

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: Outstanding (1.33)

Project Rating:

Project	Title	Average Score
0865701	Critical Roles of Transcription Factor Foxp1 in Thymocyte Development and Peripheral T Cell Function	Outstanding (1.00)
0865702	Impact of Latent Cytomegalovirus Immediate Early Protein Expression on Embryonic Development	Favorable (2.00)
0865703	Frequency, Fate and Significance of MicroRNA Editing	Outstanding (1.33)
0865704	Diagnosis of Lung Cancer from Peripheral Blood Using Genomics	Outstanding (1.00)

Project Number: 0865701
Project Title: Critical Roles of Transcription Factor Foxp1 in Thymocyte Development and Peripheral T Cell Function
Investigator: Hu, Hui

Section A. Project Evaluation Criteria

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

Strengths:

Outstanding progress was made for this project. The investigators set out in Aim 1 to determine the molecular role by which FoxP1 influences thymocyte differentiation and activation. Aim 2 focused on the characterization of FoxP1 in peripheral T cell survival, function, and generation of two transgenic mice expressing the two major isoforms of FoxP1, 1A and 1D. The project met all the goals set out in the initial application and even proposed to generate two new mouse models to evaluate in this FoxP1 pathway in conditional mice in the future. The questions were clearly laid out, there were sound and justified experimental plans, and execution was performed for each aim and experiment proposed. The only minor criticism I have with the data presented is that in year one the CFSE data demonstrated that the deletion of the FoxP1 gene from SP thymocytes did not yield proliferation, yet effector cytokine (IFN γ) was produced.

The authors did not have a control sample in this plot to demonstrate what the CFSE profile of an unstimulated population would be; therefore, they determine that the data presented either did or did not proliferate following challenge. The only conclusion they can make is that there was no difference between the control and the KO cells. In fact, it seems quite likely that these cells did proliferate as in year two. They demonstrated that the KO cells proliferate at a higher capacity in the periphery compared to WT cells but do not make interferon gamma, only IL-2 following challenge (data not shown). This idea is also intriguing given the high level of IFN-g from the SP thymocytes in the year one report, and I predict the differential cytokine and proliferative capacities, if they exist, would be explained by the gene array data. This is a very minor point, as the remainder of the science and data presented are outstanding, and the authors set out with an aggressive plan and stuck to it.

The data provided were sufficient to determine that substantial progress was made for all aims with one exception - the gene array data. There were only reports of the four different groups and the trends of genes, but no genes or gene families had been identified that may help to explain the activated phenotype associated with the cells that had lost FoxP1. Again, this is a minor point, but a weakness as it is impossible to determine the progress made without seeing the gene array data.

Reviewer 2:

A major strength of this project is that most, if not all, of the proposed objectives have been met, including the generation of new knock-in mouse models. It should be noted that a significant part of the data in the second part of the project was submitted for publication (*Blood* 115:510, 2010) only 10 days after the start of the second project indicating that a substantial amount of the work was done prior to the funding period. Nevertheless, the data in general are excellent, novel, and presented in a quality publication.

There are only a couple of weaknesses. One major concern is that the microarray analysis of CKO and Ctl thymocytes (Fig. 4) is incomplete and unsophisticated. This is a glaring deficiency compared to the quality of the other parts of the project. A second less severe weakness, but potentially problematic, is the use of the ROSA26-locus to drive the expression of the alternative forms of Foxp1. This approach will likely lead to very unphysiological levels of Foxp1 forms, making interpretation difficult. An alternative approach might have been to engineer a conditional knock-in that would result in the deletion of one or the other forms of Foxp1. Despite these weaknesses, the overall progress of the project is considered to be outstanding.

Reviewer 3:

Objectives in the project, "Critical roles of transcription factor Foxp1 in thymocyte development and peripheral T cell function," were well encountered. The experimental design and methodology were well planned and implemented. Solid and interesting data were clearly presented to fulfill the stated objectives.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

Strength:

The impact that this project has on general health and improved health outcomes is substantial; and is a critical area of immune research to better understand T cell homeostasis. The significance of this project is to understand the role of a critical transcription factor for T lymphocyte biology. This is critical for both the differentiation of T cells into different classes and also functionally critical as the loss of this factor results in an activated phenotype. Critical regulation of Foxp1 may be involved in the regulation of T effector cells or T regulatory cells during the pathogenesis of autoimmunity or even extend to immune responses against infectious agents. A better understanding of FoxP1 regulation will be important to understand and maintain T cell homeostasis and prevent or treat autoimmunity using novel therapeutic targets of this pathway. Future work described in the application is outstanding and the investigators have already generated two new mouse models to study this pathway. Outstanding beneficial impact will come from the basic science and progress made from this group.

Reviewer 2:

There is no direct benefit of the project on improving health; however, T cells are critical players in combating pathogens, tumor surveillance, transplantation, and autoimmunity. Thus, a greater knowledge of the biology of this major lymphocyte subset may indirectly lead to benefits in the

future. This kind of rationale is applicable to all basic research and is based on solid past experience.

Reviewer 3:

This is a very timely and focal project with a key impact on our understanding of the function and development of immune responses. The significance of this project for health improvement can be quite prominent since it provides a fairly good explanation on the molecular mechanisms underlying T cell development and activation. This knowledge can be applied in treatments from cancer to autoimmune diseases.

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:

Investigators made outstanding progress for Criterion 3 of the grant. The authors planned to secure additional funding and achieved this goal. They report National Institutes of Health (NIH) funding of \$250,000 in 2010. The status of this grant is unknown, as this grant did not appear as a funded grant in the NIH Crisp reporter at the time of this review.

Weaknesses:

None.

Reviewer 2:

The applicant plans to submit grant applications to the NIH and other private foundations based on data generated in this project.

Reviewer 3:

The outcome and data from this project were used to submit a new application, "Transcriptional regulation of T cell activation by FOX family transcription factors," to the National Institutes of Health (NIH) in March 2011. It is still pending.

Dr. Hu is also planning to expend his research by submitting another grant to NIH and the American Cancer Society.

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:

Weakness:

No publications have resulted from these studies at the time of submission of the Final Progress Report. The authors intend to submit a publication from these to report the studies performed.

Reviewer 2:

The principal investigator (PI) states that a manuscript is in preparation. Some of the data has already been published. No licenses, patents, or commercial development opportunities were obtained as a result of this grant.

Reviewer 3:

The researchers currently have one manuscript in preparation. There were no major discoveries, new drugs, inventions, patents or commercial development opportunities created by this project.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

Strengths:

There are no reports of improved infrastructure; however, a new investigator was recruited and added. Dr. Xiaoming Feng has joined this group. Funds were used to support both pre- and post-doctoral students.

Weaknesses:

None.

Reviewer 2:

This grant enhanced the grantee's institution by making it possible for the PI to conduct state of the art research at this facility. Notably, this grant supported five researchers at various levels, including a scientist from out of the state, and made it possible to generate novel mouse models. Publications that will likely be forthcoming will enhance the prestige of the institution. The PI participated in training undergraduate students from the Community College of Philadelphia.

Reviewer 3:

During this research a new mouse model was created, which enhanced the quality and capacity at the grantee's institution. One pre-doctoral student, three post-doctoral students and one undergraduate student were supported by the present study. Additionally, one out of state researcher was introduced and brought to Pennsylvania to carry out the study.

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 authors report that they collaborated with professors at the University of Pennsylvania and the University of Texas at Austin.

Weakness:

The authors did not list the collaborators, either new or old.

Reviewer 2:

Collaborations were established with professors at the University of Pennsylvania and the University of Texas at Austin.

Reviewer 3:

As a result of this study, several collaborations were made with fellows outside the institution, including the University of Pennsylvania and the University of Texas at Austin. One undergraduate student was engaged in training from the Community College of Philadelphia.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

None.

Reviewer 2:

1. More sophisticated analysis of the microarray data is recommended, perhaps obtaining a collaborator with bioinformatics expertise, if there is not already one on board.

Reviewer 3:

None.

Project Number: 0865702
Project Title: Impact of Latent Cytomegalovirus Immediate Early Protein Expression on Embryonic Development
Investigator: Maul, Gerd G.

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 proposed an interesting approach to investigating the mechanism of brain damage in neonates, suggesting that the effects seen with IE1 in tissue culture cells would negatively impact brain development in a transgenic mouse. The proposal is based on the assumption that a single viral protein (or two) is solely responsible for developmental defects in the brain. This is a high risk/potentially high-reward idea; however, the results do not support the assumption. The project met the stated objective of constructing a mouse model with inducible IE1 expression. However, no deleterious effect of IE1 was seen as assayed by the number of offspring produced by the transgenic mice.

The research design and methods were adequate. The data obtained were in line with, and applicable to, the original research proposal. Unfortunately, the results obtained did not support the hypothesis, namely that IE1 expression would alter brain development in an adverse manner.

Reviewer 2:

The objective of this proposal was to determine whether inactivation of the host's repressors and gene-silencing proteins by the MCMV IE1/3 gene products affected differentiating neuronal cells in the developing embryo. To evaluate these effects, the PI would develop a transgenic mouse system that allowed conditional expression of the IE proteins during different stages of development and in large portions of the brain. They had two specific aims: 1) to develop this mouse model of inducible expression and 2) to analyze the effect of IE1 expression on mouse brain development.

In order to accomplish Specific Aim 1, the investigators had to construct targeting vectors, create ES cells containing an integrated vector, and then breed to homozygosity animals that had the IE expressing construct, which also expressed the CRE enzyme that was used to induce expression of the IE proteins. Along the way, they checked for functionality of their constructs, which appeared to be working as planned. They appeared to have constructed their mouse system.

Difficulty developed when they tried to assess whether there were any effects of IE expression on embryonic development and they essentially found no changes. After multiple tests, too

numerous to discuss here, the PI finally concluded that there were just not enough cells actually expressing the IE proteins to exert a negative effect on development.

The inherent problem in the design of the initial experiments was the utilization of the endogenous MIEP promoter/enhancer to drive expression of the IE proteins. It has been known for some time that this promoter, in particular, is under tremendous influence for silencing by the host cell. Indeed, they found that there were multiple types of silencing going on of their transcript in their cells. In an analysis of adult mice derived from the transgenic litters, it was very clear that only a very small fraction of cells was actually expressing the transgene. Unfortunately, the project was doomed from the start.

Reviewer 3:

This was a highly ambitious, and well conceived project. However, due to experimental issues and largely negative results, the major specific aim of establishing an experimental transgenic animal model, wherein inducible MCMV IE expression produces significant developmental brain abnormalities, was not achieved. Specifically, there is no evidence to support the hypothesis that IE1/IE3 expression in the developing brain of transgenic mice will produce any identifiable pathology or deleterious effects.

As proposed in the Strategic Plan, the experimental design and methods described were appropriate and adequate for the successful completion of the project. Preliminary results, provided in the original application, indicated that essential aspects of the experiments had been completed prior to the start of this project. Most significantly, the construction and validation testing of the vector with the full major immediate early transcription unit with the transcription/translation stop sequence flanked by loxP sequences, had been "completed" before this grant was funded. Consequently, given the principal investigator's established experimental expertise with the required research methods, and his 85% time commitment, considerable progress in achieving the stated goals of the project was to be anticipated.

A well defined set of experiments, with unambiguous objective measures and tests to be employed, was proposed. Several of the required transgenic mouse lines were established during the period of support. However, beyond the brief descriptive summaries results in the Progress and Final Reports, no detailed data representations of the embryonic development of the CNS in these transgenic mouse lines has been provided. Moreover, no future publications or grant applications relating to the specific aims are planned or in preparation.

The description of the results indicate some progress was made towards meeting the specific aims identified; however, data confirming cell line responses and embryonic mouse CNS development were not provided, and the very limited data examples presented in the Annual and Final Progress Reports on adult transgenic mouse brain expression patterns lacked sufficient resolution and detail to allow for an objective evaluation of the data. Notably, absent was any discussion of intra- and inter-subject variability for the data examples shown in the figures.

In summary, the data and information provided in the reports were relevant to the project objectives listed in the Strategic Research Plan. However, progress towards satisfying the specific aims was quite limited; the results were largely negative; the hypothesis of IE-induced

developmental brain pathology proposed for the mouse model was not substantiated; and sufficient data to objectively evaluate the claimed experimental results were not provided.

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 impact of this project is small. Mouse cell lines and transgenic mouse lines were created that may serve as useful resources in the future to answer other related, but different questions. The overall goal of establishing a mouse model to test efficacy of blocking compounds was not able to be accomplished as the transgenic mice appeared normal.

Reviewer 2:

Due to the fact that this project was not successful in establishing a functional model system, the impact will most probably be very small, if measurable at all.

The one potential outcome, as the PI mentions, is that the MEFs derived from these embryos may be useful for studying other mechanisms of silencing of the MIEP not currently known. They also may be useful for studying what signals might be involved in reactivation of the MIEP during transplantation.

Reviewer 3:

The budget information provided was inadequate to objectively evaluate the proposal. It was stated several times that no NIH funds were sought or received, but on page two of the Final Progress Report, Box 10 indicates that co-funding of \$250,000 by NIH was received during the project period. Consequently, it is not possible at this time to judge the progress of this program in light of actual dollars budgeted and received.

In the Final Progress Report, the principal investigator states no publications on embryonic development of the transgenic mouse lines generated are currently planned; no future grant applications are contemplated; and no leveraging of the allocated funds was attempted. Given that Box 22 indicates "Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment: None," the decision not "to apply for additional funding in the future to continue or expand the research (Box 11)" is appropriate.

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 funds were leveraged nor were additional grant applications submitted as a result of this project.

Reviewer 2:

The project did not leverage any funds or put in any additional applications due to its lack of overall success.

Reviewer 3:

As stated in the Strategic Plan, NIH funding was being sought, but no NIH or other funding support for the research project was available during the period of funding. Apparently, no new grant support of any kind was obtained during the funding period. Moreover, according to the Final Progress Report (Box 11), there are no plans to apply for additional funding in the future to continue or expand the research. However, it has been proposed that the transgenic mouse lines established by this program will be made available (Final Report, Box 12) to "Dr. William Britt in Alabama and Dr. Jonjic in Croatia" for possible future studies. No formal collaborative arrangements with Drs. Britt and Jonjic have been provided.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

The investigators are in the process of writing a manuscript.

Reviewer 2:

No papers or patents were submitted due to the lack of overall success. A manuscript was listed as being in preparation, but nothing has been published in the literature to date.

Reviewer 3:

No publications relating directly to the specific aims are in progress or contemplated (Final Report, Box 20). A publication on "Characterization of the Full MCMV Major Immediate Early Gene Expression in the Adult Mouse" by G.G. Maul, O.V. Vladimirova, D.G. Negorev and N. Dahmane has been proposed, but a manuscript/draft was not included in the Final Progress Report.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

One post-doctoral fellow was involved in the project. The project enhanced the research of a collaborator at The Wistar Institute.

Reviewer 2:

These funds apparently allowed Dr. Maul to keep a post-doctoral fellow who was working on this project. There was also apparently some interaction between Dr. Maul and one of his colleagues at Wistar (Dr. Dahmane) in evaluation of the histological specimens. This reviewer does not deem these as substantial impacts.

Reviewer 3:

No changes to the infrastructure were identified. No outside researchers were brought to the institution to help with this research. No funds from this formula grant were used to pay any pre- or post-doctoral students. Notably, the funding source for senior technician, Dr. Vladimirova (100% effort), and research associate, Dr. Negorev (75% effort), has not been identified.

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:

Two new collaborations were established, including one with Dr. William Britt, another highly respected researcher in the field.

Reviewer 2:

Dr. Maul has transferred these mice to two other investigators (Dr. Bill Britt at the University of Alabama at Birmingham and Dr. Stipan Jonjic in Croatia) to potentially continue investigations. Since there does not appear to be substantial use for these mice in their current status, it is unclear whether a true collaboration will be established.

Reviewer 3:

No active collaborations with research partners outside of the institution have been identified. However, given the PI has stated there is no intention of pursuing this research, it is appropriate that the established transgenic mouse lines generated by this program will be made available (Final Report, Box 12) to "Dr. William Britt in Alabama and Dr. Jonjic in Croatia" for possible future studies.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

1. The impact of cytomegalovirus on brain development is being actively investigated by multiple labs around the world. Published work, "Murine Cytomegalovirus Infection of Neural Stem Cells Alters Neurogenesis in the Developing Brain," Manohar B. Mutnal, Maxim C.J. Cheeran, Shuxian Hu, and James R. Lokensgard, demonstrates that the murine model system can be used to investigate the effects of the virus on the brain. The effects of targeted mutant viruses are likely to be more informative than the overexpression of a few viral genes.

Reviewer 2:

1. Unfortunately, the MIEP was used in these transgenics to drive the expression of IE1 and IE3. It appears that Dr. Maul was correct in his assumption that he should use an inducible construct to control the timing of expression; however, a construct under the control of a constitutive promoter that he knew would be expressed once recombination occurred, should

also have been constructed. At this point, once the negative situation was encountered, these animals could have been brought into play.

Reviewer 3:

1. This was an ambitious and well-reasoned proposal; however, progress was very limited, and detailed data confirming any of the results reported relating to the studies with cell lines, or the investigation of transgenic mouse embryonic brain development described in the Progress and Final Reports, has not been provided. The inclusion of additional data to clarify and confirm results presented in the reports would have been appropriate.
2. Data relating to various organs in the adult transgenic mouse lines lacked appropriate detail and resolution to allow for an objective evaluation of the stated results. Additional figures, identification of labeled cells using immunophenotyping, and other relevant supporting data as proposed originally in the Strategic Plan should have been included.
3. The proposed immunohistochemical, Western blot, in situ hybridization, and morphological analyses proposed for studies of the developing embryos from the various transgenic mouse lines generated have not been conducted. Notably, no attempts to clarify the identity of GFP expressing cells by immunophenotyping was conducted as planned. Given limited expression of GFP in cells, immunostaining with anti-GFP antibodies would have been a useful approach to boost cellular signals.
4. Due to overall negative results, and the absence of any significant findings, the principal investigator has appropriately elected not to pursue further funding for this line of research.

Generic Recommendations for the Wistar Institute

Reviewer 2:

A major recommendation would be to have some sort of preliminary review process for these grants. Had this proposal been reviewed by colleagues in the field, many of the issues that developed with the transgenics would have been anticipated and potentially addressed prior to the start.

ADDITIONAL COMMENTS

Reviewer 2:

It is very unfortunate that these transgenic mice did not really give us any information regarding the potential role of IE1 and IE2 in embryonic perturbations. It is this reviewer's hope that in the future, a different tact can be taken that would lead to a mouse model with higher levels of expression to more fully explore this issue.

It should be noted, however, that Dr. Maul did what he stated, to construct these transgenics and test their effects.

Project Number: 0865703
Project Title: Frequency, Fate and Significance of MicroRNA Editing
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:

Strengths:

Sufficient data was obtained and analyzed to support the fact that the project met its stated objectives with acceptable progress. The research design and methods were successful.

Reviewer 2:

There were two specific aims that were proposed and completed. The investigators surveyed an miRNA gene transcript for editing and determined the frequency of this occurrence. They also determined the effects of pri-miRNA editing in vivo by miRNA processing using recombinant complexes. The proposed projects were designed appropriately and were completed. There was sufficient data presented in the Final Report to indicate that the project met its objective and made substantial progress. This project worked on a new, emerging area so it is still at the basic investigation stage.

Reviewer 3:

The project met the stated criteria, in that the research questions that were posed in the specific aims were very well addressed. The researchers have performed Aim 1, i.e., they have systematically investigated the presence of edited sites within miRNAs. They then made excellent progress in addressing Aim 2, whether editing would affect miRNA processing. Surprisingly, it did, suggesting that editing may be a mechanism of regulating miRNA expression rather than the mature form of the miRNA. This result is surprising but is very interesting in its own right. In addition, sequence and secondary structural determinants of editing were also identified. Excellent progress was made and very interesting questions opened with regard to the regulation of miRNA expression. The strengths of the application were the significance of the subject and the creative approach to studying miRNA maturation and regulation.

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:

Weaknesses:

Although the results of the project are described in considerable detail, more discussion regarding the translational impact of the study findings would have been helpful. The PI did not comment on the value of the research towards eventual improvement in health outcomes. One could envision this, but the PI does not address it sufficiently.

Reviewer 2:

Future plans include dissecting the significance of microRNA editing. The investigators discovered that viral miRNAs are also subjected to RNA editing. The investigators will determine the significance of these specific types of editing in virus replication and latency. There is the possibility that future studies will lead to a broader understanding of miRNA editing and its role in human disease.

Reviewer 3:

The project was highly significant for health. miRNA expression is aberrant in a variety of disease conditions, from cancer to inflammation, yet the mechanisms by which their expression is regulated are only beginning to be understood. The work reported here strongly suggests that RNA editing is a mechanism of regulation at both nuclear and cytoplasmic maturation steps, though the detailed biochemical mechanisms by which this occurs are still unclear. These results open all kinds of future questions, both with regards to the mechanism of regulation and to the biological impact of editing that will hopefully be addressed by the investigator in the future. Suggestions for focusing future efforts are the elucidation of the mechanism by which editing affect miRNA processing and, especially, to understand whether this mechanism of regulation is physiologically significant, as it is likely to be, and in what context it is.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

Strengths:

The PI leveraged the funds from this project to support the work of post-doctoral and undergraduate students. The PI has a long-standing NIH grant on the control of cardiogenesis by microRNA editing. The results of the present project, assisted the PI in getting the NIH R01 grant renewed for funding.

Reviewer 2:

Leveraged funds were obtained. A grant from the NIH was awarded for a total of one million dollars on control of microRNA editing. This was a direct result from the support of this project. Additional funds will also be applied for.

Reviewer 3:

A proposal was submitted to NIH - which may be an R01 extension. This project clearly is NIH-fundable and is quite interesting and unique, in a very significant area of research. I expect a successful outcome, though with NIH funding rates being what they are, it is difficult to predict.

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:

The only publication listed by the PI was a review paper. The PI indicated plans to submit a manuscript to *Nucleic Acids Research*. Presumably, this would be an original research article describing the results of this project.

Reviewer 2:

No outside collaborations or commercial developments were indicated. No community involvement and no licensing fees were noted. There was a reported review published at the time of the Final Report. Upon examining *PubMed*, the principal investigator has published a significant number of papers and reviews relevant to the supported project.

Reviewer 3:

Only one peer-review publication is listed, which is a review in a very major journal. However, I expect one or perhaps two publications will be forthcoming based on the results presented in this report.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

Strength:

Funding from this project released PI salary funds that were used to pay for research performed by undergraduate and post-doctoral students.

Weakness:

The project noted no improvements to infrastructure and no new investigators brought to the institution.

Reviewer 2:

Two post-doctoral fellows were supported on this project. In addition, four undergraduate university students were supported. They were not from out of state.

Reviewer 3:

Funds were used to support post-doctoral researchers, but it does not appear that any significant new infrastructure was added.

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:

Weakness:

There were no new collaborations from this research.

Reviewer 2:

There were no reported outside collaborations.

Reviewer 3:

It does not appear that significant new collaborations were initiated.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

1. Although the results of the project are described in considerable detail, more discussion regarding the translational impact of the study findings would have been helpful. The PI did not comment on the value of the research towards eventual improvement in health outcomes.
2. The only publication listed by the PI was a review paper. The PI indicated plans to submit a manuscript to *Nucleic Acids Research*. An update regarding plans to publish the results of this project as an original research paper, would be helpful.

Reviewer 2:

None.

Reviewer 3:

None.

Generic Recommendations for the Wistar Institute

Reviewer 3:

This is a fine project in a very significant area of research. What I found most impressive is that the PI was able to find a new angle and unique insight in what is a very important but also very crowded area. I would recommend continued support, if at all possible.

Project Number: 0865704
Project Title: Diagnosis of Lung Cancer from Peripheral Blood Using Genomics
Investigator: Showe, Louise C.

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 its stated objectives and documented excellent progress. The research design and methods were quite adequate, although based on differences in the quality of PMBC samples acquired from various locations, blood samples are now collected with the more easily standardized PAXgene system. The basis for this change was explained and justified. They also have established new collaborations/institutions for sample collection in order to increase sample size and the power of the analysis.

The data were rigorously tested and analyzed and were sufficient to answer the research questions posed. Additional samples are needed to fully confirm the underlying hypothesis, and further funding is being requested to conduct these studies.

Reviewer 2:

This research project was designed to test the hypothesis that a specific and diagnostic gene expression profile for Non-Small Cell Lung Cancer (NSCLC) can be identified in the peripheral blood mononuclear cells (PBMC) of lung cancer patients. They proposed two specific aims to approach the projected objectives:

- 1) To identify gene profiles in PBMC on Illumina microarrays that accurately distinguish early stage NSCLC patients from the at-risk controls; to assess the effects of gender, race and smoking history on molecular classification; and to evaluate changes in the cancer signature as a function of surgical removal of the cancer by comparing pre- and post-surgery PBMC gene expression profiles.
- 2) To validate the study using samples from additional locations and reassess biomarker panel generated on new samples collected over the grant period. The research design and methods were adequate and reasonable in light of the project objectives. The project has made reasonable progress and met the proposed objectives.

For Aim 1, they collected more than 400 samples and processed about 300 on arrays for analysis. They identified a 29-gene expression signature using Illumina gene microarrays that could distinguish NSCLC patients from an at-risk control population of smokers and ex-smokers with

non-malignant lung disease with 91% sensitivity and 80% specificity. They analyzed differential gene expression profiles in the PBMCs taken before and after removal of NSCLC and found a number of significant alterations in pathways associated with the innate immune response in post-surgery PBMC samples. They also analyzed miRNA transcription profiles that differed in pre- versus post-surgery samples and found expression of five miRNAs to be universally upregulated in pre- compared to post-surgery samples. They developed specific signatures that take into account cancer subtype and stage and improved accuracy of these signatures in distinguishing benign from malignant nodules. For Aim 2, they validated the generalizability of their 29-gene signatures on independent subsets of case and control samples from external validation sites. Validation studies are continuing with samples being collected at various locations at the time of the final progress reporting.

Reviewer 3:

The project aims at identifying gene expression profiles from blood cells as biomarkers for early stage lung cancer. The investigators have collected over 400 samples and about 300 of them have been processed on array for gene expression analysis. The investigators were able to achieve the stated milestones by successfully collecting biosamples and initiating biomarker analyses. Also, the project has generated promising results and the first paper was published in *Cancer Research*. It is of note that the investigators have included microRNA profiling analysis in their approach, which will be analyzed together with coding gene expression and will greatly enhance the proposal. Overall, the investigators have done an excellent job achieving the stated objectives of the proposal.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

The results of the project remain somewhat preliminary (more samples and analysis of these samples are required), but the overall findings have potential for substantial impact on lung cancer diagnosis, treatment, and survival.

The results of this particular project are incremental. There was very strong preliminary data and additional data is required to fully document the usefulness of these findings. Future plans are to seek additional funding to further test the approach.

Reviewer 2:

Results generated from this study demonstrated that the gene expression signatures in peripheral blood could be used to diagnose NSCLC with reasonable accuracies similar to more invasive procedures like bronchoscopy and to gene expression profiles generated from sputum samples. Their results also suggest that analysis of pre- and post-surgery blood samples from patients with NSCLC may contain information that could be predictive of outcome and recurrence. Their findings suggest that it is possible to develop a non-invasive test for the presence of lung cancer using gene expression in PBMC samples. This test may be used in conjunction with present standards for detection and may help to reduce the large numbers of false positive results

presently resulting from CT scans and to improve lung cancer survival by detecting malignant tumors in high-risk patients at an early stage.

Reviewer 3:

The overall goal of the project is to develop a noninvasive and inexpensive test for early detection of lung cancer. The potential beneficial impact is high since there is no screening test available now for the detection of early stage lung cancer, and this early detection and subsequent therapies is the only effective treatment for lung cancer. Their preliminary results have associated several candidate genes with altered expression levels detected in blood cells that can distinguish early stage NSCLC patients from controls. If the proposed research was completed, findings will result in a fast blood test for detecting early stages lung cancer and may ultimately improve the ability to develop novel surgical intervention. The study may also have potential great impact on public health because there are a large numbers of smokers and ex-smokers who are at increased risk of lung cancer.

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:

A grant application was submitted to NCI for funding. As of this date, the grant does not appear as a funded grant in the NIH reporter system. Additional funding was also requested from the Science Center QED Program. Prospects for funding from either source are not described in the Final Progress Report.

Reviewer 2:

The findings generated from this grant allowed investigators to apply for additional funding to continue and expand their research projects. They submitted an NIH grant entitled “Signatures for diagnosis & prognosis of NSCLC from gene expression in blood” in 2010 and another grant entitled “Clinical test for lung cancer using whole blood samples” to the Philadelphia Science Center QED Program in 2010. They are also in contact with two companies for sponsored research support to continue this work.

Reviewer 3:

As a result of this project, the investigators have submitted a grant to NIH and another to the Philadelphia Science Center QED Program to expend and continue the current work. The investigators also have contacted two companies for sponsored research support.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

A high quality/impact paper describing this work was published in the December 2009 issue of *Cancer Research*. A patent was filed in December 2008 (technically just before this grant was funded). This patent was issued in July 2010.

Two additional manuscripts were listed as in preparation for submission.

Reviewer 2:

The research projects are productive. They published a paper in *Cancer Research* in 2009 (Showe, M.K, et al. Gene expression profiles in peripheral blood mononuclear cells can distinguish patients with non-small cell lung cancer from patients with nonmalignant lung disease, *Cancer Research*, 69:9202-921,2009). Their results showed the feasibility of using peripheral blood gene expression signatures to identify early-stage NSCLC in at-risk populations. They are also preparing two manuscripts for publication: 1) Differential Expression of Coding and Non-Coding Genes in PBMCs of NSCLC Patients after Tumor Removal, and 2) Predicting Outcome in NSCLC Patients Based on Gene Expression in Peripheral Blood.

One patent application, PCT/US2008/013450 (“Method for Diagnosing Lung Cancers using Gene Expression Profiles in Peripheral Blood Mononuclear Cells”) was filed in 2008. National phase applications have been filed in the United States and Europe. This patent application, jointly owned by The Wistar Institute and the University of Pennsylvania, claims compositions for and methods of diagnosing and staging lung cancer using purified white blood cells (PBMC) and includes multiple claims of sets of genes used in the diagnosis and/or staging of the cancer. The European patent application was filed at the request of a local venture firm that is interested in commercializing this test.

Reviewer 3:

According to the Annual Progress Report, the project has published one peer-reviewed article in *Cancer Research*. The investigators also indicated that they have plans to publish several more papers. The investigators have filed a patent based on the findings and have had a marketing assessment on the clinical utility. Further, they are in the process of negotiating with biotech companies to commercialize their patent. This is very satisfactory given the relatively short period of the current project.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

Although explicit improvements in infrastructure were not documented, the research enhances the bioinformatic capabilities and data base.

The grant supported two pre-doctoral (MS) students. Out of state researchers were not recruited directly into the project, but new collaborations were initiated outside of the state.

Reviewer 2:

This project allowed for the development of new approaches to identify gene expression information from clinical samples with diagnostic and prognostic utility and led to new collaborations with other laboratories interested in applying the approaches to their own samples of interest. This project also funded two master's degree students at the institute.

Reviewer 3:

No significant improvements in infrastructure or personnel were made as a result of this project. However, the funds were used to pay for research performed by master's degree students or post-doctoral fellows.

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 included collaborations, and the results have generated new out-of-state collaborations with the New York University Medical Center and Brigham and Women's Hospital.

Reviewer 2:

They have established collaborations at the University of Pennsylvania, New York University Medical Center, and Brigham and Women's Hospital in Boston for external sample collection and data analysis.

Reviewer 3:

The current Progress Report indicated that the project has led to collaborations at the University of Pennsylvania, New York University Medical Center, and Brigham and Women's Hospital.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

None.

Reviewer 2:

None.

Reviewer 3:

None.