

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 (1.78)

Project Rating:

| Project | Title | Average Score |
|----------------|---------------------------------------------------------------------------------------------|----------------------|
| 0865201 | Role and Regulation of Focal Adhesion Kinase in Melanoma | Outstanding (1.33) |
| 0865202 | Prolactin and Growth Factor Signaling in Breast Cancer | Outstanding (1.33) |
| 0865203 | Stat5 and ErbB2 in Prostate Cancer | Favorable (1.67) |
| 0865204 | Targeting the IGF-1 Receptor in Cancer | Favorable (2.33) |
| 0865205 | The Role of MicroRNA (miRNA) Gene Expression in Therapy Resistance of Human Breast Cancer | Outstanding (1.33) |
| 0865206 | Mechanisms for Metastasis Suppression through Kisspeptin Regulation of the Microenvironment | Unfavorable (2.67) |

Project Number: 0865201
Project Title: Role and Regulation of Focal Adhesion Kinase in Melanoma
Investigator: Aplin, Andrew

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:

While this project did not meet the objectives stated during the initial application, there was reasonable progress made during the funding period as evidenced by three original research articles published during this time period. Originally, the main outcome (on my reading of the application) was to have "identified a protein that plays a critical role in melanoma cell invasion and understand what regulates its properties," thus allowing the development of a biomarker that could identify invasive and aggressive forms of melanoma. This broad objective was not met, although the investigators do have interesting data that has been presented in their publications as well as in progress reports from previous years.

Upon reviewing the history of their progress reports, it appears that in the middle of the funding cycle the investigators made a decision to focus primarily on some findings related to the development and mechanisms of resistance to targeted BRAF inhibitors (BRAFi) while eschewing the focus on FAK that was put forth in the original application. This decision was not unreasonable, since this new class of drugs (recently FDA-approved as first-line treatment for patients with activating BRAF mutations in their tumors) has been the subject of intense clinical and laboratory research efforts in melanoma. The authors did indeed make some interesting observations regarding the role of certain proteins in BRAFi-resistant tumors, and while these studies have not led to the development of prognostic markers as previously hoped, they have contributed to the growing body of literature on BRAFi resistance and lent new insight into how cells may develop resistance to BRAFi.

Specific strengths: The investigators came up with some important and novel findings, and their progress is supported by the publication of three original research articles in journals that are well-respected in the field of melanoma and cancer research (*Oncogene*, *J Invest Derm*, and *J Biol Chem*). The quality of the data presented throughout the funding cycle was excellent, and almost all of it was publication quality, which is a testament to their technical excellence and expertise in this area. The authors were among the first to make observations regarding the activation of MAPK signaling in NRAS-mutant melanomas when cells with wild-type BRAF were exposed to BRAFi.

Specific weaknesses: This proposal did not appear to be designed to meet the broad objectives stated in the original proposal, since there was no incorporation of even a small subset of patient specimens for pilot studies of potential biomarkers. The studies were entirely performed using established melanoma cell lines in laboratory (in vitro) assays. That being said, the range of budget for this grant would not have permitted more significant experiments such as mouse models or clinical trials.

The investigators were side-tracked from their original focus on FAK, which became somewhat of a bystander protein in their subsequent publications on BRAF resistance. The focus on FAK in the original proposal was quite rational, as was the approach to study effects of FAK on various aspects of cellular function including invasion, cellular migration, and focal adhesion turnover. However, the focus of the project was diverted to the larger goal of BRAFⁱ resistance before the goals of the original proposal were entirely addressed. Based on what I have seen from the previous progress reports, the role of FAK in regulating these cellular aspects of melanoma cells in this model still remains unanswered.

Reviewer 2:

Two aims were proposed. The project met the stated objectives.

The research design and methods are adequate. The PI is highly experienced and used necessary and appropriate designs and methods.

The data presented and the peer-reviewed publications clearly indicate that the project met its objectives.

The PI and his team are clearly pursuing a strategic research plan to understand the molecular basis for BRAF resistance of melanoma. The data presented on regulation of FAK by BRAF and the role of FAK in melanoma aggressiveness are therefore clearly in line with this plan.

Reviewer 3:

Strengths: Results obtained from this study verified that phosphorylation of FAK at serine 910 regulates FAK localization and turnover in focal adhesions, and cellular migration and invasion.

The proposed research questions were appropriately addressed, and information provided met the objectives of the project.

Weakness: Proposed research design and methods were adequate, but results from proposed Fluorescence recovery after photobleaching (FRAP) were missing in the report. An explanation should be given.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

This project had very high significance with regards to potentially improving health, since melanoma remains a significant health problem worldwide. The goal of finding ways to augment treatment or understand the development of resistance has tremendous value towards improving outcomes for this disease, which had until recently very limited treatment options. The research completed made significant contributions to our mechanistic understanding of the events that underlie acquisition of resistance to BRAFi, which may ultimately contribute to the development of therapies that can circumvent this phenomenon. It does appear that the authors have submitted an NIH R01 proposal to try and continue working on this project, although they do not provide an NIH grant number in their final progress report.

Strengths: The overall area of study has high scientific and clinical value to understanding melanoma. The authors did indeed make some significant contributions to the literature, evidenced by three original research articles published in respected journals. They made the now accepted observation that MAPK signaling is activated in NRAS-mutant (BRAF WT) melanoma cells with BRAFi, and were among the first to publish on this significant finding (along with several other groups).

Weaknesses: The studies performed are valuable and interesting, but still quite preliminary with respect to being able to draw any stronger conclusions due to the lack of any in vivo (mouse model) data or patient data. This weakness was present at the time of the original proposal, and does not reflect any failures on the part of the investigative team during this proposal.

Reviewer 2:

Resistance to BRAF inhibitors has become an important clinical challenge. This project addresses the mechanisms in this resistance.

This is highly significant for eventually improving the treatment of BRAF-positive melanoma patients.

The results of this research could impact the treatment of melanoma patients.

The future plans include in vivo studies of BRAF resistance mechanisms.

Reviewer 3:

Strengths: It provided potential therapeutic options to overcome resistance to the inhibitors of BRAF mutation, PLX4720, a promising medicine for metastatic melanoma treatment.

It elucidated the effect of NRAS^{Q61K} on PLX4720 resistance, and the role of SHOC-2/Sur-8, a RAS-RAF scaffold protein, on NRAS mutation induced PLX4720 resistance.

It determined the acquired resistance to PLX4032/4720 involves ERK1/2 pathway re-activation and ERK1/2-independent silencing of BH3-only proteins.

It also explored the combined treatment of HDAC inhibitors and MEK inhibitors to correct PLX4032 resistance.

Weakness: Although the proposed study has translational potential, the likely beneficial impact is small without convincing data from animal models and human related studies.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

The investigators were able to obtain extramural funding through the Department of Defense, which offered a year of support. The investigators noted that they were planning to submit an NIH grant, although no additional details were included in the final progress report regarding this planned application.

Reviewer 2:

The project leveraged a federal (Department of Defense DoD) grant. The researchers have already submitted an NIH R01 grant application. Both leveraging of DoD funding and applying for additional funding are strengths.

Reviewer 3:

Strengths: With this support, the investigator was successfully awarded a DoD grant, entitled “Novel mechanisms of resistance to RAF inhibitors in melanoma.” They also applied a new R-01 application early in 2013.

Weakness: None noted.

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:

Six peer-reviewed publications were sent with the final progress report, of which three represent original research articles. There are two reviews and one commentary. There are no plans for further publications related to this project.

Reviewer 2:

The completed research resulted in two excellent peer-reviewed publications and 3 reviews or commentaries between 2010 and 2012 in high-impact journals. No more publications are planned.

Overall, this is excellent productivity.

Reviewer 3:

Strengths: With this support, the investigator and his team successfully published 3 original research articles and 3 review papers. All published papers are with better quality.

Weakness: None noted.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

Training of two post-doctoral researchers was supported with funds from this award.

Reviewer 2:

No improvements were made to infrastructure. This is appropriate.

No new investigators were added. No researchers were brought into the institution.

Funds from the project were used to pay for one pre-and two post-doctoral students. This project offered excellent opportunity for students to perform outstanding research.

Reviewer 3:

Strength: This funding provided support for post-doctoral fellows and doctoral student.

Weakness: None noted.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

No collaborations, past or future, were noted for this proposal.

Reviewer 2:

The research performed did not involve any collaboration and the researchers do not describe any plans for future collaborations. This is appropriate since the research has demonstrated that they have the necessary expertise.

Reviewer 3:

Not aware of any collaborative work has been established/initiated with this funding support.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

1. While the investigators did secure a small extramural grant, it does not appear that they made a larger effort to secure larger extramural grants (such as the NIH R-series) except in passing mention in the final progress report. The final progress report was designed to allow reporting of grants that were submitted and not funded, and if the investigators did indeed apply for more extramural funding (even if unsuccessful); these grants should be listed in the final progress report. Furthermore, the mention of an NIH grant submitted in February 2013 does not include relevant information including grant title, RFA, NIH institute where grant will be reviewed, and grant number if assigned.

Future recommendations: I would recommend more aggressive efforts to leverage funding, and inclusion of relevant grant data for submitted applications regardless of funding outcome.

2. For a grant whose goal was to elucidate biomarkers that might have prognostic value, there was no clinical collaboration or use of in vivo models (either at TJU or through collaborative efforts). For future grants with this objective, I would recommend that the investigators seek collaborators who can work with them to either secure patient samples for biomarker testing or to confirm their findings in mouse models (either transgenic or xenograft-based).

Future recommendations: I would recommend establishing collaborations to increase the chances of obtaining more pre-clinical data if the objective is indeed the establishment of clinical biomarkers.

3. For many of these experiments, a limited number of cell lines were used (likely due to limited resources). Given the limitations of laboratory melanoma models in replicating the human disease, future studies should consider use of a broader set of lines (genetically characterized to some extent, i.e., BRAF/NRAS mutation status, PTEN status, CDKN2A status, etc.).

I would recommend using a broader panel of genetically characterized cell lines for all experiments.

4. While six publications were associated with the final progress report, only three represent actual original research that is directly relevant to this award. The other three reviews, while interesting and also related in part to the research, are not publications I would link directly with this award.

I would (as a reviewer) prefer focusing primarily on publications that report original research funded through this grant proposal.

Reviewer 2:

None.

Reviewer 3:

It would improve the beneficial impact of this study, if either antibody directed against p-FAK ser-910 or small molecule inhibitor specific to p-FAK ser-910 are developed and tested in animal models.

Generic Recommendations for Thomas Jefferson University

Reviewer 1:

I am unaware if this grant was made with matching funds from the institution, but given the rather ambitious scope of this application, similar future applications could benefit significantly from some type of either linked supplement or matching funds to help finance the research.

Project Number: 0865202
Project Title: Prolactin and Growth Factor Signaling in Breast Cancer
Investigator: Rui, Hallgeir

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 PI completed the proposed research and, as predicted, identified a potential diagnostic and prognostic indicator of breast cancer as well as a novel therapeutic target to prevent progression of breast cancer. This project has met its stated objectives. The PI provided an excellent description of the progress his lab has made during the funding of this project. The achieved goals very closely matched the original specific aims of the project.

The most solid piece of evidence of the PI's success is the number and quality of manuscripts published. The PI was able to publish his findings in numerous top-quality journals. The PI lists five publications (in outstanding journals) in his progress report, but a quick search of PubMed indicates that he had two additional data publications and a review article since submitting the progress report. This is outstanding progress.

There are no major weaknesses.

Reviewer 2:

Breast cancer mortality is associated with metastatic spread believed to arise from the loss of cellular differentiation and epithelial-to-mesenchymal transition (EMT). In this proposal, the PI presents preliminary data that prolactin-mediated Stat5 signaling normally acts in a tumor suppressive manner to maintain breast epithelial differentiation so that EMT observed in breast cancer may, at least in part, involve loss of prolactin-Stat 5 control. Specifically, the central hypothesis is that EGFR/Her2 signaling inhibits the prolactin receptor-Jak2-Stat5 signaling pathway in breast tissue. Suppression of the prolactin receptor-Jak2-Stat5 signaling pathway, then, results in EMT and loss of ER expression. Consequently, two specific aims were proposed. Aim 1 would determine whether the prolactin receptor-Jak2-Stat5 signaling pathway is itself regulated by EGF signaling. Aim 2 would seek to determine whether Stat5 (re)-activation can overcome the dedifferentiation, EMT and loss of ER caused by dysfunctional EGFR/Her2 signaling in human breast cancer. The use of cell cultures and mouse xenograft models are proposed to show that EGFR/Her2-induced EMT and loss of ER could be reversed with Stat5 (re)-activation in breast cancer. Likewise, cell culture models and IHC examination of archival breast tissue specimens are proposed to show that EGFR/Her2-positive breast cancers with persistent Stat5 signaling will be of lower grade and have improved prognosis. Over the course of this project the following results were found:

Aim 1: PTP1B, a tyrosine kinase activated by EGF, is a negative regulator of the prolactin receptor-Jak2-Stat5 signaling pathway; BCL6, which promotes EMT and is associated with poorly differentiated breast cancers, is inhibited by prolactin through Stat5; extracellular acidosis disrupts the prolactin-Stat5 signaling pathway as indicated by GLUT1 expression; prolactin suppresses chemoresistant progesterin-induced CK5 expressing cells through Stat5 and; progesterin induces BCL6 expression.

Aim 2: Nuclear localization and phosphorylation of Stat5 serves as an independent prognostic marker in node-negative breast cancer as well as indicator for antiestrogen therapy; levels of nuclear Stat5a, but not Stat3 predict prognosis and response to anti-estrogen therapy and; clinical correlation between BCL6 and CK5 expression.

The major strengths of this project were that the objectives were clearly stated from the start and the research designed to test the central hypothesis followed a logical stepwise manner. A thorough statistical analyses plan was provided and substantial data were generated to understand the role of prolactin receptor-Jak2-Stat5 signaling pathway in breast cancer.

Weaknesses: Though many findings consistent with the original hypothesis are described, it is unclear through the course of this grant if all the molecular signaling experiments as originally outlined (e.g., EGF signaling experiments) were performed.

Reviewer 3:

The project was originally designed to determine whether i) the mammary epithelial cell pro-differentiation/anti-tumor progression cascade initiated by the prolactin-Stat5a axis can be inhibited by EGFR/Her2-induced epithelial-mesenchymal transition (EMT)-promoting factors in the tumor microenvironment and ii) Stat5a activation can suppress the EGFR/Her2-induced de-differentiation, EMT and loss of ER α observed in breast cancer. During the project period, progress was made in terms of identifying the potential tumor-suppressive role played by prolactin/Stat5a signaling with regard to the inhibition of BCL6 expression (Cancer Res 70: 1711, 2010), the identification of PTP1B as a tumor promoter by suppressing the prolactin/Stat5a axis (Am J Path 177:297, 2010) and the prognostic value of Stat5a versus Stat5b and Stat3 in breast cancer (Am J Cancer 1:347, 2011; J Clin Oncol 29:2448, 2011 and Breast Cancer Res 14:R130, 2012). However, none of these studies actually addressed in a direct way the original aims of the project. Neither the role of the microenvironment, the EGFR/Her2 axis nor Stat5a activation was examined in terms of their ability to affect breast cancer de-differentiation, EMT or ER α suppression. From this reviewer's reading of the final progress report as well as the interim reports, no explanation was given for the lack of progress regarding the original aims or the apparent shift in direction of the project. As such, the data and information provided by the applicant were not directly applicable to the project's original objectives listed in the strategic research plan. Further, the data were not developed sufficiently to answer any of the research questions posed. Nevertheless, in terms of characterizing the prolactin/Stat5a axis as a tumor suppressor in tandem with carefully correlated clinical data, the final conclusions and published reports do represent a substantive contribution to the literature. That having been said, the applicant does list at least two NIH R01 grant awards as well as a Komen for the Cure Promise Grant for translational breast cancer research, and with the lack of progress made in the original

objectives, it is not clear to what degree the accomplished goals overlap with pre-existing 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:

Decoding the underlying basic science of breast cancer is the first step in developing new diagnostic and prognostic indicators of the disease. In addition, these types of studies help identify potential molecular targets for new drug development. Identifying the loss of STAT5 activity as a potential biomarker for the diagnosis and prognosis of breast cancer is an important first step for clinical analysis.

The future plans for this study include transitioning these molecular findings to analysis in patient samples to clarify the extent to which these findings can be used for a clinical diagnosis. In addition, the PI proposes to target STAT5 to inhibit the development of breast cancer. There are no major weaknesses.

Reviewer 2:

Strengths: The results of these experiments detail the role of prolactin receptor-Jak2-Stat5 signaling pathway in the maintenance of a differentiated breast cancer phenotype with improved prognosis and responsiveness to anti-estrogen therapy. Specifically, prolactin receptor-Jak2-Stat5 signaling pathway suppresses BCL6 and inhibits progesterin-induced CK5-positive drug resistant cells such as Stat5 prevents progesterin-mediated de-differentiation and emergence of drug resistance. Loss of Stat5, but not Stat3, expression is an independent prognostic maker. Loss of Stat5 may serve as a physiological switch to facilitate histological de-differentiation, thereby promoting an invasive (EMT) phenotype. Loss of Stat5 may occur through EGF-mediated PTP1B activity and/or extracellular acidosis. Taken together, levels of or changes in Stat5 expression in breast tumor tissue may provide diagnostic and prognostic clinical information. Lastly, these studies suggest that agents which (re)-activate prolactin receptor signaling or which inhibit BCL6 could represent novel avenues of therapeutic intervention. This, in turn, may provide women with increased health options to reduce the high mortality associated with invasive breast cancer. Going forward, then, further research is anticipated with clinical trials to target re-activation of prolactin receptor signaling and/or inhibition of BCL6.

Weaknesses: No major weaknesses were noted.

Reviewer 3:

The project's major accomplishment is in identifying nuclear-phospho-Stat5 and protein levels of Stat5a protein as independent prognostic markers in node-negative breast cancer. This is a significant, but relatively modest, advance to the field. It is "reasonable" given the dollars budgeted (the issue of overlapping goals with other existing grants notwithstanding). At this juncture, it is difficult to evaluate the importance of these findings in terms of diagnostic impact until such time that the loss of nuclear phospho-Stat5a has been confirmed as an independent

marker in prospective studies or the value of loss of Stat5a as a predictor of response to anti-estrogen therapy has been validated in randomized clinical trials.

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 additional funding was listed on the progress report or in NIH reporter. However, the PI reports that he is making a continual effort to solicit more funding. Unfortunately, in this funding climate this is a challenge. Based on the number of high quality publications, the PI is on the right track for securing funding in the near future.

It is a weakness that additional funding was not secured, but the quality of work warrants future funding. It seems reasonable that funding will be obtained in the future.

Reviewer 2:

Strengths: The project does not appear to have garnered any additional funds during this funding period. However, of significance, the PI has already submitted 3 new grant applications (1x DOD, 2x NIH) and 2 additional NIH R01 submissions are in preparation. The data derived from the current grant has likely provided strong preliminary data for all these submissions. These new grant submissions represent continued investigations by the PI into mechanistic studies on the role of prolactin in breast cancer.

Weaknesses: None noted.

Reviewer 3:

No leveraging funds were identified and while the applicant is planning to submit new grants to pursue aspects of this work, these applications have not yet been submitted.

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 PI has an excellent publication record. He had five publications during the funding period and two additional data publications since submitting the progress report. All publications are in high-quality journals. This is an outstanding measure of the PI's productivity.

There are no major weaknesses.

Reviewer 2:

Strengths: Though there has been no commercial developmental opportunities initiated, this project has been very successful and productive in the publication of at least 5 papers within the

4-year period of this grant. These publications have been in either relatively high-impact journals or appropriate journals representative of this project's subspecialty. It is also likely that additional manuscripts will be prepared from the data collected during this project. Consequently, there has already been public disclosure of data obtained from this project.

Weakness: None noted.

Reviewer 3:

The applicant lists 5 publications in medium level-impact journals. The number of publications is reasonable, though again, each of the papers cite multiple granting sources and the stated objectives of the original project are not the direct subject of any of these papers.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

There are no reports of improvement to the institution's infrastructure. The findings generated by this funding were an asset to the scientific community at Thomas Jefferson University. The PI was able to support/train several researchers (3 post-doctoral scientists and 3 pre-doctoral students) and staff members (2 research technicians).

There are no major weaknesses.

Reviewer 2:

Strengths: A major strength of this project is the training and/or support of 5 students and 3 post-doctoral fellows during the course of this study. This support allowed for the students and post-doctoral fellows to become well-versed in many powerful cellular, molecular and animal techniques. In this way, these grant monies helped to train our next generation of researchers. Weaknesses: No new faculty investigators were recruited to this university/research group as a result of this study and no apparent changes were made to the infrastructure.

Reviewer 3:

No identifiable improvements were made to the infrastructure. Funds were used to enlist the support of one undergraduate student, one master's student, three pre-doctoral students and three post-doctoral students. In overview, no substantive gains could be identified in terms of enhancing the quality or capacity for research at the grantee's institution, though the efforts certainly reinforced collaborative efforts.

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 research resulted in several collaborations with investigators outside the university. Several were relatively local (the Philadelphia/Washington D.C. area) and one was from the University of Nebraska.

There are no major weaknesses.

Reviewer 2:

Strengths: A significant benefit of this project was the collaborative interactions initiated between the PI and researchers both within and outside of Thomas Jefferson University. Within the PI's home institution, this project strengthened collaborative interactions between researchers at Thomas Jefferson University by bringing new technologies and research concepts to a group of researchers and colleagues at Thomas Jefferson University. Outside interactions include the establishment of research collaborations between the PI and the University of Pennsylvania (Dr. Fuchs), Walter Reed and the Joyce Murtha National Military Cancer Center of Bethesda, Md. (Drs. Shriver and Hooke), the University of Nebraska (Dr. Wagner) and MDR Global Inc., Pa. In this way, additional performance sites and researchers were included in this study, thereby expanding the penetration of the studies throughout Pennsylvania as well as establishing an inter-institutional and national research network.

Weaknesses: None noted.

Reviewer 3:

It is not clear that any new collaborations are in the planning stages and whether the collaborative studies extended beyond the collection and/or analysis of clinical materials. The project did, nevertheless, lead to collaborative efforts outside of the institution for analyzing clinical materials.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

None.

Reviewer 2:

None.

Reviewer 3:

Weaknesses: The major criticism is the lack of any progress on the stated objectives in the project, particularly with regard to the potential interface between the EGFR/Her2 axis and the prolactin/Stat5a signaling cascade. Nevertheless, the applicant did succeed in identifying BCL6 as a potentially important target of prolactin signaling as well as identifying PTP1B as a potential negative modifier of Stat5a signaling. The clinical studies identifying Stat5a as a predictive factor are also interesting, but not related to the original objectives. In the absence of information regarding the stated goals of the other funding sources listed on each of the published papers, it is not easy to identify how funds from the Pennsylvania Department of Health expedited progress on these projects.

Recommendations:

1. New studies would be needed to identify and characterize interactions between EGFR/Her2 signaling and the prolactin/prolactin receptor/Stat5a axis.
2. Characterize whether EGFR signaling and ERK1/2, Jnk or Akt kinase hyper-activation correlate with Stat5a inactivation.
3. Characterize the role of Stat5a activation in restoring ER α expression in ER α -negative/Her2-positive tumors in vitro and in vivo.
4. Characterize the role of IGF-1 in regulating the PRLR-Stat5 axis, examine Her2, Her3, Her4 expression in human breast cancer patients with inactive Stat5.
5. Characterize the role of Stat5 activation in regulating breast cancer function in 3D culture systems.

Generic Recommendations for the Thomas Jefferson University

Reviewer 2:

Additional sustained funding to support students and/or post-doctoral fellows would allow for additional data collection and young investigator training.

Reviewer 3:

Require the applicant to either execute the stated projects or provide a timely rationale for the marked changes in goals and approaches. Apparently, grantees need to be evaluated on a yearly basis by external reviewers.

Project Number: 0865203
Project Title: Stat5 and ErbB2 in Prostate Cancer
Investigator: Nevalainen, Marja

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:

Overall, the project met its goals.

Looking at the milestones, these were met:

- Determine whether ErbB2 activates Stat5a/b in a set of human prostate cancer cell lines and clinical prostate cancers.
- Determine whether ErbB2 inhibition leads to apoptosis of human prostate cancer cells and decreases prostate xenograft tumor growth through inhibition of Stat5a/b.
- Determine whether active Stat5a/b combined with phosphorylated ErbB2 provides a better prognostic indicator of poor clinical outcome than active Stat5a/b alone.

Together these met milestones cover the core issues - Is Erb2 and Stat5 activation a guide to outcome, can they be causally linked, and will the suppression of this pathway lead to a cell death? The data offered in support of their completion are not very extensive, but they do address the central issues.

However, these two milestones do not appear to have been addressed by the data offered:

- Test whether Stat5a/b activation determines whether primary clinical prostate cancer responds to ErbB2-inhibition by growth suppression and cell death using ex vivo organ cultures of clinical prostate cancers as the testing system.
- Determine whether Stat5a/b is potentially the mediator of the synergetic interaction between ErbB2 and AR in prostate cancer cells.

It is not clear why these questions were not addressed experimentally, or if they were, why the results were not available for presentation.

Reviewer 2:

The project did meet the stated objectives that were to illuminate the role of Erb2 and Stat5 signaling in prostate cancer.

Strengths include the research design, which for the first time systemically examined the Erb2 partners in a variety of human prostate cancer cell lines, and also examined the downstream signaling events leading to Stat5 activation. Moreover, the ability to link the function of Stat5 activation to prostate cancer viability was also examined and found to be an important determinant of cell growth. Indeed, blocking Stat5 resulted in prostate cancer cell apoptosis. Importantly, the PI was able to demonstrate that active Stat5 signaling is associated with high-grade prostate cancer and has negative impact on overall survival.

Sufficient data was supplied to support the goals. There were no weaknesses observed.

Reviewer 3:

Strengths: The project clearly managed to keep pace with proposed statement of work and produced data that matched the stated goals of the proposed research plan. The acquired data supported the PI's hypothesis and served as the basis for subsequent grant submissions to the NIH. The acquired data were sufficient to meet the goals and expectations of the proposed research. The research proposed for all three aims was addressed and data acquired for all aims. Overall, given the scope of the proposed research, the data acquired were sufficiently complete to allow for appropriate conclusions to be made for most aspects of the project.

Weaknesses: There was a significant departure from the proposed research with regards to the number of cell lines to be used. The initial proposal stated 5 cell lines would be examined while the data produced show only three maximally for any given experiment. No explanation was made. This is considered a minor weakness as the case could be made that three cell lines were adequate. That said, subsequent figures focus on results from CWR22Rv cells without justification in any project update. Considering the focus on erbB1 and erbB2 interactions, using CWR22Rv cells that have the lowest erbB1 expression seems odd. Finally, the original application stated that several (3) EGFR (erbB1) ligands would be tested. Data were presented only for EGF. No explanation was provided for omission. Finally, all immunoprecipitations (IP) were performed with erbB2 to look for binding partners. However, the data clearly indicate another binding partner for EGF induction, especially in CWR22Rv. IP with other erbB family members followed by erbB2 immunoblot may have revealed additional binding partners.

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?

COMMENTS ON STRENGTHS AND WEAKNESSES

Reviewer 1:

The key finding is that EGFR/ErbB2 signaling activates STAT5, and that the activation state of this pathway appears to correlate with outcome.

Strength: This finding is of value, in confirming that this pathway is clinically relevant in prostate cancer, and has prognostic significance.

Weakness: The project does not advance knowledge very far. The Her -Stat pathway has been described in many systems. Translation of the finding to the clinic relies on identifying a good agent, and that has not occurred to date. Furthermore, many markers correlate with outcome without lending themselves either to being useful in clinical management or as the basis for therapy development. Neither of these possibilities was tested in the work shown.

Productivity was also not very high given the number of people working on the research, and the time and funds invested. A paper has yet to be published.

Reviewer 2:

Strengths: The significance of this project was high. Although inhibitors of Erb2 have been tested in prostate cancer patients with little success, it is conceivable that Erb2 was only driving tumorigenesis in a handful of the patients treated. The PI therefore proposes the use of active, phosphorylated Stat5b as a surrogate marker for Erb signaling in prostate cancer in order to stratify patients to receive anti-Erb-based therapies. The potential value for this research to improve health is high, given that there are FDA approved drugs that block Erb signaling. The future plans for the research include the development of Stat5b and Erb2 as makers to stratify patients to receive anti-Erb based therapies.

One weakness centers on the lack of proof-of-principle experiments using xenograft models to determine the effect of Erb2 blockade in quelling prostate cancer growth in the setting of a complex tumor rather than in cell culture models.

Reviewer 3:

Strengths: The project has identified that strong stat5 nuclear staining, when used in conjunction with erbB2 staining in prostate cancer specimens, portends faster progression of the disease as determined by time to biochemical failure. That said, stat5 staining alone was better than any erbB2 staining at predicting relapse, although the time required to see this difference was much longer. The data supported the possibility that stratifying patients by stat5 and erbB2 might provide a clinical trial that has a higher likelihood for success with anti-erbB2 drugs. The project produced sufficient preliminary data to apply for and receive an NIH R01 research award. From an infrastructure perspective this is more than 3X the research dollars received from direct costs on this award mechanism. Further, indirect costs will benefit the PI's home institution to at least double the investment made by this award mechanism. The project is now NIH-funded to proceed.

Weaknesses: To date there are no univariate or multivariate immunohistological tests used to stratify patients for treatment of prostate cancer despite numerous attempts to develop such tests. Veltri et al, in particular, demonstrated several markers that assisted in erbB2 stratification of patient survival. Statistical associations of Stat5/erbB2 in disease progression and survival were not as strong as one would hope.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

The PI indicates that an NIH grant was obtained. She does have an R01 active in this area (STAT5 in Prostate Cancer) but this grant started in 2005 (although it was renewed in 2013): http://projectreporter.nih.gov/project_info_details.cfm?aid=8460816&icde=18056028
It appears that the funds provided by the PA mechanism were used for the renewal, which is not a leverage opportunity. R01 projects are in general self-sustaining. Therefore, I evaluate that no leveraging occurred. The PI does indicate the intent to apply for additional NIH funds in the future.

Reviewer 2:

Strengths: Funds were leveraged to renew an NIH grant from the NCI. In this difficult funding environment, this is a testament to the PI's impact in this field. The PI also plans an NIH application on the related topic of Erb2-Stat5b in prostate cancer metastasis.

Weaknesses: It was unclear why the PI did not try to submit an application to the DOD prostate cancer research fund.

Reviewer 3:

Strengths: An NIH application was submitted and awarded. Therefore, the role of stat5 and erbB2 in prostate cancer will continue to be examined for the mechanistic details; in particular, cross talk and synergy with AR pathways and developing better erbB2 antagonists.

Weaknesses: None.

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 papers were published, although one is planned. No patents or licenses emerged. This lack of productivity is a weakness.

Reviewer 2:

Strengths: A manuscript is listed that will be submitted after the grant has been completed.

Weaknesses: There are several published papers on the topic of this grant that could have acknowledged funding from Pennsylvania public health department but did not. It is not clear if this was an oversight by the PI or if the papers published were not germane to this grant in the eyes of the PI.

Reviewer 3:

Strengths: At the time of submission of the final report there were plans to submit a publication. No commercial development opportunities were considered a part of this research project.

Weaknesses: No information was provided on the journal of submission for the paper.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

The project did support research, and so helped support the work of the PI's lab and the institution. However, no remarkable or unique enhancements were noted.

Reviewer 2:

Strength: The funds enabled training of a pre-doctoral student and post-doctoral researcher.

Weakness: The funds were not used to launch any new collaborations with researchers at Jefferson and this was a missed opportunity.

It is also unclear from the research project expenses how the money was used to fund personnel. For example there is 100% support listed for Ellsworth (research tech A) for year 2 with an associated cost of only \$14,308? This seems low for a full time technician salary, unless this is only part time, but then it should not be listed as 100%.

Reviewer 3:

Strengths: The research dollars were used to fund both a post-doctoral fellow and a graduate student as well as two technical slots although not all concurrently. Therefore, four lab personnel were employed in this project at various times throughout the grant. However, the project contributed significantly to the training mission of TJU. By adding two positions to the lab, this project was advanced significantly. This will be increased through recently acquired NIH funding. Infrastructure improvements at TJU will come through indirect costs associated with the awarded R01 on this research topic as a consequence of this funding mechanism and preliminary data generated.

Weaknesses: It is unclear whether or not employees were acquired from out of state or from other institutions.

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 funds supported ongoing collaborations with laboratories in Finland, where the PI trained.

Reviewer 2:

The funds did foster collaborations with Drs. Mirtti and Bergh of the University of Finland and Sweden, respectively.

Reviewer 3:

Strengths: Three outside collaborations were formed and internal collaborations with pathology were enhanced. Clearly, the investigator is excited about these collaborations.

Weaknesses: The role/nature of the collaborations established is poorly described particularly how they tie into the current project.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

1. The main weakness regarding progress is that work did not progress as far as it could have, given the resources and time provided. The researchers did address 3 of their 5 stated milestone goals, and so did examine the questions they set out to answer. However, they were not able to make sufficient progress to publish a paper and to get a grant. It is unclear from the progress report whether they encountered technical difficulties or had personnel issues or what the reasons were.

Recommendation: Require the PI to explicitly address any problems encountered in the pursuit of their work to allow more comprehensive assessment of progress.

2. It appears that this project was closely related to an existing (since 2005) R01 that the PI had. I don't know whether overlap was considered during the initial award of the funds, but I would recommend a close look at how this is addressed. The abstract from 2006 in the NIH database includes the following text, which suggests some overlap at least: "...three specific aims: 1: Determine upstream mechanisms of constitutive Stat5a and/or 5b activation in human prostate cancer. 2: Determine whether active Stat5a and/or 5b inhibits homotypic adhesion of human prostate cancer cells and stimulates heterotypic adhesion, motility and invasion of prostate cancer cells, in vitro and in vivo. 3: Determine the prognostic values of Stat5a and Stat5b in human prostate cancer with disease recurrence as endpoint. "
http://projectreporter.nih.gov/project_info_description.cfm?aid=7100589&icde=18066748

Recommendation: Request a statement from the PI about overlap of funding at the time of application.

3. The scope of the work proposed and performed is not very exploratory. In essence the PI proposed to examine specific signaling pathways, rather narrowly, confirming what is known elsewhere. The opportunity for new insights and the creation of new areas of investigation is therefore quite limited. This also negatively impacts leveraging opportunities.

Recommendation: Allow/encourage hypothesis generating research, including -omics investigations, which can lead to many new insights and hypothesis-driven investigations, and provide opportunities for leverage.

Reviewer 2:

1. It would seem important to develop a pre-clinical model of Erb2 dependent prostate tumorigenesis to obtain proof of principle that anti-Erb2 therapy can show efficacy in vivo in prostate cancer driven by the Erb-Stat5 axis.
2. Developing a set of validated antibodies to monitor Erb2 and Stat5b expression and activation state would be required to translate this work into the clinic. As it turns out, the approaches for validating these antibodies were not well articulated. I would suggest going to the human protein atlas web site and looking over their stringent criteria for antibody validation and selection in future applications. Overall, the strengths outweigh the weaknesses.

Reviewer 3:

None.

Project Number: 0865204
Project Title: Targeting the IGF-1 Receptor in Cancer
Investigator: Baserga, Renato

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 some of the proposed milestones, which were largely focused on studying the acetylation of IRS-1 and the effects of this on its biology, but was not able to go as far into this aspect of the regulation of IRS-1 as anticipated.

However, several other areas were investigated, including a strong focus on the role of miRNA145, the interplay with other signaling pathways such as c-Met, etc., suggesting that the PI adjusted the goals as the work proceeded, which is standard and good practice in science. The milestone to publish the studies completed was not accomplished.

Since no papers resulted, and no primary data is provided it is hard to judge exactly how much was accomplished. The progress reports describe advances in a fairly broad and general way.

Reviewer 2:

Two specific aims were indicated: the first focused on IRS-1 acetylation and its effects on IRS-1 function and the second on the role of IRS-1 in oncogenesis and its relationship to the EGFR. The first aim was related to colon cancer and the second to breast and lung cancer.

The report did not identify how the material presented related to the specific aims nor were detailed results presented. There were no tables or figures or actual data presented in the text. Some proteins (oncogenes, etc.) were mentioned but specific cell lines used were not indicated.

Reviewer 3:

The overall objective of this proposal was to define the mechanisms leading to resistance to antibodies that target the IGF-1 receptor (IGF-1R) in order to develop measures that enable the maintenance of cancer cell sensitivity to anti-IGF-1R antibody therapy. Two specific aims were designed in order to attain this goal. Aim 1 will examine the acetylation of insulin receptor substrate 1 (IRS-1), sites of acetylation of IRS-1 and the role of this acetylation on nuclear transport of IRS-1 and its influence on transcriptional events. Aim 2 will examine the role of IRS-1 in mediating resistance to antibody therapy, using EGFR targeting as a model.

Strengths: This project confirmed that IRS-1 is acetylated.

In order to demonstrate low levels of IRS-1 are responsible for invasive/metastatic signaling, the PI developed methods for silencing its expression in cell lines. To that end, the suppressor microRNA, miR-145 was employed, based on its known ability to down regulate both the IGF-1R and IRS-1.

A common mechanism leading to acquired resistance to antibodies targeting receptor tyrosine kinases (RTKs) or to small molecule tyrosine kinase inhibitors (TKIs) is the activation of compensatory signaling pathways. In that context, the MET receptor is often up-regulated and takes over for the inhibited RTK. The PI demonstrated that IRS-1 and MET interact with one another and are inversely regulated; overexpression of IRS-1 causes MET down-regulation and when MET is overexpressed, IRS-1 is down-regulated. This is consistent with the concept that elevated IRS-1 is responsible for growth and survival signaling in cancer cells while low levels of IRS-1 are seen in metastatic cells. This decrease in IRS-1 is expected to coincide with elevation of MET and its regulation of cell invasion/metastasis.

In a collaborative study, the PI described the paradoxical finding that IGF-1 treatment increases DACH1 expression. DACH1 is a known tumor suppressor, whereas IGF-1R signaling is typically growth promoting. However, in cells stimulated with IGF-1 DACH1 had no demonstrable suppressor activity; its typical suppressor activity is to down-regulate EGFRs and cyclin D1 levels. This led to the publication of 1 manuscript.

Weaknesses: The PI was unable to determine the specific residues in IRS-1 that become acetylated. A considerable amount of work was proposed regarding acetylated IRS-1 including its role in nuclear targeting of IRS-1 and its binding to other proteins and its effect on ubiquitination of IRS-1. However, no additional analysis of IRS-1 acetylation or its actions on IGF-1R targeting antibody sensitivity/desensitization were described.

The use of miRNA is a suboptimal choice when one wishes to conclude that its effects are specific to a single target, given that miRs typically have multiple targets. Indeed, miR-145 has multiple targets besides the IGF-1R and IRS-1 whose silencing could cause the same effects observed. Moreover the PI describes a miR-145-resistant IGF-1R that could not rescue colon cancer cells while a resistant IRS-1 was capable of rescue. These results are complicated by the variety of signaling proteins affected by miR-145.

A number of studies proposed in the original award were not detailed in any of the progress reports submitted. Some of the findings reported represent studies that represent changes to the original aims of the proposal.

There were no data figures provided in any of the progress reports. Based on the final progress report, there was little evidence of substantive progress. This was not discussed by the PI making it difficult to know whether studies were conducted and no publishable findings were made or if the studies were not conducted.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

It is hard to evaluate the concrete benefit of the work completed, beyond enriching our knowledge. There is a clinical connection in that antibodies to IGF-1 are being used clinically, and so understanding the biology of associated signaling pathways may well be important in ensuring their best use. However, this is not very clearly articulated in the progress reports.

Reviewer 2:

The project could improve health with respect to cancer treatment. The goals are to understand aspects of treatment resistance, which can be a serious problem. However, the lack of details presented and the fact that there are no publications make it difficult to determine how the results obtained will impact clinical practice in the future. No plans for the future are presented.

Reviewer 3:

Strengths: The hypothesis being tested is novel and has strong potential to lead to the development of new therapeutic targets for the treatment of breast cancer as well as other cancers driven by IGF-1 receptor (IGF-1R) signaling.

The development of acquired resistance is a significant therapeutic problem. This grant seeks ways to re-sensitize tumor cells to the effects of antibodies targeting the IGF-1R.

Weaknesses: While there was modest progress during the award period, the work done has provided some leads that could be followed up on in the future.

There do not appear to be plans for further work on this project.

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 funds were leveraged. The PI retired.

Reviewer 2:

There was no leveraging of funds nor are there any plans to pursue additional funding as the PI is retiring.

Reviewer 3:

There were no additional sources of funding reported. There are no plans for future funding 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:

No peer-reviewed publications are listed in Item 20 of the final report. The PI is a prolific author with over 400 papers published, making this somewhat surprising. It is a significant weakness, and leaves one wondering what was accomplished specifically with the PA funds, given the large number of areas addressed in a general way in the progress report but the lack of even a single paper related to these funds. Given the significant support and time, at least one publication would be reasonable.

Reviewer 2:

No publications were listed as either already published, in press or submitted (weakness). In Item 17 of the final report it is indicated that publications are planned for the future.

I did find one publication from June 2011, which appears to be related to Specific Aim 2.

Reviewer 3:

Four manuscripts and one review article appear to be related to the work accomplished on this proposal. If they resulted from this project, these represent solid publications in mid-tier scientific journals.

There are no plans for future publications, patents, licensing or commercial development.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

It is hard to evaluate, but from what is provided no specific enhancement beyond providing support for one or two scientists for 3 years is evident. No specific resource or capability was generated by this investment.

Reviewer 2:

No infrastructure related activities resulted from this grant. No new investigators were brought to the institution.

Fifty percent of a post-doctoral fellow was included in years 1 and 2 (strength).

Reviewer 3:

There were no reported improvements to the grantee institution, no new investigators were recruited to work on this project and no pre-doctoral students were supported on 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:

No new collaborations appear attributable to this specific grant support.

Reviewer 2:

There did not appear to be any outside collaboration during the undertaking of the project. Since the PI is retiring, it does not appear that there are plans on any future collaborations. Overall, this would be considered weaknesses.

Reviewer 3:

While there was collaboration with the Pestell laboratory at the same institution, there were no collaborations with investigators outside of the PI's institution.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

1. This project's accomplishments are marginally favorable. While the intended area of investigation was apparently well-explored, the specific milestones put forward were incompletely explored. Significantly, no publications were cited in the final report and plans for transfer of the work to another PI and the seeking of further funding are not evident. Therefore, the goals of the investment, to enhance research and support the creation of future projects, were not fulfilled.

Recommendation: Specify whether the work of the retiring PI will be carried on by someone else.

2. It was difficult to assess the progress made, as the progress report consisted of very general statements of findings made, and insights gathered. There was little cohesion or connection between different parts of the final report, which was created by combining elements from prior reports. No data were shown, or experiments described.

Recommendation: The PI should more concretely describe the experiments/studies performed and the findings obtained, since this cannot be seen in a published paper or even a submitted manuscript.

Reviewer 2:

1. No actual data were presented in the report making it difficult to evaluate how the results obtained help in reaching the original objectives. Presentation of tables and/or figures summarizing findings would strengthen the report.

2. No publications were listed. Some were to be submitted and more information on this would be helpful. I did find publications mentioned in one of the earlier annual reports, however. If results are not published then it will clearly not have any impact on future clinical applications of the alleged findings.
3. In the final report, there is no indication of how results obtained relate to previous work by the investigators and others. No citations are included.

Reviewer 3:

1. The determination of specific sites of acetylation should be discontinued. These are difficult analyses and require a mass spectrometry expert spending full time on the project. It might be useful in the future to combine this analysis with determination of additional post-translational modifications, including ubiquitination, phosphorylation (Ser/Thr/Tyr) and O-GlcNacylation. The miR-145 studies represent an area for future investigations in order to determine which targets are being affected besides IGF-1R and IRS-1. The studies on DACH1, which were not part of the proposed studies, may also be an area worthy of future examination.
2. As indicated above, although the overall progress on this project was modest, some key areas of future studies have been developed. These could be developed by one of the co-investigators on the project.
3. Because there were no additional sources of funding reported or plans for future funding of this project, it is difficult to make a recommendation. Overall, the project objective is of significant interest, such that one of the co-investigators may be interested in pursuing this line of research.
4. In the final report, there were no plans for publishing any of the findings from this study in the future. If there are still findings that have not been published, I strongly encourage the PI/co-investigators to submit these data for publication.
5. Given that this project appears to have been terminated, there are no recommendations. If one of the co-investigators is still at the grantee institution, they would be the likely individual to continue this work in the future.

Generic Recommendations for Thomas Jefferson University

Reviewer 3:

It would appear from the progress reports submitted over the years, that progress was slow on this project. It may be useful to intercede at an earlier point to determine whether there are problems with personnel, etc., in order to determine whether funding should be terminated prior to the end of the funding cycle.

Project Number: 0865205
Project Title: The Role of MicroRNA (miRNA) Gene Expression in
Therapy Resistance of Human Breast Cancer
Investigator: Pestell, Richard

Section A. Project Evaluation Criteria

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

This project's stated objectives were to interrogate the roles of miRNAs in breast cancer invasion and metastasis. Data presented in the form of progress reports suggest that significant progress was made to identify key miRNA drivers that regulate breast cancer. Additionally, data demonstrate a key role of cyclin D1a in regulating dicer.

A second goal of this project is to communicate the results in the form of peer-reviewed publications. In this regard, the project did not achieve its goals. Only one peer-reviewed research report was published. All other publications associated with this project are in the form of reviews. There are several manuscripts listed as in preparation or submitted, but these have not been accepted for publication.

Reviewer 2:

The authors present a comprehensive and clear review of their research progress. The project has done an outstanding job of meeting the aims of the proposal. Towards Aim 1, the authors have published a strong paper in the high-impact journal PNAS, which details their findings on the specific miRNA 17/20 and its role on the tumor microenvironment, a novel and particularly important area of study. For Aim 2, the authors describe very exciting results which are being submitted for publication identifying a miRNA based clinical signature of breast cancer which hold significant prognostic promise. They have also taken their findings from Aim 1 and translated these with an important application in therapeutic response. The results of their work towards Aim 3 are also highly impactful, as they have identified a novel mechanism for Cyclin D1a in controlling the Dicer enzyme, which is responsible for miRNA processing. This fundamental work also holds particularly impactful clinical promise, and a manuscript regarding these results is currently under review.

The research methods and protocols used were reasonable, and as they made use of commercially-available reagents and systems, can be replicated, which is extremely desirable. They have made good use of model systems (both cell lines and murine) as well as human studies to examine their hypotheses, and this has aided their potential significance.

Reviewer 3:

Strengths: The project met its main objectives over the course of the funding period. The study investigators were able to determine that miRNA 17/20 inhibits cellular invasion and metastasis in breast cancer, suggest that the cyclin D1 regulated miRNA 17/20 is important in tamoxifen and chemotherapy induced apoptosis, and identify a mechanism by which Cyclin D1 is involved in breast cancer stem cell expansion.

Weaknesses: None.

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 impact of these studies will likely be modest. There is a plethora of data on the roles of miRNAs in cancer, and specifically in breast cancer. None of these studies have resulted in improved patient outcomes. These studies will help to understand further the complexities in breast tumorigenesis but they will not likely be translatable directly into a clinical outcome.

Reviewer 2:

Many of their findings are highly relevant to human breast cancer biology, treatment, and prognosis, and it is certainly possible that their findings regarding miRNA profiles and treatment response differences could be applied clinically. In addition, the secreted factors they have identified hold therapeutic promise. I would encourage the researchers to seek patent protection of their work and to work towards commercialization, so that these findings can become incorporated in clinical practice.

Reviewer 3:

Strengths: The overarching goal of this project is to identify new targets for breast cancer treatment by understanding the molecular mechanisms underlying tumor progression and resistance to current breast cancer therapies. The investigators made significant progress towards their stated aims.

Weaknesses: The project was very basic in its approach, with no clear plan of how it will be moved forward in the translational or clinical breast cancer setting.

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 occurred. The PI submitted an R01 application to the NIH but this was not funded. The PI plans to resubmit.

Reviewer 2:

The authors have utilized the data obtained from this project to apply for additional NIH funding to continue this line of research. A revised proposal is being prepared.

Reviewer 3:

Strengths: The PI did apply for an R01 based on this work. It was not funded; however, this suggests a clear plan to continue along this line of investigation.

Weaknesses: None.

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 was a good, solid peer-reviewed publication (research) in PNAS and several reviews in modest journals. Four manuscripts were submitted or planned.

Reviewer 2:

The authors have published or are preparing for publication a number of research publications of high quality. Their published work is found in high-impact journals, a testament to the work's quality.

Reviewer 3:

Strengths: The funding to date has led to a high-impact original research publication (PNAS) and 5 review articles. The PI and his co-investigators were highly productive and have several additional original research publications under review or in press.

Weaknesses: None.

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 obvious improvements to infrastructure. This project supported junior faculty. It appears that several were brought in specifically for this project. In this sense, the award significantly enhanced the research effort at the institution. No pre-docs were supported.

Reviewer 2:

The funding has enhanced the capabilities of the researcher's specific laboratory to recruit and train students and post-doctoral fellows, which should be commended. The finding did not alter the overall infrastructure of the institution, but that does not appear to have been the intent.

Reviewer 3:

Strengths: The funds provided were used to support many post-doctoral fellows and junior faculty members, which enabled these young investigators the opportunity to conduct high quality research under the mentorship of the PI. This funding was used to significantly enhance the capacity of research at the PI's institution in regards to the career development of these junior investigators.

Weaknesses: None.

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 collaboration was reported.

Reviewer 2:

The researchers have done a good job of communicating their research through presentations at national and international meetings and it appears that collaborations are being pursued.

Reviewer 3:

Strengths: The project led to the recruitment of 8 researchers from outside of the PI's institution. These researchers came from 4 different countries.

Weaknesses: It does not appear that this project led to outside collaborations directly; however, the PI was invited to present this research at many different venues, so it could possibly lead to additional research collaborations down the road.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

Publication record is low. Recommendation: Publish.

Reviewer 2:

None.

Reviewer 3:

None.

Project Number: 0865206
Project Title: Mechanisms for Metastasis Suppression through
Kisspeptin Regulation of the Microenvironment
Investigator: Peiper, Stephen

Section A. Project Evaluation Criteria

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

This project set out to identify the cell types mediating the antimetastatic effects of Kisspeptin. Previous work had shown specific antimetastatic properties.

In wound healing assays, the investigators were not able to show any beneficial effects of natural or synthetic Kiss peptides. This led the investigators to revisit the original assays used to identify kisspeptin as antimetastatic. In a very elegant and careful study, the investigators identified artifacts due to the previous experimental design. These studies were published in 2012.

Because of the clinical association with Kiss1 and GPR54 on beneficial outcome in ovarian cancer, the investigators set up an ovarian model. No data on the role of Kiss peptides in ovarian metastasis are presented. These are all strong, well-developed and executed studies.

A weakness is the seemingly unrelated studies on IL8 and ROS. No direct connection was made with these studies and the proposed studies and goals.

Another weakness is the slow progress with ovarian cells. It appears that almost nothing was done on Kiss in the last year of funding.

Reviewer 2:

Strengths: The PI has pursued a number of experimental directions and has published.

Weaknesses: The hypothesis for this proposal was based on weak preliminary data. The PI performed a number of experiments which ultimately demonstrated that the hypothesis was incorrect and that the experimental models were unreliable. The methods that were proposed were unlikely to have succeeded given the complexity of the experimental models.

Reviewer 3:

The objectives of this proposal were to measure the impact of KISS1 on the physiopathological process investigated, detect the presence of GPR54 in the remodeled tissue, and identify the cells expressing GPR54. The applicant's findings show that the in vivo xenograft model that they

chose to use was actually flawed and thus did not work for them in the desired setting of melanoma. They spent far too much time and space showing that they could validate the reagents that others have used. No need to show the standard curve and melting curve for q-PCR four times in the write-up. Nearly half of the figures do not contain a legend, making it extremely difficult to judge what the experiment is detailing. Statistics are completely missing from numerous figures, making it impossible to determine significance.

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 likely beneficial impact is to report the artifacts associated with the melanoma model that was used to identify Kiss1 and GPR54. This is extremely important so that other investigators do not follow a dead end path.

It is unlikely that the results of this project will have any beneficial impact on human health.

Reviewer 2:

The impact is minimal as most of the data was negative. The PI did publish a paper so that others will not waste time pursuing this line of experimentation.

Reviewer 3:

The significance of the project is limited since the melanoma model did not work as published. The work in the ovarian system is promising though. It looks like much work could be done to shore this up and move it forward in the future. It is not stated by the applicants what direction they would take this work though, especially since they do not indicate that they will be submitting for long-term funding. However, a single grant is listed as applied for, although not funded.

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 leveraged funds were promised and none were used. The studies with ovarian cells are incomplete; the PI states that if positive results are obtained then an R01 would be submitted. Given the lack of any positive data, at this point it seems highly unlikely that anything fundable will come of this work.

Reviewer 2:

The PI has not received additional funding.

Reviewer 3:

No other funds were used and a single grant has been applied for but not yet funded.

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:

One publication describing the artifacts associated with the melanoma model resulted from that work. No other publications are planned.

Reviewer 2:

The PI published one manuscript. Although this is good, the overall research plan was weak and the investment quite high for this level of productivity.

Reviewer 3:

A single paper was published in July 2011 in the specialized mid-tier journal, Melanoma Research. This publication is largely based in negative data showing that KISS1 is not a metastatic suppressor in the melanoma model. The ovarian data has not been written up or submitted for peer review. This is very low productivity for a three-year grant at a high level of funding.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

The project supported multiple investigators who were either technicians or junior faculty. No pre- or post-docs were supported.

Reviewer 2:

No.

Reviewer 3:

No improvements to the infrastructure at the institute and no pre-doctoral students listed. No post-doctoral students listed either. Funding provided for two assistant professors, one professor, and one research assistant II.

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.

Reviewer 2:

The PI had several collaborators.

Reviewer 3:

This work did not lead to any collaborations inside or outside of the institute.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

There was low productivity on the ovarian model and no test of any of the central hypotheses in this model. It was clear after year 1 that the central hypothesis was in jeopardy of being disproven. The PI should have refocused sooner.

Reviewer 2:

1. The hypothesis for this proposal was based on weak preliminary data.
2. The PI performed a number of experiments which ultimately demonstrated that the hypothesis was incorrect and that the experimental models were unreliable.
3. The methods that were proposed were unlikely to have succeeded given the complexity of the experimental models.
4. The experiments that were performed to study another problem were not convincing and perhaps also unjustified.

Reviewer 3:

1. No long-term funding achieved. The PI should submit more external long-term grants to the NIIH, Department of Defense, etc., based on preliminary findings.
2. No pre-doctoral or post-doctoral students were trained. The training of students should be a priority for this research and funding mechanism.
3. There was only a single publication peer reviewed over three years ago in a mid-tier specialized journal. These data presented largely negative findings regarding the role of KISS1 as a metastatic suppressor. The ovarian data should be published to strengthen the argument that KISS1 has a functional role in this process.

4. Many figures completely lack a legend other than the title and many are missing statistics. These must be included in any future write-ups.

Generic Recommendations for Thomas Jefferson University

Reviewer 2:

The decision to include this project in funding was questionable even before the experiments were performed. This grant was based on weak evidence and proposed an ambitious set of experiments using complicated techniques. Although the PI showed that the basic premise was flawed, the number and types of experiments did not merit this level of funding.