

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 (1.83)

Project Rating:

Project	Title	Average Score
1087901	Isoform Specific p73 Regulatory Networks in Neurogenesis	Favorable (2.33)
1087902	SECTM1 is a Novel Mediator of Melanoma Tumorigenesis and Progression	Favorable (2.00)
1087903	Regulation of EBV Infection and Latency by Editing of Viral MicroRNAs	Favorable (1.67)
1087904	Laboratory Renovation Research Infrastructure	Outstanding (1.33)

Project Number: 1087901
Project Title: Isoform Specific p73 Regulatory Networks in Neurogenesis
Investigator: Davuluri, Ramana

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 partially met the stated objectives. The research was well designed for the project objectives.

Strengths: ChIP-seq assays were performed on undifferentiated cells and RNA-seq was performed on undifferentiated and aggregated cells. Select genes were tested in p73 isoform knockdown cells with quantitative polymerase chain reaction (qPCR). Basic bioinformatic analysis was performed on the results, including peak calling and binding counts for the p73 isoforms. Interesting differential patterns were documented.

Weaknesses: No assays appear to have been finished on differentiated cells at seven days. Genome-wide assays were not completed for p73 isoform knockdown cells. The bioinformatics are primarily limited to data processing, rather than analysis of results. There does not appear to be clear progress on the development of computational models. More data collection and analysis is necessary to determine if the original research question has been adequately addressed.

Reviewer 2:

The PI presented two specific objectives: 1) to conduct ChIP-seq for TP53, TAp73 and DNp73 and mRNA-seq experiments in differentiated cells at day 0, 4, and 7 after treating the P19 cells with retinoic acid, in a) wild-type P19 cells, b) TAp73-knock down P19 cells, and c) DNp73-knock down P19 neuronal cells; and, 2) to develop novel computational methods to model the combinatorial interaction of p73 isoforms (TAp73 and DNp73) with p53 family members and other TFs and decipher isoform regulatory networks of each isoform in controlling the expression of target genes during neuronal differentiation, under normal and perturbed cellular conditions.

The PI met the first objective by carrying out the stated experiments, and the experimental design seemed appropriate. There were not any significant changes to the original research protocol. However, the project did not meet the second specific aim objectives in that the PI did not conduct any novel tool development. In the final progress report the only mention of the “novel computational methods” is, “Using an integrative approach, that involves analysis of the ChIP-seq data from day0 P19 cells, mRNA-seq data from 8day 0 and specified day 4 P19 cells,

identification of p73 binding sites and their neighboring TFBS, we predicted several TF partners that included YY1 and AP1 proteins.” This does not match the strategic plan in which the PI indicates that they “will apply innovative statistical modeling approaches that combine state-of-the-art meta-classification algorithms, such as Naïve Bayes Tree, Logistic Model Tree, Bagging and LogitBoost, with Random Forest feature selection to classify different types of target promoters with good classification accuracy and reduced instability, in order to infer the cis-regulatory modules (CRMs) that can explain target genes of one isoform versus the other.” Furthermore, given the large number of candidates that show TP53, Tap73, and DNp73 binding via ChIP-seq there is not a great amount of detail showing how the PI honed in on the handful of candidates that were experimentally validated. Therefore, it is hard to take a global systems biology view of the data since the results focus on a small subset of genes.

Reviewer 3:

Progress in meeting stated objectives was modest.

Aim 1: The data shown are not described well enough to discover if the results are believable. If there is only one replicate at each time point, then the apparent smooth trajectories could just be the result of dividing by a set of initial measurements. In that case, there is no real support in the data for the assertion that the two isoforms of p73 have opposite trajectories in neurogenesis. Also, the P19 cell line may not generate typical neurogenesis, due to its origin in embryonic carcinoma, so confirmation is required.

Aim 2: If this aim was addressed, there are no details in the report.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

Strengths: The use of alternate isoforms of important proteins like p73 is of central importance in understanding tumorigenesis and cancer progression. Understanding the roles of isoforms TAp73 and DNp73 in development is therefore of interest, and the project is a well-designed study of how these isoforms differentially contribute. Identifying critical “switches,” such as this difference in isoform use, could provide improved understanding of cancer biology and potential therapeutic targets.

Weaknesses: In light of the dollars budgeted, progress on the project has been too limited to assess the long-term impact.

Reviewer 2:

The overall beneficial impact of this proposal is to identify the regulatory network of p73 isoforms under the notion that this will result in targeted therapeutics and personalized treatments. However, none of the predicted targets were tested to be responsive to drug treatment, and this was not conducted in patient samples (or validated across human cohorts); so, the translational impact will not be immediate.

Reviewer 3:

Alternative splicing in cancer is an important area of research, and both aims are relevant to progress in this. While the research is more basic than translational, it has eventual implications for cancer diagnosis, treatment and prognosis.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

Strengths: Data from this project has been used in an NIH R01 grant application that has been granted a very good priority score. Pending the availability of federal funds, the expansion of this research looks promising.

Reviewer 2:

The PI applied for three grants, one of which was not funded; the other two are pending. The PI intends to apply for additional funding.

Reviewer 3:

They have one successful R01 application, "Informatics platform for mammalian gene regulation at isoform-level," which is along the lines of Aim 2, as well as several other applications. They have leveraged the funds well.

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:

Weaknesses: To date, the project has only produced a literature review article. As stated above, the project requires additional experimentation and/or data analysis to achieve publication. However, this is not unusual given the limited time frame and the scale of the proposed experiments.

Reviewer 2:

One review article materialized from this project. There were not any commercial developments.

Reviewer 3:

They have one publication. They do not yet have a publication specifically on isoforms of p73, the nominal subject of the project. This aspect is a bit disappointing.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

Strengths: Resources and expertise for ChIP-seq, RNA-seq, and ChIP-PCR are all vital contributions to the grantee's institution.

Reviewer 2:

The only form of enhancement was that the funding was used to support two post-doctoral fellows.

Reviewer 3:

It appears that sequencing infrastructure has been improved, which could have a substantial positive effect.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

Strengths: The research has led to external collaborations and joint funding applications.

Reviewer 2:

The research led to collaborations with Dr. Donald O'Rourke (director of Pennsylvania Brain Tumor Tissue Bank) and Dr. Vivek Mittal.

Reviewer 3:

There were several collaborations coming from this project:

- 1) With Dr. Donald O'Rourke, director of Pennsylvania Brain Tumor Tissue Bank and neurosurgeon at the University of Pennsylvania. This collaboration has led to submission of an R01 application to NIH.
- 2) With Dr. Vivek Mittal, an internationally recognized cancer researcher and the director of the Lehman Brothers Lung Cancer Laboratory at Weill Cornell Medical College, Cornell University, New York, NY.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

1. The bioinformatics analysis has been very limited to date. Additional analysis of the target genes for the different p73 isoforms could improve the understanding of the roles of these proteins. The current status report lists summary statistics for differential binding events but does not substantially delve into the differential function. Some gene functions are listed, but the methods and significance of these findings are not discussed in any detail. A fuller analysis and specific hypotheses that arise therefrom would greatly improve the significance of the work.
2. There is no discussion of the development of computational models that might explain the cis-regulatory modules and target promoters. This is an interesting problem, and the grantee has designed a study that makes such modeling tractable. The current work is more descriptive than predictive, but developing models would greatly improve potential impact.
3. Although a substantial amount of data has been collected and analyzed, the missing data substantially limits the interpretability of the study. Global data for isoform knockdowns and differentiated cells, as originally proposed, would be helpful.

Reviewer 2:

1. The significant concern is that the PI did not provide detailed information about the computational methods for Specific Aim 2. Develop the computational models specified in Specific Aim 2 to better understand the combinatorial regulation for the different P73 isoforms.
2. Expand the integrative analysis to prioritize candidates for subsequent validation, since it is unclear how candidates are currently chosen.
3. Increase the translational impact by identifying potentially actionable interactions in human disease.

Reviewer 3:

This is a strong researcher with an excellent track record.

1. I recommend that a publication be prepared with specific results on isoforms of p73 using P19 cells with replication, as well as with other cell lines or experimental platforms, to support the assertions of the differential roles of TAp73 and DNp73.
2. I recommend that computational methods developed under this proposal be described in a peer-reviewed publication.

Project Number: 1087902
Project Title: SECTM1 is a Novel Mediator of Melanoma Tumorigenesis and Progression
Investigator: Kaufman, Russel

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 entitled, "SECTM1 is a Novel Mediator of Melanoma Tumorigenesis and Progression," has demonstrated reasonable progress and met most of the objectives stated in the original application. In Specific Aim 1, researchers proposed to assess the significance of SECTM1 protein on MAMs (melanoma associated macrophages) during melanoma progression. Researchers have been able to test the hypothesis in *in vitro* settings and support it by a significant body of work. The comprehensive tissue microarray consisting of primary melanoma, melanocytic nevi and metastases has been analyzed for SECTM1 expression. It was noticed that metastases had higher expression than primary tumor and were characterized by an increase in inflammatory infiltration. From this correlation authors conclude that there is a causal relationship between infiltrating immune cells and SECTM1 production. This observation potentially strengthened the hypothesis. Nevertheless, it has a potential flaw relevant to widespread expression of SECTM1 in many different types of cells including normal monocytes, macrophages and melanoma cells, as well as increase in expression of SECTM1 by T cells. Thus, an increase in SECTM1 staining in metastases and primary melanomas infiltrated by immune cells might be due to infiltration rather than increased expression of SECTM1 by melanoma itself. The source of SECTM1 in outlined experiments, as well as its form, i.e., secreted, cytoplasmic or transmembrane, was not specified.

The authors further demonstrated that SECTM1 was capable of inducing differentiation of monocytes into macrophages. Thus, increased macrophage infiltration at the melanoma site might be due either to SECTM1-induced monocyte differentiation or SECTM1-induced attraction of macrophages and activation of T cells. It was shown that SECTM1 could activate T cell through the CD7 receptor and produce INF-gamma, which in turn induces production of SECTM1. CD7 also is present on monocytes, and the effects of SECTM1 on differentiation of monocytes are CD7-dependent. It was also shown that both classes M1 and M2 macrophages were produced through the SECTM1-induced differentiation. Since M1 and M2 macrophages have opposing effects on melanoma progression, it is unclear how the mixed population nevertheless leads to increase in melanoma cells' invasion and migration. This study proposed the following *in vivo* models to test the hypothesis of SECTM1's role in melanoma progression *in vivo*: 1) 3D skin reconstruction, and 2) B16 mouse melanoma model. Unfortunately both *in vivo* models failed due to unresolved technical problems.

Reviewer 2:

Strengths: Two specific aims were proposed to determine the significance of SECTM1 on melanoma-associated macrophages in the melanoma microenvironment and to determine the effect of SECTM1-induced macrophage in the tumor microenvironment. Most of the stated objectives were met. The overall quality of the data developed and the methods are adequate and appropriate. The project followed a focused direction, and the data and information provided are highly applicable to the project objectives. Explanation was provided for not completing the 3D skin reconstruct and B16 mouse melanoma experiments.

Weakness: There are problems with the rationale for some experiments. For example, what is the rationale for overexpressing SECTM1 in melanoma cells that are already expressing SECTM1? Given the migratory nature of the macrophages and the sensitivity of primary macrophages to *in vitro* culture, the problems with the 3D model may be insurmountable. A knockdown strategy instead of overexpression should be considered for the *in vivo* mouse melanoma experiments.

Reviewer 3:

The project largely met its stated objectives and resulted in three publications (two published and one accepted for publication). It appears to have advanced the state of melanoma research at the PI's center, leading to a new institutional program on tumor microenvironment. The prospect exists for R01 funding to be generated from this research, but a weakness has been the lack of progress with developing a 3D organotypic model for studying tumor-associated macrophages. A murine model involving the B16 melanoma also has thus far failed to work out. If either or both of these models' problems can be overcome, it is likely that the area of research will continue to be fruitful and profitable.

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:

Based on the presence of SECTM1 in the serum of about 50% of tested melanoma patients, it is reasonable to suggest that SECTM1 could be considered a potential prognostic marker of melanoma or other cancers. Further studies need to be performed to gauge the depth of the correlation with particular stage of cancer progression or other biological parameters. Increase in SECTM1 might be directly linked to inflammation and activation of regulatory T cells (T_{regs}) which could have a role in activating the immune system in certain immunodeficiency states. No additional health or diagnostic features relevant to incidence or treatment of the disease or risk factors were developed under this project. The accomplished research has some potential value for improvement in health outcome. Future plans include development of an animal model to test the SECTM1 effects in prevention of cancer growth and metastasis.

Reviewer 2:

Strengths: The aims of this project are to investigate the role of a secreted protein that activates T cell activation and differentiation of monocytes to melanoma-associated macrophages. The project has potential to improve the health of patients with melanoma. It can be rated as highly

significant, specifically in generating new scientific knowledge that in the long term could eventually improve health outcomes of melanoma patients.

Weakness: The likely benefit in terms of how the scientific knowledge will be applied for improving health has not been articulated. The future plans do not include any specific ideas on how the discoveries will be translated to improve diagnosis, prognosis or treatment of melanoma.

Reviewer 3:

The interaction between tumors and their immediate surrounding environment of nonmalignant cells (the microenvironment) is an important and understudied area in cancer biology. Most of the research in this area has focused on tumor-infiltrating lymphocytes; but monocytes and macrophages constitute an important component of the immunologic microenvironment, and the investigators have significantly advanced progress in this area. If they can perfect model systems to study more accurately the interaction between melanomas and the nearby monocytes and macrophages, this could have theoretical and practical implications for our understanding of melanoma biology and could direct the development of local (i.e., intralesional) and systemic therapies aimed at manipulating this microenvironment. Overall, the value of the research was commensurate with the dollars budgeted.

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:

Additional funds were not secured during the current funding period. The researchers plan to submit an R01 application after the additional preliminary data are developed.

Reviewer 2:

Strengths: The preliminary data generated in this project will be used to develop an NIH R01 grant application for submission in 2013 to continue and expand the research.

Weakness: This project was entirely carried out using the Health Research Grant.

Reviewer 3:

The project has not yet leveraged additional funds or submitted any additional grant applications; but the researchers plan to do so if they can develop an appropriate 3D organotypic model. If they can do so, it is considered likely that they will be able to leverage their research to additional funding that will continue or expand their efforts.

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 current project resulted in three peer-reviewed publications. No patent, licenses or commercial development opportunities were filed under the current funding period.

Reviewer 2:

Strengths: This project resulted in three peer-reviewed publications, one each in *Journal of Leukocyte Biology* (JLB), *Pigment Cell & Melanoma Research* (PCMR), and *Frontiers in Biology* (FB). Three publications are a reasonable output for the project, and two of the three papers are in well-respected specialty journals (JLB and PCMR). One other manuscript is planned to be submitted.

No plans, either short-term or long-term, are described for any licenses, patents or future commercial opportunities.

Reviewer 3:

Two publications have been published in peer-reviewed journals of high quality, and one has been accepted for publication. This is considered appropriate and reasonable in light of the amount of funding received. No licenses or patents were applied for and none is considered likely in the immediate or foreseeable future of this research.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

These studies have helped create a perspective on the tumor microenvironment that will be the center of a new program on tumor microenvironment in the PI's cancer center. One researcher, Dr. Yingbin Ge, MD, Department of Physiology, Nanjing Medical University, China, was brought into the institution to help carry out this research. The funds were used to pay for research performed by six students.

Reviewer 2:

Strengths: Two undergraduate students and a master's student participated in the project. A post-graduate researcher from China was recruited and contributed to the project, as evidenced by the primary authorship and co-authorship on published/accepted papers.

Weakness: Based on the results presented, the impact of this project on research capacity and quality is modest, specifically on its potential to nucleate a new program on tumor microenvironment at the cancer center.

Reviewer 3:

According to the grantee, this project is said to have advanced the state of melanoma research at the PI's center, leading to a new institutional program on tumor microenvironment. Multiple junior-level research personnel were paid for using funds from this grant, and one visiting researcher from China was recruited to Pennsylvania to participate in the research using funds provided by this grant.

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 researchers were not planning to begin any collaboration as a result of the research. No new collaboration with research partners outside of the institution or new involvement with the community were established during the current funding period.

Reviewer 2:

Weakness: No collaboration with research partners or any involvement with the community has resulted from this project. No plans to begin any collaborations are described.

Reviewer 3:

No new collaborations are described; but if the planned institutional program on tumor microenvironment comes to fruition, this will certainly lead to new and potentially highly productive collaborations.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

1. Systematic analysis of SECTM1 production (expression in cytoplasm, cell surface, and secreted form) in the panel of up to ten melanoma cell lines is highly recommended in order to establish the general phenomenology of SECTM1 expression in melanoma and establish the localization and secretion pattern. The expression and functionality of CD7 receptor on the surface of melanoma cells need to be shown. It is clear that some melanoma cell lines such as B16 cannot tolerate overexpression of SECTM1, and the potential biology behind this needs to be defined.
2. The project lacks the direct evidence that SECTM1 is able to induce differentiation of monocytes to macrophages. The factors from melanoma-conditioned media (MCM), some of which express SECTM1, were capable of this; but the results regarding SECTM1 alone are

missing. Thus, it is not completely clear if SECTM1 by itself could induce differentiation and what additional factors are required.

3. It was shown that factors from MCM-induced macrophages (MCMi-M) express both M1 and M2 macrophage markers, but microarray analysis revealed that the majority of genes expressed by this mixed population were associated with tumor invasion. Since M1 and M2 have opposing influences on tumor progression this conflict of microarray data and macrophage marker expression needs to be discussed. Additionally, the macrophages produce factors which inhibit melanoma-specific T-cell proliferation, while SECTM1 induces robust proliferation of T cells and IFN-gamma production. These two opposing effects in the melanoma microenvironment also need to be discussed, especially in the prospective attempts to inhibit SECTM1 production during tumor progression.
4. The further development of *in vivo* models is strongly encouraged in order to test applicability of obtained knowledge in clinical practice. This reviewer suggests using NSG immune compromised mice, since they lack functional T, B and NK immune cells and defective macrophages, but have monocytes to test the hypothesis of differentiation of monocytes into functional macrophages through expression of SECTM1 by melanoma. This model could be humanized and populated with selected classes of human immune cells, including macrophages which could be labeled with GFP marker or produced from GFP mice to test and visualize the infiltration of implanted 3D skin reconstruction model of melanoma by injected GFP-macrophages or monocytes. The production of SECTM1 in melanoma cells could be silenced by anti-SECTM1 targeting shRNAs (constitutive or doxycycline inducible) thus allowing researches to gauge the requirement for SECTM1 production in order to get macrophages and monocytes into primary site.
5. Introduce SECTM1 in B16 cells under inducible promoter to allow for fine manipulation in expression levels and opportunity to induce expression after the tumor cells were injected into animals.

Reviewer 2:

1. The overall rationale for the project is weak. If SECTM1 activates T cells and induces macrophage differentiation, it is not clear how it promotes tumor progression. The researchers need to provide a better mechanistic explanation for the observed effects of SECTM1.
2. If SECTM1 is highly expressed in melanoma cell lines and tissue lesions (as shown in Figure 1), the rationale for overexpression of SECTM1 is not clear. The researchers should consider genetic knockdown strategy to investigate the role of SECTM1 on biology of melanoma cells per se.
3. The overall writing and presentation of the project is weak. For example, the experimental design and methods section of the strategic research plan is poorly written with many typographical errors, such as "mass spectrum analysis." The PI's biosketch appears to be incomplete with no list of publications and ongoing grant support, etc. A better proofreading

of the writing will help the reviewer to better understand the researcher's intent and research plans.

Reviewer 3:

The most evident weakness is the failure to date to establish a working 3D organotypic model involving SECTM1 overexpression that is associated with survival of the macrophages. Secondly, a B16 mouse melanoma model also failed due to lack of macrophage survival. At least one and ideally both of these models must be developed into a successful model system for the current research to have a high likelihood of obtaining R01 or equivalent funding. However, nothing is seen that indicates the problems encountered to date are insoluble.

Project Number: 1087903
Project Title: Regulation of EBV Infection and Latency by
Editing of Viral MicroRNAs
Investigator: Nishikura, Kazuko

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 partly met its stated objectives. However, the reported research did not address the goals of Specific Aim 1, which envisioned “overexpressing or knocking down ADAR1,” the key RNA editing enzyme, in EBV-positive BL and NPC cells. The project did largely address the goals of Specific Aim 2 by generating data suggesting that the level of Dicer expression affects EBV latency. However, they did not show that regulation of endogenous Dicer expression by endogenous miR-BART6 was functionally significant, since all experiments involved ectopic overexpression of miR-BART6 or anti-Dicer shRNAs. Therefore, while some progress was made, no clear conclusions can be drawn from this work. This is presumably why this research remains unpublished.

Reviewer 2:

Strength: The grantee conducted a research project entitled, "Regulation of EBV Infection and Latency by Editing of Viral MicroRNAs," which was funded by a 1.5-year Health Research Grant and an NIH grant. The main objective of this project is to investigate the biological role of EBV-encoded microRNA BART6 (ebv-mir-BART6) in EBV's life cycle/pathogenesis as well as the regulation of ebv-mir-BART6 in human cells. Overall, this research project was well designed and conducted and met its stated objectives. During the grant period, the grantee accomplished both of the proposed aims and important conclusions were made: 1) post-transcriptional regulation of BART6 by A-to-I RNA editing has a negative impact on the overall activity of this microRNA; and, 2) ebv-mir-BART6 plays a significant role in maintaining viral latency and preventing lytic replication, which is critical for EBV's pathogenesis.

Reviewer 3:

The investigators completed both of the aims stated in the proposal. All of the methods used to obtain data to address the aims were appropriate, and sufficient data was presented to evaluate progress. The work proposed for this project follows up on some novel observations that at least one EBV miRNA can be modified by cellular editing machinery. The effects of editing on the EBV miR-BART6 are explored. Thus, new knowledge has been generated from work proposed in this application, which may lead to development of new strategies for treating EBV-associated malignancies.

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:

I do not foresee that this research will have any beneficial impact on improving human health. While the budget was fairly small, and some progress was made, this was less than envisioned in the original proposal.

Reviewer 2:

Strength: The grantee's discoveries have advanced the current knowledge of how EBV interacts with its host cells to achieve the homeostasis of its life cycle, a good contribution to the EBV research field.

Reviewer 3:

The investigators have found a new pathway by which EBV may regulate its own gene expression through modulation of one or more cellular pathways. As a consequence, this project has identified new pathways that may be explored to develop novel therapeutics for EBV-associated cancers.

There is a discrepancy between the stated aims and what the investigator proposed to do to address the aims. So while the details of what he proposed to do were achieved, the significance of the findings remains unclear. For example, the data indicate that miR-BART6 may play an important role in suppressing the expression of some latency genes, such as EBNA2. However, this was only measured at the level of RNA, and there is no indication that this had any effect on expression of the EBNA2 protein. Moreover, it remains unknown whether any of the changes in gene expression as a result of miR-BART6 expression (or editing) affect the actual growth or viability of EBV-associated tumor cells.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

The applicant states that funds from NIH were used during this project, but no details are provided. The applicant states that she has submitted a related grant to NIH, based on this work, and that she will submit a Defense Advanced Research Projects Agency (DARPA) grant application in the near future.

Reviewer 2:

Strength: This research project has led to a new NIH grant application. Meanwhile, the grantee has been planning to apply for additional funding to continue this research.

Reviewer 3:

No leveraging of new funds materialized, but preliminary data has been generated for new applications to agencies such as NIH. The investigator has at least one application pending with NIH.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

The applicant reports one manuscript--a review article in a book. However, this seems only modestly related to the current application. No primary, original manuscript in a peer-reviewed journal was reported. No licenses or patents were submitted.

Reviewer 2:

Strength: This research project led to publication of a peer-reviewed book chapter entitled "Post-transcriptional gene regulation by an Editor: ADAR and its role in RNA Editing."

Reviewer 3:

No peer-reviewed publications were generated that directly were the result of research proposed in the application. A general review article on the topic of RNA editing has been accepted as a book chapter. The investigators plan to submit a manuscript this year on the work generated from this grant.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

The applicant states that these funds were used to train two undergraduates and pay part of the PI's salary. No infrastructure improvements were made or new investigators recruited.

Reviewer 2:

Strength: This research project enhanced the quality for research at the grantee's institution. Analysis of RNA-editing of viral transcripts, a novel and challenging topic in EBV research, can now be successfully conducted at the grantee's institution.

Reviewer 3:

No improvements were made to infrastructure. Funds from this grant were used to support 50% of a post-doctoral fellow and two undergraduate research students for at least one semester.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

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

Reviewer 2:

Weakness: This research project did not lead to any collaboration with research partners outside of the institution or new involvement with the community.

Reviewer 3:

No new collaborations with other researchers appear to have been forged as a result of this research.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

While the applicant made some progress on Specific Aim 2, no work on Specific Aim 1 was reported; and there were not any significant publications based on this work. The applicant needs to design experiments that look at endogenous miR-BART6 function using miR-BART6-specific antagomirs and/or EBV viruses lacking miR-BART6. Indeed, the widely used laboratory EBV variant B95-8 lacks miR-BART6. Experiments looking at ADAR function, and more precisely demonstrating whether miR-BART6 editing is significant, are also needed.

1. The applicant needs to address the function of endogenous, virally encoded miR-BART6 in BLs, NPCs and LCLs using antagomirs and in EBV strain B95-8 derived LCLs by rescue using physiologically relevant levels of miR-BART6, e.g., expressed from a lentivector.
2. Experiments using RNAi to examine the role of ADAR1 in miR-BART6 function need to be performed, as originally proposed in Aim 1.
3. Experiments using physiological levels of miR-BART6 expression need to be performed to examine how miR-BART6 affects Dicer expression.

Reviewer 2:

None

Reviewer 3:

There is a discrepancy between the stated aims and the actual specifics of what the investigator proposed to do, which were somewhat narrower than stated in the aims. In the aims, the investigator stated that she will determine the significance of pre-MiR-BART6 editing and miR-BART6 on latency and lytic replication, but she stopped short of fully realizing these outcomes by only looking at changes in latent or lytic viral gene RNA expression. So while the details of what she proposed to do were achieved, the significance of the findings remains unclear. For example, the data indicate that miR-BART6 may play an important role in suppressing the expression of some latency genes, such as EBNA2. However, this was only measured at the level of RNA, and there is no indication that this had any effect on expression of the EBNA2 protein. Moreover, it remains unknown whether any of the changes in gene expression as a result of miR-BART6 expression (or editing) affect the actual growth or viability of EBV-associated tumor cells. The significance and importance of these studies could be enhanced if the investigators can develop functional assays to address this last issue. The investigators need to publish the results generated from this grant proposal.

Project Number: 1087904
Project Title: Laboratory Renovation Research Infrastructure
Investigator: Altieri, Dario

Section A. Project Evaluation Criteria

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

This is a space renovation project. The goal of the project was achieved by providing for the renovation of a main research laboratory and tissue culture laboratory space for two researchers on the first floor of the 1894 Building covering 2,271 square feet. A laboratory for a new researcher was built with the renovation project. The information provided in the reports is applicable to the project objectives listed in the strategic research plan. The information in the report supports the fact that the project has completed and that the space renovation as proposed has met the stated objectives. No weaknesses were identified.

Reviewer 2:

The renovation was successful and the laboratory space upgraded on time and apparently on budget.

Reviewer 3:

This project is to renovate an outdated research laboratory into a modern laboratory for cancer research. Wistar Institute provided \$435K matching funds for the renovation. The project was successfully executed and met the original objective. The renovation work truly improved the space for proposed research.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

The newly renovated laboratory and its infrastructure were stated in the reports to be instrumental for the recruitment of new biomedical researchers to the Wistar Institute, which will be successfully completed by the beginning of 2013. The newly renovated laboratory space will be occupied by a new research team focused on ovarian cancer immunotherapy. The renovated space and the new senior investigator will contribute to the establishment of a tumor immunology research program in line with the long-standing interest of the institute in vaccine and other forms of immunotherapy. The new research will be expected to enhance

ovarian cancer patients' immune systems to oppose tumor growth *in vivo*. No weaknesses were identified.

Reviewer 2:

Successful recruitment of a high-level scientist will be highly beneficial and allow launching of a new research program on the immunotherapy of ovarian cancer.

Reviewer 3:

This new space will benefit the cancer research at Wistar Institute and will promote collaboration with other institutions. It will also help to recruit an established researcher. The impact is high. This infrastructure improvement will promote cancer research which may greatly improve health.

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 grants were submitted or planned. Wistar Institute put into the project a significant amount of internal funds. No weaknesses were identified.

Reviewer 2:

This was not expected.

Reviewer 3:

Although there is not yet any new proposal derived from this project, when the established investigators are recruited, more proposals may be derived from 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, licenses, or patents, were published or submitted/filed. These outcomes were not expected from the space renovation project.

Reviewer 2:

This was not expected.

Reviewer 3:

Since this is an infrastructure improvement project, we did not expect any publication or invention directly from this project. However, when the senior investigator is recruited, the research performed by the senior researcher may produce many publications and some new inventions. The possible impact is high.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

The availability of new research space in the 1894 Wistar building allows the thematic expansion of a new research initiative on ovarian cancer centered on potential new immunologic approaches for treatment options in women with advanced and recurrent disease. The project clearly enhanced the quality and capacity for research at the grantee's institution. No weaknesses were identified.

Reviewer 2:

New and modern renovated laboratory space was built. The description of the various components is excellent.

Reviewer 3:

This is an infrastructure improvement project, which enhanced the research space, quality and capacity for research at Wistar Institute. A new senior investigator will be recruited to advance cancer research activities. The funds were used for renovation only, not for personnel support.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

The reports stated that the research project completed with the allocated funds enabled a broad set of collaborative studies between Wistar Institute and the Helen Graham Cancer Center, a member of the Christiana Health Care System in the state of Delaware, with which the Wistar Institute has recently signed a broad collaborative agreement. No weaknesses were identified.

Reviewer 2:

It is too early to tell. This needs to be evaluated in about 2015 or two years after the new investigator arrives/is recruited.

Reviewer 3:

This project led to collaboration with research partners outside of the Wistar Institute. This new space will greatly improve cancer research and support collaboration.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

None

Reviewer 2:

It would have been useful to know in evaluating this proposal whether their recruitment was successful.

Apparently their lead candidate had visited four times and was pleased with progress. They anticipated finalizing recruitment by early 2013. Did a new investigator arrive in early 2013?

Reviewer 3:

This renovation project was well planned and executed. This new space helped to recruit a senior researcher in cancer research. The Wistar Institute match helped to move the project forward.