewer follicles compared to wild-type mice. In addition, homologous chromosomes separate prematurely during meiosis I. The phenotype of *Chtf18*-null females closely resembles conditions afflicting women that impact on their fertility. Therefore, study of our mouse model provides the opportunity to gain a better understanding of fundamental processes regulating both folliculogenesis and meiosis, as they relate to oocyte quantity and quality, respectively, in women. Studies to address a mutation in *Chtf18* in humans are planned (with mouse studies still ongoing). However, even if a mutation in *Chtf18* itself in humans does not contribute to human subfertility, information obtained from this project will still shed significant light on the molecular basis of defects leading to meiotic errors (already known to be a common cause of chromosome mis-segregation and aneuploidy) in humans. In addition, *Chtf18* may play an indirect but still important role in human subfertility through interaction with other proteins in its pathway or related pathways.

Defects other than those observed previously in *Chtf18*-null mice are also being examined. The underlying causes of ovarian follicle defects and the potential role of *Chtf18* in ovarian aging are being studied. The functions of *Chtf18* in DNA double strand break repair and crossover will be determined by examining and quantitating both meiotic recombination and chiasmata, and by hotspot analysis. The mechanism of premature disjunction of *Chtf18*-null homologues and the

likely role *Chtf18* plays in chromosome cohesion will be examined by immunoprecipitation, co-immunoprecipitation, and oocyte microinjection experiments. Analyses of metaphase, anaphase, the meiotic spindle, and TUNEL are being utilized to determine the consequences of *Chtf18* deficiency on chromosome alignment and segregation.

Future studies will have greater numbers of animals in each group; Dr. Berkowitz is now working with Dr. Edward Gracely, a biostatistician and Associate Professor of Family, Community, and Preventive Medicine at Drexel University College of Medicine and Drexel University School of Public Health to ensure that appropriate statistical methods are used and that studies are adequately powered.

Drexel University Response:
Work completed on the CURE grant was necessary as first steps to characterize defects in *Chtf18*-null females. The work has been continued, advanced and has been presented at local and national meetings. The current and future studies are now more mechanistic. The data generated from the CURE Award led to a competitive DeWitt Pettit foundation grant and a recently submitted NIH R21 proposal that seeks to understand the molecular basis of defects observed in *Chtf18*-null female mice. This R21 grant proposal was recently reviewed (February 2013) and competitively scored.

Dr. Berkowitz has formed a collaborative relationship with Dr. Richard Schultz at the University of Pennsylvania, an expert in the fields of oocyte biology and maternal age-related aneuploidy to help her explore the role of *Chtf18* in female fertility and meiosis. He is a consultant, and Dr. Berkowitz meets with him to discuss the work every 2-3 months.
Dr. Berkowitz also has a weekly joint lab meeting with Dr. Eishi Noguchi, an Associate Professor in the Dept. of Biochemistry and Molecular Biology at Drexel University College of Medicine. He has overlapping research interests with Dr. Berkowitz, and her worked is presented and discussed at this meeting on a regular basis. The Dept. of Biochemistry and Molecular Biology also holds monthly faculty chalk talks where research and grant proposals are vetted by the faculty.

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

Response:

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

Since the time of the final progress report of the CURE grant, Dr. Berkowitz has published a paper, entitled "Disruption of *Chtf18* Causes Defective Meiotic Recombination in Male Mice", *PLoS Genetics*, 8, e1002996 (2012).

Another manuscript describing the *Chtf18* mutant female mouse phenotype will soon be submitted for publication.

Since the completion of the CURE grant Dr. Berkowitz has applied for and obtained additional research support (Pending and Completed listed below). Both the R01 an R21 grant applications received competitive scores and "Just in Time" information was requested for both of these by the NIH.

Pending Research Support

1 R01 GM106262-01 Berkowitz (PI) 4/01/2013 – 3/31/2018
NIH/NIGMS
"Regulation of meiotic recombination and chromosome segregation in mammals"
The goal of this project is to elucidate the roles of *Chtf18* in mammalian DNA double-strand break repair and chromosome segregation during meiosis.

1R21HD078189-01 Berkowitz (PI) 7/01/2013 – 6/30/2015
NIH/NICHD
"CHTF18 Function in Female Germ Cell Development and Fertility"
The major goal of this project is to use *Chtf18-null* mice as a model system to elucidate the molecular basis of follicle atresia and oocyte aneuploidy in females.

Completed Research Support

Mary DeWitt Pettit Fellowship Award Berkowitz (PI) 6/1/2011- 5/30/2012
"Genetic defects associated with female infertility and pregnancy loss"
The goal of this project is to elucidate the role of *Chtf18* in female fertility and embryonic viability in mammals.

Dept. of OB/GYN Research Grant Berkowitz (PI) 7/1/2011 – 6/30/2012
"The Roles of *Chtf18* in Mammalian Meiosis"
The goal of this project is to investigate the role of *Chtf18* in male meiotic recombination and fertility.

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

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

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

<u>Reviewer 1:</u>

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.

<u>Reviewer 2:</u>

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.

<u>Reviewer 3:</u>

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.

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

PI Response:
We agree with the recommendations provided by reviewer 1. In fact, we have continued with our studies in our novel liver model system and are beginning to incorporate human hepatocytes. While our initial focus was on ritonavir, a focus necessitated by the short duration of the proposed project, our longterm goal is to expand our use of this system as a novel tool for studying drug toxicity of approved as well as potential new drugs. These studies will include analyses of dose-dependent, species-specific, and zonally-dependent hepatotoxicity. Once we have completed our construction of a completely humanized mini-liver system, we will contact potential industrial partners for our toxicity studies.

In addition to toxicity studies, we also proposed to use our miniliver system to study general liver biology and thus, as suggested, plan to characterize not only hepatocyte and endothelial biology in this system but also the biliary axis.

Since completion of the final report for this proposal, we have continued our creation of the miniliver device and have been able to demonstrate co-culture of primary rat hepatocytes and primary rat liver sinusoidal endothelial cells. Additionally, we have now been able to maintain these co-cultures with continual dynamic fluid flow for at least 60 days. We are now poised to begin drug toxicity studies and plan to start these types of studies very soon.

We have made considerable progress in creation of layered co-cultures of primary hepatocytes and liver sinusoidal endothelial cells in a microfabricated microchannel exposed to continual fluid flow and thus have already addressed many of the concerns or recommendations of reviewer 3:

We have perfected standard protocols for collagen coating of glass coverslips and can now use these, if necessary, in our devices.

In addition to detection of mRNAs of hepatocyte and endothelial cell specific markers, we have now conducted longterm hepatocyte function assays in our co-culture system. For these studies, we analyzed secreted albumin and urea synthesis over a 50-day time course and demonstrated that albumin and urea continue to be synthesized by hepatocytes. We also showed that the co-culture system is superior to a single culture microchannel system for maintenance of hepatocyte specific functions.

Now that we have completed hepatocyte function assays, we are proceeding to drug toxicity studies; as originally stated in our final report, this delay was due to complications with leakage of our device that required extensive revisions of our original construction protocol. Because our system is now complete, toxicity studies will be conducted in primary hepatocytes and not HepG2 cells as we believe that primary hepatocytes are more physiologically relevant for drug toxicity studies. The focus on ritonavir in our proposal was based on the 1-year time frame

allowed; our longterm goal is to use our system to test the liver toxicity of various drugs including novel drugs and drugs already available in the clinic.

We have followed the work of multiple groups who are also attempting to make 2D and 3D liver models. To date, none have been similar to our model, which we believe more accurately reflects the 3D environment of the liver. However, as recommended, we will continue to monitor liver model systems that are described in the literature to ensure that we are not simply replicating already-existing models.

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

Response:

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

Response: We have made considerable progress generating a "miniliver" as described in our original proposal. In addition to our original publication describing the generation of microchannels and incorporation of hepatocytes into these channels, we are now in the final in-house editing stages of a manuscript that describes our layered co-culture of primary hepatocytes and endothelial cells and are in the process of writing a second manuscript that describes similar layered co-cultures maintained under dynamic fluid flow and the influence on longterm maintenance of hepatocyte specific functions.

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

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

Reviewer Comment on Specific Weakness and Recommendation (*Copy and paste from the report the reviewers' comments listed under Section B - Specific Weaknesses and Recommendations)*:
<u>Reviewer 1:</u>

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 <u>both</u> 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 5five 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.

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

PI Response:

We currently have a paper under review at The Journal of Biological Chemistry entitled "HER2/neu (erbB2) Requires HIF-1 for Mammary Tumor Growth and Anoikis Resistance" that is based on the research performed in this grant.

I agree with the reviewer that the ramifications of MNK2 induction need to be explored more deeply. We are particularly interested in the role of the MNK2 mediated eIF4E phosphorylation on breast cancer development.

We analyzed more than one hypoxic target. We knocked down 4 hypoxic targets and examined the effect on Bim expression and none had an effect on Bim expression. The targets tested were: Lysyl Oxidase (LOX), Integrin α5 (ITGA5), Map Kinase Interacting Serine/Threonine Kinase 2 (MNK2), and Angiopoietin-like 4 (ANGPL4). We also used an inhibitor of LOX and it had no effect of DCIS formation. Furthermore we assayed an MNK inhibitor but it targeted both MNK1 and MNK2. This inhibitor reduced overall cell growth which made it difficult to interpret the 3D cultures.

It is clear that an *in vivo* model system would strengthen our grant proposal. To this end, Dr. Reginato has started a collaboration with Dr. Tiffany N. Seagroves (University of Tenn.) who has a HIF1α -/- MMTV breast carcinoma mouse model.

We are very interested in understanding the regulation and roles of HIF1α and HIF2α in breast cancer development under hypoxia. We do not believe that this regulation is cell line specific because we have observed the same pattern in the prostate cancer cell line, PC3. The molecular mechanism responsible for the loss of the HIF1α mRNA has been under investigation in the Johannes laboratory. Several trans-acting factors and miRNAs interact with the HIF1α mRNA that may affect its half-life. We have not examined the role of microRNAs but that is an excellent idea. We do believe that the HIF1 anti-sense plays a pivotal role.

We have shown that the Endothelin-2 (ET2) mRNA induction by hypoxia is not affected by HIF1α knockdown. In contrast, HIF2a knockdown completely abolished ET2 mRNA induction by hypoxia, indicating it requires HIF2. Chromatin immuno-precipitation (ChIP) experiments were performed to demonstrate binding of HIF2 to the ET2 promoter but they were not successful. This may reflect a technical issue or may indicate that ET2 is not a direct transcriptional target of HIF2. Thus at the induction of ET2 by hypoxia may involve an indirect effect mediated by HIF2. The mechanism involved with HIF2's contribution to the hypoxic induction of ET2 has not been elucidated. We have considered using cells derived from the HIF1α and HIF2α knockout mice but due to funding issues we have not pursued this investigation further.

I agree with the reviewer that an *in vivo* system is required to generate a competitive grant application focused on the regulation and expression of HIF1α and HIF2α in breast cancer development. Toward this end, I am developing collaborations to incorporate these studies into a mouse model system.

It should be noted that this grant was for 1 year (we carried over unspent funds for the following year). We did not suddenly change the aims in year 3. I made the decision to alter the aims after we determined that knockdown of 4 target genes had no effect on Bim expression, indicating that the targets were not involved with DCIS formation under hypoxic conditions. I felt that it would be more productive to pursue the novel regulation of HIF1α and HIF2α that was discovered while pursuing aim 1. We are currently examining this phenomenon and are hopeful our grant resubmission will be successful.

We have submitted a paper to JBC, entitled: HER2/neu (erbB2) Requires HIF-1 for Mammary Tumor Growth and Anoikis Resistance, that included data obtained from this grant. Based on the data obtained from this grant, Dr. Reginato (Co-PI) has begun a collaboration with Dr. Tiffany N. Seagroves (University of Tennessee) who has HIF1a knockout breast carcinoma mouse model.

As we pursue new funding opportunities we will be sure to fully justify the correlation of hypoxia with DCIS formation and breast cancer development. We will also focus on the molecular mechanism(s) involved in these processes

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

Response:

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

Response:

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

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

Reviewer Comment on Specific Weakness and Recommendation (*Copy and paste from the report the reviewers' comments listed under Section B - Specific Weaknesses and Recommendations)*:
<u>Reviewer 1:</u>
The applicant indicates that he created a prostate cancer tissue microarray. No diagnostic tools for prostate cancer resulted from the current studies.

<u>Reviewer 2:</u>
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.

<u>Reviewer 3:</u>
None

Generic Recommendations for Drexel University

<u>Reviewer 3:</u>
This research looks extremely promising and should be supported as long as external funding is being actively sought.

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

PI Response:
One prostate cancer tissue microarray was created. However, due to poor quality of available O-GlcNAc antibodies at the time we did not feel confident in using this antibody in our experiments. We are currently testing specificity of other commercial available antibodies and they will be tested.

This is an excellent recommendation that will be pursued if commercial antibodies do not work.

This is an excellent suggestion. However, downstream target of OGT, the transcription factor FoxM1 has already been shown to be elevated in prostate cancers, thus analysis of this specific target may not offer new data. Once we have an optimal O-GlcNAc antibody we would like test whether an association exists between elevated O-GlcNAc and FoxM1 in same prostate tumors.

Using Oncomine database we found four independent microarray gene expression studies showing elevated O-GlcNAc transferase (OGT) mRNA expression patterns in human prostate carcinoma compared with normal adjacent tissue samples. A survey of the National Center for Biotechnology Information Gene Expression Omnibus indicated a positive correlation (r2 _ 0.996) between high OGT expression and metastatic progression in normal, primary tumor, and metastatic prostate tumor tissues. Furthermore, an additional study of 94 patient tumor samples, when stratified by the level of OGT expression, indicated that disease-free survival 5 years post-treatment for prostate cancer was higher (75% probability of survival) in patients with a low OGT expression profile compared with patients with increased OGT expression (25% probability of survival). These results were published recently in J. Biol. Chem VOL. 287, NO. 14, pp. 11070–11081, March 30, 2012.

We will continue to actively seek additional funding for this project.

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

Response:

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

Response:

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

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

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

<u>Reviewer 1:</u>

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.

<u>Reviewer 2:</u>

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.

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

Response to Reviewer 1:

1. Regarding additional experimental work to address stability, durability, and deployability

   Studies on the piezoelectric microcantielver sensor (PEMS) and its successor, piezoelectric plate sensor (PEPS) are ongoing. To make PEMS/PEPS more stable and durable, we have the thoroughly examined the methodology of electrical insulation, and the fabrication of the piezoelectric layer. The electrical insulation improvement is critical; it reduced the electrical noise by three orders of magnitude. As a result, we could reliably carry out in-liquid detection (see publication 2 below). The materials property study of the piezoelectric layer is important in that it allows us to reproducibly optimize and control the detection frequency shift. As a result, we achieved an unprecedented sensitivity of direct, in situ DNA hybridization at 1×10^{-18}M without DNA extraction and amplification (see Publications 1 and 3 below).

2. Regarding additional focus on publication, grants, collaboration

Studies of PEMS and PEPS are on-going. Particular emphasis has been on the durability, stability in in-liquid detection and the sensitivity enhancing mechanism. Example publications, patent applications, and grants are listed below.

Example Publications:

1. Q. Zhu, W. Y. Shih, W.-H. Shih, "Enhanced Dimethyl Methylphosphonate (DMMP) Detection Sensitivity by Lead-Magnesium Niobate-Lead-Titanate/Copper Piezoelectric Microcantilever Sensors via Young's Modulus Change, *Sensors and Actuators B*, in press
2. M. Soylu,* W.-H.; W. Y. Shih, "Insulation by Solution 3-Mercaptopropyltrimethoxysilane (MPS) Coating: Effect of pH, Water, and MPS Content," Industrial & Engineering Chemistry Research. Ind. Eng. Chem. Res. 2013, 52, 2590−2597.
3. W. Wu, C. Kirimli, W. Y. Shih, and W.-H. Shih, "Real-time, In Situ DNA Hybridization Detection with Attomolar Sensitivity without Amplification Using (Pb(Mg1/3Nb2/3)O3)0.65-(PbTiO3)0.35 Piezoelectric Plate Sensors," *Biosensors and Bioelectronics*, 43, 391–399 (2013)
4. L. Loo, J. A. Capobianco, W. Wu, X. Gao, W. Y. Shih, W.-H. Shih, K. Pourrezaei, M. K. Robinson, and Gregory *P. Adams, "Highly Sensitive Detec*tion of HER2 Extracellular Domain in the Serum of Breast Cancer Patients by Piezoelectric Microcantilevers," *Anal. Chem*., 83, 3392–3397(2011)
5. L. Loo, W. Wu, W. Y. Shih, W.-H. Shih, H. Borghaei, K. Pourrezaei, G. P. Adams, "A Rapid Method to Regenerate Piezoelectric Microcantilever Sensors (PEMS)," *Sensors*, 11, 5520-5528 (2011).

Example patent applications/disclosures:

1. W. Y. Shih, Qing Zhu, and Wei-Heng Shih, "Enhanced Sensitivity with Piezoelectric Sensors," US National Phase Application No. 12/921,756 filed October 20th, 2010, Drexel University Dock No: 08-0910D.
2. W. Y. Shih, M. Soylou, W.-H. Shih, "Dense (3-mercaptopropyl)trimethoxysilane (MPS) coating with enhanced insulation property," Disclosure, Drexel University Dock No: 12-1447D
3. W. Y. Shih, W. Wu, M. Soylou, C. Kirimli, W.-H. Shih, "Piezoelectric plate sensor (PEPS)," disclosure, Drexel University Dock No: 12-1422D.
4. W. Y. Shih, M. Soylou, C. Kirimli, W. Wu, and W.-H. Shih, "Direct Detection < 100 copies/ml of Bacterial Genetic Signature in Stool in 30 min without Isolation, Concentration or Amplification using Piezoelectric Plate Sensor (PEPS)," Disclosure, Drexel University Dock No: 12-1402DP.

Example Grants applications

1. NIH STTR grant, "Rapid genotypic detection of toxigenic Clostridium difficile from stool at point of care," to be submitted 4/5/2013. PI: W. Y. Shih, Co-PI: W.-H. Shih, S. Joshi, R. Hamilton, M. Saks, C. Emery.
2. Coulter Translation grant, "Rapid, Low-cost, Genetic Assay of Shiga Toxin-producing *Escherichia coli* for both Food Safety and Clinical Applications," to be submitted to be submitted 4/1/2013. PI: W. Y. Shih, Co-PI: W.-H. Shih, G. Paoli.
3. NIH STTR grant, "Rapid and Cost-Effective Hepatitis B (HB) Detection and Chronic HB Monitoring," to be submitted 8/5/2013. PI: W. Y. Shih, Co-PI: W.-H. Shih, C. Emery.

Response to Reviewer 2:

Regarding the antibody to differentiate MRSA and MSSA.

> We completely agree with the reviewer's concern about the specificity of the antibody. In the past few years, we have realized PEPS could detect DNA with PCR-like sensitivity (see publication 3) without DNA extraction and amplification. As a result, we have shifted our focus to genetic detection. Presently we are using the Shiga toxin genes, stx1 and stx2 to detect Shiga toxin producing E coli (see proposal 2), which has been shown by PCR to be highly sensitive and highly specific for Shiga toxin producing E coli. We believe using a similar strategy to target the genes related to antibiotic resistance to allow better differentiation of PRSA from MSSA.

Regarding the economic benefit of this instrument.

> It is rapid (30 min) and low-cost (The measuring impedance analyzer is only $500, it needs no expensive reagents) and yet has PCR-like sensitivity but without the need of DNA extraction and amplification. The economic benefit is low-cost compared to PCR and it is rapid. It is particular useful in providing low-cost, accurate diagnosis of acute infections.

Regarding using a variety of MRSA/MSSA strain background to validate the sensitivity and specificity of the instrument. We completely agree with the reviewer.

Response to Reviewer 3:

Regarding more strains to be tested:

We completely agree with the reviewer; it should be tested in more strains. For example, in the E coli detection proposal, we are working with our USDA collaborator who has more than 100 strains of Shiga toxin producing E coli.

Regarding whether the antibodies made against MRSA are adequate.

We completely agree with the reviewer's concern about how specific the antibodies can be against MRSA. As mentioned above, our focus has shifted to genetic detection. We believe detecting genes instead of the surface antigen will make MRSA detection more specific.

We agree with the review and we will perform the pre-absorption experiments with mixtures of S. aureus and MRSA with the anti-MRSA rabbit polyclonal ab20920 (AbCam).

Generic Recommendations

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

Response:

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

Response:

We completely agree with the reviewers' concern about the specificity of antibody-based MRSA detection. As we have discussed above, we have shifted out focus to genetic detection. The niche of the PEMS and its successor, PEPS is that its detection resonance frequency shift is enhanced by three orders of magnitude due to the stress induced by the binding of the target molecules on the PEMS/PEPS surface. This enhancement is further amplified in DNA detection as the stress generated is much larger by the highly charged DNA molecules. As a result, PEPS can detect DNA with PCR-like sensitivity without the need of DNA extraction and amplification. In addition, the measurement unit of PEPS is only $500 and the measurement time is <40 min. This makes PEMS/PEPS genetic assay uniquely suitable for sensitive and specific acute infection detection.

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

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

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

<u>Reviewer 1:</u>

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.

<u>Reviewer 2:</u>

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 effectiveCCR2-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.

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

The funds were used to support salaries of the co-investigators, a graduate student, and a research technician to the extent indicated in the final report. The remainder of the budget was used to purchase supplies that were used in the performance of reported experiments.

The reviewer is correct in identifying incomplete knockdown of CCR2 as a weakness in the results of this project. We explored several different miRNAs and assayed results at the level of expression of CCR2 mRNA (by RT-PCR) as well as expression of the receptor on the cell surface (by FACS). We concluded that effective knockdown of CCR2 on the cell surface might require considerable time to allow for turnover of existing proteins. Additional miRNAs that target regions unique to CCR2 in this family of receptors must be tested for more effective silencing, even at the RNA level. Testing of the effects of CCR2 knockdown on the migration of cultured cells and clinically relevant cells awaits better results in knocking down CCR2 expression at the cell surface.

In light of our difficulties in finding a strong suppressor of CCR2, we undertook a more complete characterization of the CK-TAR promoter in order to move the project forward. This promoter can be useful to drive the silencing of viral or cellular genes in addition to CCR2. We believe that in some circumstances, this promoter could be a better choice than the HIV-LTR, that is also Tat-inducible, because use of an active LTR increases the potential for vector mobilization, which is considered a problem in many therapeutic applications.

The work resulted in the following publication:

Sanghvi, V.R. and Steel, L.F. 2010. Expression of interfering RNAs from an HIV-1 Tat-inducible chimeric promoter. Virus Research 155, 106-111.

We thank the reviewer for the suggestion that we pursue IP protection for these ideas. We will consult our Technology Transfer Office for further advice on this matter.

Drexel University internally audits the CURE accounts to ensure that the funds allocated for the research projects are used for that purpose. It is unclear why Reviewer 1 suggested that the funds were not utilized for this project when indeed the funding was spent to support salaries of the co-investigators, a graduate student, and a research technician.

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

Response:

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

Response:

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

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

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

<u>Reviewer 1:</u>

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 lyzates 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.

<u>Reviewer 2:</u>

Additional preliminary data would be of benefit.

<u>Reviewer 3:</u>

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.

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

It is true that as our results came in, the direction of the research changed to take advantage of interesting results. In my experience this is typical of the scientific process.

The forskolin-dependent LTP deficit was actually found first, whereas we later found no deficits in other types of LTP.

As mentioned by one reviewer, our research plan was overly ambitious.

We would concur that the limited budget of the grant limited our ability to analyze a large number of animals.

These results comparing basal levels of cAMP in various hippocampus lyzates from WT vs. SST3 were very consistent, surprising, and very robust. Proper normalization was done.

We began a collaboration with Dr. Lippa in the Neurology Department at DUCOM, unfortunately this was interrupted when our term at DUCOM ended.

Thank you for your constructive criticism. I appreciate the reviewer's comments. Since they were mostly positive and criticisms were minor, I will only say that I will focus on improving my grantsmanship in the future.

I would agree that additional preliminary data is always useful, and will attempt to provide more in the future.

I received extremely good scores on both an NIH challenge grant application (12th percentile) and the Alzheimer's Association (mostly 9s and 10s on a scoring system of 1 [worst] to 10 [best]), unfortunately the limited funds in both programs did not allow my grants to be funded.

We did begin a collaboration with Dr. Lippa in the Neurology Department at DUCOM, unfortunately this was interrupted when our team left Drexel University.

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

Response:

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

Response:

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

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

Reviewer Comment on Specific Weakness and Recommendation (*Copy and paste from the report the reviewers' comments listed under Section B - Specific Weaknesses and Recommendations)*:
<u>Reviewer 1:</u>
None

<u>Reviewer 2:</u>
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.

<u>Reviewer 3:</u>
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

<u>Reviewer 1:</u>
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.

<u>Reviewer 3:</u>
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.

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

We have indeed performed a microarray analysis using the Kis-RNAi reagent with the funding from the NIH R21 grant that we obtained. Using the data from this analysis, we have found a number of candidate genes whose expression is regulated by Kismet function. We are pursuing two of the most promising candidates, and have recently received a 4 year NSF grant beginning April 2013 to continue pursuing these**.**

This is an interesting approach. As mentioned above, the NSF proposal that currently supports this work is focused on identifying the mechanistic link between two Kismet target genes in regulating axon pruning in the brain, but if sufficient funds are leveraged in the future, this would be an excellent approach in order to identify any other gene mutations associated with CHARGE syndrome patients.

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

Response:

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

Response:
I thank the reviewers for their positive comments and feedback for the work. We are in the process of preparing two manuscripts for submission that are directly spawned from the initial seed money provided by this grant. We anticipate completion of submission by the end of August 2013.

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

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

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

<u>Reviewer 1:</u>

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.

<u>Reviewer 2:</u>

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.

<u>Reviewer 3:</u>

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.

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

We agree that our approach needs additional testing. This effort is underway. We do intend to process and analyze many more specimens. For example the study of Oncotype DX utilized 668 tumor blocks[1] and another study on correlating tumor morphology with survival utilized 576 specimens[2]. These are reasonable numbers of samples to demonstrate the efficacy of our approach. We intend to process and analyze approximately 600 specimens in a future study. Ultimately a large-scale clinical trial would be needed to validate the approach for clinical use, a long-term goal of our work.

The mean tumor size in our cohort is approximately 2 cm, which is large enough for our current technique to effectively determine metastasis status. We have recently shown that tumors with a size as small as 0.6 cm can also be used to positively predict lymph node metastasis with a Region Of Interest (ROI) 3 mm^2 in size[3]. Though the issue of minimum tumor size needed for our technique to be effective is still to be determined and will be explored in the future.

Additionally, we have begun to develop techniques for finding the most predictive ROI for a patient via scanning all of the 2 to 6 slides available per patient. Ultimately we will determine which 3 mm^2 portion of a tumor provides the most reliable prediction.

We agree that the statistical techniques of our original study needed improvement. In our most recent work[3] we have significantly improved the statistical analysis of our dataset.
We now utilize a 2-stage classifier with SVM in the first stage and a weighted majority algorithm for the second stage. Additionally we ensure a strict separation of the test and training data during analysis.

As mentioned above we intend to process and analyze many more specimens.

No control slides were required in our study because we had the ground truth metastasis status for each of the specimens. Since we have a known ground truth for our specimens the focus of our work is to create a computational classifier based on this ground truth. Since the dataset

incorporates both known N0 and N1 specimens we are conducting a classification based on the characteristic image features derived from each class of specimen.

Since our work utilized an automatic immunostainer (Ventana Benchmark Ultra) with FDA-approved, immuno kits to stain our samples, there are no significant variations between runs. We also use external and internal controls to standardize the scoring.

In the future we intend to perform our analysis on H&E stained specimens. In this case we do understand that the quality of the staining can vary between slides. In this case we will address the issue of histology variation and standardization. The effect of staining variation on our predictions will be explored and quantified in future work. A major focus of this work will be to improve the segmentation algorithms in order to mitigate the effect of staining variability on our resulting predictions.

In our current work we only analyze a single ROI from one slide for each patient. We utilize the slide/staining that gives the highest number of stained nuclei to maximize segmentation of malignant nuclei. The ROI is chosen as the region with the highest density of stained nuclei.

Our current research[3] using the same data from this study shows that we are able to predict metastasis status from a single immunostained slide with an ROC area under the curve of approximately 0.9, indicating high sensitivity and specificity. This result was produced with fewer features than our original study (11 instead of 17), potentially improving the generality of the algorithm.

Since a number of high resolution slides with different stainings are available for each patient, we intend to determine which ROI within all of these multiple slides provide the most reliable prediction. This should lead to finding correlations between image features and predictiveness.

We agree that our dataset size should be increased, and will be increased in future studies. The dataset of our study is quite heterogeneous and includes a variety of Nottingham histologic grades from 1 to 3, tumor sizes that span 0.5 to 3.5 cm, ages that span 30 to 80, and all four molecular subtypes (Luminal A, Luminal B, HER2 and Triple negative). We do need to more systematically explore how these variations correlate with predictive capacity of the approach.

As stated above we plan on conducting additional studies with significantly larger cohort sizes, with approximately 600 specimens.

The study did include a practicing pathologist, Dr. Fernando U. Garcia. He continues to lead this effort.

Looking back on the proposal we agree that the statement of the study's hypothesis was not as clear as it should have been in the proposal text.

The hypothesis of our project is that metastasis status of a breast tumor may be predicted by image analysis of the histology of the primary tumor. The goal of our work is to evaluate the sensitivity and specificity of our algorithms when predicting metastasis status. Our initial and

current work shows high sensitivity and specificity as demonstrated by the high AUC produced in our results. Therefore we believe that we have demonstrated that our approach can be used to predict metastasis status of a breast tumor via analysis of only the histology of the primary tumor. The specificity and sensitivity of our computational prediction should be the measure of the success of our project. We do recognize that in this initial phase of the study the number of samples used is low to confirm success and robustness, but the results are encouraging and indicate the potential of our approach.

We have continued to work on this project and have new, unpublished results. In our current work we have improved our ability to assess the reliability of the prediction. We have now developed a confidence measure for our predictions, which provides diagnostic thresholds to evaluate the reliability of the prediction. Our most reliable results now provide predictions with a 98% correct rate. Over 50% of the cases in our original study are considered "reliable", i.e. they meet the diagnostic criterion for reliability.

The gold standard for our approach should be the ability to always predict the correct metastasis status of a tumor via analysis of its histology. It is not clear what the gold standard for predicting metastasis should be in a clinical setting. Any one test cannot be the sole determiner for patient treatment. We see our approach being another tool that will contribute to the diagnosis/treatment decision-making process.

The goal of our work is not to detect metastasis directly in lymph nodes, but rather to develop computational techniques for predicting metastasis status from analysis of the primary breast tumor. The core of these techniques is a supervised machine-learning module that is tuned using the known metastasis status of breast carcinomas. These statuses are determined by inspection by a pathologist of the resected lymph nodes associated with the tumor. We do assume that the clinical diagnoses of our training specimens are accurate and correct. Detecting metastasis in lymph nodes via direct imaging, scanning or other biomarkers is not the focus of our research.

We can also limit our analysis to cases that are clinically determined to be N0.

We believe that our approach has merit and deserves further study. We see the value of our approach in settings, i.e. rural or third world hospitals, where advanced imaging and molecular tests are either unavailable or unaffordable. Since the technique is only based on computational analysis of standard histology slides, any health facility that handles and diagnoses breast carcinoma specimens could be able to perform the prediction, obviating the need for expensive molecular testing and advanced imaging. We also imagine the development of a web-based application to process the scanned slides from underserved areas that may not have the needed computer/software resources.

A publication describing our latest results is being prepared for Science Translational Medicine. We have begun discussions with Drexel's Technology Commercialization Office about the possibility of patenting and licensing our techniques.

1. S. Paik et al., "A Multigene Assay to Predict Recurrence of Tamoxifen-Treated, Node-Negative Breast Cancer," N Engl J Med 2004; 351:2817-2826.

2. A.H. Beck et al., "Systematic Analysis of Breast Cancer Morphology Uncovers Stromal Features Associated with Survival," Sci Transl Med 9 November 2011: Vol. 3, Issue 108, p. 108.

3. M. Zarella, M.A. Reza, D.E. Breen, Y. Gong and F.U. Garcia, "Lymph Node Metastasis Status in Primary Breast Carcinoma Can Be Predicted Via Image Analysis of Tumor Histology," Modern Pathology, Vol. 26 (Suppl. 2), p. 383A, February 2013.

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

Response:

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

Response: