

# **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 (1.87)

#### **Project Rating:**

<b>Project</b>	<b>Title</b>	<b>Average Score</b>
0863401	A Growth-Regulating Protein Tyrosine Phosphatase	Outstanding (1.00)
0863402	Characterization of the Role of MTAP Gene in Tumorigenesis	Favorable (2.00)
0863403	The ARF Tumor Suppressor and Autophagy	Outstanding (1.33)
0863404	Anti-Glucose Transporter-1 Antibodies as a Novel Treatment against Human Cancers	Unfavorable (2.67)
0863405	Regulation of Human Somatic Wee1 by Cyclin A/Cdk2 Complexes	Favorable (2.33)

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**Project Number:** 0863401  
**Project Title:** A Growth-Regulating Protein Tyrosine Phosphatase  
**Investigator:** Chernoff, Jonathan

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### *Section A. Project Evaluation Criteria*

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

#### **STRENGTHS AND WEAKNESSES**

##### Reviewer 1:

- The project met the stated objectives.
- The research design and methods were suitable for the proposed objectives.
- The data were adequately developed in context of the proposed research.
- No apparent changes were made to the experimental protocol.
- Adequate information was provided to thoroughly evaluate the success of the project.
- Data and information provided were applicable to the project and presented thoroughly.

##### Reviewer 2:

- The project met the stated objectives.
- The research design and methods were adequate in light of the project objectives.
- The data were developed sufficiently to answer the research questions posed and were developed in line with the original research protocol. Two papers were published in high-quality journals.
- No changes were made to the research protocol.
- 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: Not applicable
- Sufficient data and information were provided to indicate or support the fact that the project met its objectives or made acceptable progress.
- The data and information provided were applicable to the project objectives listed in the strategic research plan.

##### Reviewer 3:

This project, which focused on PTP1B in breast cancer, met all of its objectives. The investigator identified the localization of a modified form of PTP1B as well as specific substrates. The research was performed diligently, and progress was excellent as measured by two publications and a Department of Defense research grant. The research was performed within the research plan, and minimal changes were required. The investigator presented a clear case that the objectives were met.

There were no weaknesses.

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

## **STRENGTHS AND WEAKNESSES**

### Reviewer 1:

The potential for further understanding how PTP1B is involved in tumorigenesis could be used to further understand the mechanisms of PTP1B involvement in breast cancer progression. However, it is still quite early to evaluate properly the significance of these findings in the context of improving human health. The major weakness here is that much of the information generated in this application, especially that relating to Src, has been shown by others. The role of Sumoylation is new and potentially exciting, but it is still too early to evaluate properly.

One of the weaknesses is that the future plans were not fully articulated by the applicant. A few experiments were described; however, a broader long-term plan was not articulated. It is stated that an R01 application is planned, but the content is unclear. It is recognized that moving these types of findings into areas of clinical significance is very challenging, and thus the weaknesses need to be taken in context.

### Reviewer 2:

The major strengths of this project were that two outstanding manuscripts were published in high-tier journals that shed light on how a key protein tyrosine phosphatase, PTP1B, functions in metabolism and breast cancer.

A manuscript was published by Dr. Chernoff's group indicating that PTP1B is required for ErbB2 transformation. PTP1B plays a major role in inhibiting signaling from the insulin and leptin receptors. Recently, PTP1B was found to have an unexpected positive role in ErbB2 signaling in a mouse model of breast cancer, but the mechanism underlying this effect was unclear. Using human breast epithelial cells grown in a three-dimensional matrix, PTP1B, but not the closely related enzyme T-cell PTP, was shown to be required for ErbB2 transformation *in vitro*. Activation of ErbB2, but not ErbB1, increases PTP1B expression, and increased expression of PTP1B activates Src and induces an Src-dependent transformed phenotype. These findings identify a molecular mechanism by which PTP1B links an important oncogenic receptor tyrosine kinase to signaling pathways that promote aberrant cell division and survival in human breast epithelial cells.

Dr. Chernoff's group published a second manuscript demonstrating that PTP1B interacts with emerin, in a sumoylation-dependent manner. PTP1B is an abundant non-transmembrane enzyme that plays a major role in regulating insulin and leptin signaling. Previously, they reported that PTP1B is inhibited by sumoylation and that sumoylated PTP1B accumulates in a perinuclear distribution, consistent with its known localization in the endoplasmic reticulum (ER) and the contiguous outer nuclear membrane. In this manuscript, they report that PTP1B also is found at the inner nuclear membrane, where it is heavily sumoylated. They also found that PTP1B interacts with emerin, an inner nuclear membrane protein that is known to be tyrosine phosphorylated, and that PTP1B expression levels are inversely correlated with tyrosine phosphorylation levels of emerin. PTP1B sumoylation increases as cells approach mitosis,

corresponding to the stage where tyrosine phosphorylation of emerin is maximal. In addition, expression of a non-sumoylatable mutant of PTP1B greatly reduced levels of emerin tyrosine phosphorylation. These results suggest that PTP1B regulates the tyrosine phosphorylation of a key inner nuclear membrane protein in a sumoylation- and cell-cycle-dependent manner.

Their findings may change the perspective and treatment of certain forms of breast cancer. In particular, their work identifies two potential therapeutic targets in ErbB2-positive breast cancer: PTP1B itself, and the PTP1B target Src. There are clear implications of the research for treatment of ErbB2-positive breast cancers. The major discovery was that PTP1B, acting through Src, is required for transformation by ErbB2. Both PTP1B and Src are therapeutic targets, and clinical inhibitors of Src are available. They intend to use a combination of substrate-trapping and mass-spectroscopy methods in PTP1B-plus and PTP1B-null mammary epithelial cells to determine potential cancer-relevant substrates for the phosphatase.

Reviewer 3:

The impact of this work is that it provided insight into how a major breast cancer oncogene is regulated. Since components of the signaling network may be drug targets, this work may eventually have clinical impact. The investigator plans to continue this work under other funding. No weaknesses were noted.

***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:

Funds were obtained from the Department of Defense. The researchers propose to submit an NIH R01 application.

Reviewer 2:

A Department of Defense grant was obtained in late 2009 for \$523,500. An NIH R01 is planned.

Reviewer 3:

The PI applied for and received a Department of Defense grant. He also plans to write an NIH R01 grant on this work.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

Two papers were cited as published or in press. Both of these papers are solid pieces of work of excellent quality that are published in solid mid-tier journals. The work was of good quality and contributed to the field of PTP1B research in cancer. The major weakness, as cited above, is that

the work on the signaling aspects of HER2 on breast cancer was somewhat incremental in the context of other work and what was already known about PTP1B in HER2 signaling. I probably would have liked to have seen at least one other paper; however, the overall progress was good.

Reviewer 2:

Two papers were published. One additional publication is outlined, but no commercial activities were reported. Both papers are in high-quality journals: *Cancer Research* and the *Journal of Cell Science*.

Reviewer 3:

The PI published two papers directly related to this work. These are solid publications in respected peer-reviewed journals.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

No apparent changes were made to infrastructure. The PI hired a post-doctoral researcher who conducted experiments directly on this project. The funds were used to pay for this post-doctoral fellow's salary. The post-doctoral fellow was able to use this support to generate additional preliminary data for the successful award of a Department of Defense grant.

Weaknesses: No substantive areas can be identified.

Reviewer 2:

Five pieces of equipment were purchased: a cell sorter, a microscope, an imager and two freezers. Dr. Saha was recruited from India as a new post-doctoral associate and obtained a grant from the Department of Defense to continue the project.

Reviewer 3:

The research was of high quality and enhanced the PI's research program. The funds were used in part to pay for 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:

Collaborations were initiated with Dr. David Speicher at the Wistar Institute and Dr. Steve Seeholzer at Children's Hospital of Philadelphia. There is no indication that future collaborations are planned.

Reviewer 2:

The grant allowed collaborations with Drs. Speicher and Seeholzer of Wistar and Children's Hospital of Philadelphia, respectively.

Reviewer 3:

There were collaborations with other investigators.

***Section B. Recommendations***

**SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

Reviewer 1:

In general, the goals attained were consistent with the available resources provided.

Reviewer 2:

None

Reviewer 3:

None

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**Project Number:** 0863402  
**Project Title:** Characterization of the Role of MTAP Gene in Tumorigenesis  
**Investigator:** Kruger, Warren

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### *Section A. Project Evaluation Criteria*

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

#### **STRENGTHS AND WEAKNESSES**

##### Reviewer 1:

The investigators studied mice heterozygous for Methylthioadenosine Phosphorylase (MTAP). Such mice succumb to T cell lymphomas. The PI altered the research plan, since he received an NIH R01 in a partially overlapping topic, but good progress was made during the funding period. The PI published one paper describing these mice and the expression profile induced by loss of this gene. The PI made acceptable progress during the funding period.

A minor weakness was the publication of only one paper.

##### Reviewer 2:

The objectives changed six months after funding due to overlap with a funded NIH grant on the same topic.

New Aim 1 relates to characterizing the lymphoma cells present in MTAP heterozygotes. The researchers determined that 40% of the MTAP heterozygote mice had increased CD4+ T-cell populations, and these cells were monoclonal (indicative of a rapidly proliferating cell population derived from a single cell, consistent with lymphoma) in 60% of the animals. Some unclear results were why the two main experiments, the initial one with 525-day-old control (MTAP+/+) mice and MTAP heterozygote mice 260 days old, compared with a second experiment with significantly older mice, gave such different results in terms of lymphoid hyperplasia. One might expect more lymphoid progression in the older mice, but it was actually less. Thus, there remain questions to be addressed in characterizing the cellular consequences of partial loss of MTAP and how they relate to lymphoma. Other experiments showed that cells change in physiology to compensate for the partial loss of MTAP by secreting the MTA substrate into the media, preventing high, potentially toxic concentrations in cells.

The second aim related to creating a cell line that can express MTAP at different levels depending on doxycycline level, used as a regulator of gene expression. Results showed that cells not exposed to doxycycline did not express MTAP enzyme, whereas at 1 mg/ml of doxycycline, cells did express MTAP at concentrations that increased over time. This aim was achieved.

The third aim involved observing which genes were significantly up- or down-regulated in the cell line developed in Aim 2 to explore the physiological consequences of absence or increasing concentration of MTAP in the cells. From the results, they focused on ten genes, half up-regulated and half down-regulated, and identified as being involved in the extracellular matrix, bone development, and oxidoreductase function. One gene was focused on, SEC16B, but they did not discuss the connection between this gene and processes that relate to the MTAP pathway or lymphoma. The experimental results from two different days on induction of SEC16B were much different and not explained, except to say that MTAP expression seems to result in an alternative form of the gene product of SEC16B. There was no summary or explanation of future directions, including the roles of the other interesting genes identified. Thus, this aim was partially addressed.

Aims 1 and 3 were partially achieved, and there are questions about the cause of variable experimental results, as well as the researchers' future directions for achieving these aims.

The data was mostly well presented, except for the rationale for focusing on SEC16B in Aim 3, and the experiments were consistent with the altered aims.

The aims were altered after this grant was approved to avoid overlap with their funded NIH proposal, whose aims were not presented in the progress report. It is not clear whether this change in direction involved consultation with or review by the Pennsylvania Department of Health.

A thoughtful review of significantly altered aims would have been valuable before the revised project proceeded, given that the MTAP project would then be funded by two agencies at a significant level.

Reviewer 3:

The project partially met the stated objective. The aims were modified early in the project, since there was overlap with another NIH-funded grant. The idea is that MTAP (methylthioadenosine phosphorylase) may be a tumor suppressor and that a single alleles loss leads to mice dying prematurely of lymphoma. This is a novel observation. Their Tet inducible MTAP was a good idea, and the expression led to identification of genes that were differentially expressed. The observation that Sec16B had a 50- to 61-fold induction in a short time after inducing MTAP is a potentially important observation that will be followed up. The xenograft experiments comparing MTAP- to MTAP+ cells are not well done. The investigator stopped the experiment at 28 days (and they claim loss of MTAP leads to more robust tumors); however, had they gone longer it is possible there would have been minimal, if any, differences. However, satisfactory progress was made, and the hypothesis that MTAP may play a role in tumorigenesis is worth followup.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

This work provides insight into the role of this gene in T-cell lymphomas. This may provide more insight into this disease. The PI proposes to continue this work.

Reviewer 2:

There was prior evidence that MTAP function is related to cancer, and MTAP has been (and may still be) a target of drug discovery by pharmaceutical companies. Understanding the mechanism by which MTAP affects cell proliferation and processes in healthy versus cancer cells is important. This project has made useful advances in those areas, mainly by developing a series of biological tools for studying the role of MTAP within cells and showing that low levels of MTAP are related to the proliferation of a certain type of T-cell resulting in lymphoma.

This project contributes fundamental tools and initial biological results on the mechanism of MTAP function in healthy and cancer cells, which are necessary to assess whether it can be an effective therapeutic target for improving cancer outcomes.

The researchers' conclude that loss of MTAP resulting in rapid proliferation of CD4+ T-cells in mice provides a mechanism for how MTAP loss results in cancer. This provides a handle on which cells should be targeted for suppressing to decrease tumor formation. That could involve targeting molecules other than MTAP that are CD4+ T-cell specific.

The progress report ended rather abruptly before Aim 3 results were fully explained.

Reviewer 3:

This is still at the basic science level. Additional studies will be required to lead to development of advanced trials in humans with 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:

The PI received an R01 grant on a partially overlapping topic but has not applied for additional funds.

Reviewer 2:

Additional funds have not yet been leveraged. The NIH application that was funded, resulting in an adjustment of aims for this MTAP grant, preceded new results from the Pennsylvania Health Department grant. NIH funding supports that this is viewed as a promising area of research.

The researchers state they will apply for an additional NIH R01 grant, using data from this funding as preliminary results.

Reviewer 3:

From the original proposal an R01 NIH grant was obtained. The investigator correctly moved on to the new project and made progress. There is no indication if the PI will apply for additional funds.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

The PI published one paper in *Cancer Research*.

Reviewer 2:

One peer-reviewed publication on MTAP-deficient mice succumbing to T-cell lymphoma was published in early 2009 in the journal *Cancer Research*. It was likely submitted before the initiation of the Department of Health research project.

The researchers plan to submit a paper describing gene profile changes resulting from different levels of MTAP expression.

Reviewer 3:

There was a major paper published in *Cancer Research* and another one to be submitted. No commercial developments or patents resulted.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

This funding helped the PI make progress on this project and allowed for additional interactions with other investigators and trainees at the institution.

Reviewer 2:

There were no improvements to infrastructure, no new researchers were recruited, and no funding was devoted to training undergraduate or graduate students. Two existing post-doctoral fellows and the PI's salary were partially funded by this project.

Reviewer 3:

Funds were used for salaries and basic research. No obvious infrastructure improvements were noted. There were two post-doctoral fellows supported by this award. These were new investigators.

*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 PI had collaborators within the institution.

Reviewer 2:

There is no mention of new collaborations, and the research is not clinical.

Reviewer 3:

No new collaborations were stated.

### **Section B. Recommendations**

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

Reviewer 1:

The project was successful, but additional publications would have made this outstanding.

Reviewer 2:

1. A considerable amount of funding was provided for this project. One would expect more progress, as measured by submissions and publications of peer-reviewed journal articles, for this level of funding. The one published paper apparently derived from results obtained before the research period for this grant began.
2. The researchers should address the variability in experimental results mentioned above, in terms of pinning down the source of variability, leading to results that will be convincing and publishable. Overall conclusions and future directions should be clearly spelled out at the end of the progress report.

Reviewer 3:

It would help if additional outside funding and peer-reviewed publications were produced.

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**Project Number:** 0863403  
**Project Title:** The ARF Tumor Suppressor and Autophagy  
**Investigator:** Murphy, Maureen

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## *Section A. Project Evaluation Criteria*

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

### **STRENGTHS AND WEAKNESSES**

#### Reviewer 1:

The PI has described three completely independent aims that are related but completely without dependence on one another. The PI proposed to identify the domains of the ARF protein that determine mitochondrial localization, autophagy, induction and Hsp70 interaction first. Then a novel small molecule Hsp70 inhibitor (PAS) would be evaluated for its ability to kill tumor cells. Lastly, the PI proposed to determine if ARF and Hsp70 silencing inhibit tumor development in a xenograft model.

**Strengths:** In general, the PI has completed all three of the proposed aims in the original document. Their preliminary data associated ARF with both autophagy and mitochondrial localization and also showed that the PAS inhibitor interacted with and inhibited Hsp70 selectively. They investigated the mechanisms involved, including a mass spectroscopy study confirming that Hsp70 binds ARF directly. They demonstrated that PAS blocks Hsp70/ARF interaction and thus migration to mitochondria and ARF-associated autophagy. PAS sensitivity was also shown to be dependent on the level of ARF expression, with ARF deficient cells being most sensitive. The PI concluded that PAS may be an effective treatment for advanced stage cancer where typically ARF is high and p53 is low in expression levels.

The PI has completed identification of the active domains of ARF identifying distinct domains responsible for mitochondrial localization and autophagy. A defective and truncated form of ARF was confirmed not to possess these qualities.

The effects of the PAS inhibitor on cancer cells were also investigated. PAS clearly seems to directly suppress autophagy in tumor cells. It appears to suppress both degradation systems in cells that are dependent on Hsp70 and Hsp90, respectively. These results were interpreted to mean that PAS has potential as an anticancer agent in patients and that Hsp70 pathways offer a promising therapeutic target for cancer treatment as well. A new analogue of PAS, termed PAS-Cl, was also described. This molecule was evaluated in a mouse model, and it enhanced survival in a xenograft model.

**Weaknesses:** The description was brief but adequate, though more experimental details would be helpful. Despite the briefness of the descriptions in the final report, there was a surprisingly

high level of redundancy in the descriptions. Although the effects of the parent molecule PAS were well described, much less was provided concerning the treatment aspects of PAS-Cl or any adverse effects, and this will be critical to developing this reagent as a potential therapeutic.

Reviewer 2:

The project largely met two of the three stated objectives. The proposed work related to the putative involvement of ARF over expression in tumor survival through the promotion of autophagy. Aim 1 related to defining ARF domains associated with its localization, interaction with Hsp70 and promotion of autophagy. Studies were performed to directly address these questions, and a paper published in *Cancer Biology and Therapy* in 2011 presented the outcome of these studies. Aim 2 involved the evaluation of PAS, an Hsp70 inhibitor, in human tumor cells. A paper published in *Molecular Cancer Research* in 2011 indicated that PAS interfered with cancer survival pathways in various human tumor cell lines. Aim 3 was to determine the impact of ARF and Hsp70 silencing on tumor development *in vivo*. It does not appear that studies were performed to address the goals of Aim 3 or that work is continuing in this direction. Overall, the findings in the two published papers appear to support the premises driving the first two specific aims. Experimental approaches utilized were rigorous and appropriate. It is however somewhat disappointing that no studies were performed in animal models relating to tumor development (the focus of the third specific aim). Although no studies were performed to test the PAS compound as a potential antitumor drug candidate during the period of funding, such studies are planned for the future and appear to be in progress.

Reviewer 3:

This evaluation criterion was primarily a strength of the project. Based on the data provided and the publications available, the project met most of its overall objectives in that the researchers successfully characterized the major domains of ARF with regard to its localization and autophagy functions and successfully evaluated the PES compound as well as a related compound, PES-Cl, on their anti-tumor activity. The methodology used and the data generated were of sufficient quality. It is not clear from the available information if the third specific aim was technically achieved by silencing ARF and Hsp70 in human xenograft models; however, the additional data provided on the mechanism of the two drugs with regard to their interactions with the proteins, their effect on autophagy *in vivo*, and their anti-tumor effects provide much information that is similar in nature to the third aim, with the exception that their effect on tumor development has not been shown in actual human cells *in vivo*. Thus, overall, this seems like a reasonable departure from the last aim, given that significant progress was made on the aims and the most major impact of the project will primarily be drug development. The data and information provided seem completely applicable to the project goal, aims, and objectives listed in the strategic research plan.

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

### **STRENGTHS AND WEAKNESSES**

#### Reviewer 1:

Strengths: There is a clear strength in both the identification of the PAS inhibitor as a potential anticancer treatment, especially for difficult to treat later stage cancers, and in the identification of the Hsp70 pathway as a potential target for future anticancer therapeutic strategy development. Should they be successful, the PI and her program could have an important new cancer treatment in hand.

Weaknesses: Much less data was provided addressing the use of PAS and its derivative in whole animals than was originally proposed. The hypothesis that PAS sensitivity would be especially applicable to late stage tumors is both logical and plausible given the data presented. However, evaluation of this potential requires much more analysis to be performed; these conclusions regarding therapeutic value are at present somewhat more speculative representing a higher risk.

#### Reviewer 2:

The primary significance of this project for improving health relates to the potential development of the PAS compound as a cancer chemotherapeutic drug. There is also the potential to exploit the pathways identified using PAS as potential therapeutic targets. Future plans relating to development of PAS appear to be promising. Studies are under way to test PAS in experimental animal models of childhood and adult leukemia using patient samples, as well as to develop an understanding of drug pharmacokinetics and pharmacodynamics.

#### Reviewer 3:

This criterion is a major strength of the project. The PES compound and its enhanced PES-Cl derivative that was discovered during the duration of the project were shown to simultaneously disrupt several critical cancer survival pathways, including autophagy. Autophagy is a critical survival pathway in cancer cells, though to date there is only one FDA-approved compound currently for use in patients. The drugs seem to have significant anti-cancer activity and are effective on at least some forms of cancer in *in vivo* models. Therefore, these drugs and other drugs developed using the discovered mechanism of action have significant therapeutic potential in cancer treatment and may likely enter clinical trials eventually, which seems to be the future direction of the project as indicated by the investigator.

***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 reports no co-funding of the project during the funding period but does note two grant proposal submissions to NIH. One proposal in 2010 was not funded; however, a second proposal in 2009 for approximately \$1.8M was funded (for 2010). This appears to have

been a resubmission from 2008 before the current grant was initiated. The PI credits Pennsylvania Department of Health funds for allowing completion of three manuscripts that pushed the successful NIH R01 grant into the funded range. Lastly, the PI has stated that two different mechanisms were to be proposed in 2012, including an NIH grant in melanoma and a foundation grant in leukemia and lymphoma.

Weakness: None was noted.

Reviewer 2:

One grant proposal was submitted but not funded. Another proposal to NIH was funded that provided \$1.8 million towards the research. While this is quite impressive, it is not evident that the research supported by the Pennsylvania Department of Health could have directly contributed to this grant, since funding from the Pennsylvania Department of Health did not begin until January of 2009, and the grant was submitted in March of 2009.

Reviewer 3:

This criterion was a strength of the project. One R01 from NIH was funded in part due to preliminary data generated from the project, producing an approximately three-fold increase in invested funds. Additionally, although a second R01 was not funded (it just missed the top quartile), a P01 grant is currently in the submission process. Additionally, a Leukemia Lymphoma Society Translational Research Program application was submitted.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

Strengths: The PI credits Pennsylvania Department of Health funds for allowing completion of three manuscripts that, in turn, promoted successful funding of the NIH R01 grant.

The PI notes that the three publications are all now published from 2010 through 2011, and all appear to emanate from the funded efforts described. They include peer-reviewed papers in the *Journal of Molecular Cancer Research*, *Cancer Biology and Therapy* and *Trends in Cell Biology*, all of which are highly respected and ranked. The PI also notes plans to submit at least two more manuscripts for peer-reviewed publication. The quality appears to be excellent.

The PI also reports one invention that has resulted in a patent for the PAS and derived inhibitors. The patent has a number and appears to have been issued, although the PI notes that the invention was not “conceived or first actually reduced to practice” during the period of the Pennsylvania Department of Health funded project. No licenses or commercial activities related to this patent are reported.

Weakness: None was noted.

Reviewer 2:

The project resulted in three peer-reviewed publications, two of which presented primary data and one of which was a review article. These all appear to be relatively high-quality publications. The researchers indicate plans to submit two additional manuscripts; however, at least one paper was to be submitted in early/mid 2012, and this has not yet been published in the literature.

Reviewer 3:

This criterion was a major strength for the project. At least four publications (one is a review paper in a significant journal) have been published as of January, 2013, with the possibility of at least one other in the near future. Although these publications were not in the most prestigious of journals, the publications seem fairly significant. In addition, a patent for the HSP70 inhibitor has been filed, with the intention and potential for significant further commercialization of the PES and PES-Cl drugs.

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

**STRENGTHS AND WEAKNESSES**

Reviewer 1:

Strengths: The PI describes improvements in infrastructure that include two new equipment items. These include an Agilent QPCR apparatus and an Eppendorf refrigerated microcentrifuge to enhance the capabilities of the program. The PI also notes three pre-doctoral undergraduate students that were trained as part of this program. No post-doctoral trainees are noted.

Weakness: None was noted.

Reviewer 2:

It is not clear whether new investigators were brought into the institution to help carry out this research. Funds were used to support research by three pre-doctoral students and one post-doctoral student.

Reviewer 3:

This criterion was a strength of the project, since the funds were used to pay in part for the support of three graduate students. In addition, a post-doctoral fellow, whose salary was not directly supported by the grant, was heavily involved in the project. Thus, the training of future investigators was fully assisted by the project. A number of pieces of new equipment contributed to the infrastructure, and the project supported the Organic Synthesis Facility infrastructure at Fox Chase Cancer Center.

***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 PI notes real advances in new collaborations involving two other cancer research scientists at other institutions in the Commonwealth of Pennsylvania. These include Children's Hospital of Philadelphia and University of Pennsylvania School of Medicine. Both appear to have offered real advantages to the PI and the research group in moving the project forward.

Weakness: None was noted.

Reviewer 2:

This project led to extensive and continuing collaborations with other research groups.

Reviewer 3:

A number of collaborations were fostered with research partners outside of the institution.

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

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

Reviewer 1:

Although not a real weakness per se, there is a need to pursue the biological consequences of the use of PAS and its derivative in whole animals, including a comprehensive dose response and lethal/toxic dose study if this has not been completed. A study of the adverse effects is also essential for translation to human use.

Reviewer 2:

The primary reservation relating to this work is that the proposed animal studies relating to tumor development were not performed. Furthermore, although the papers published were of high quality, the level of productivity appears to be somewhat low considering the considerable amount of funding that has been made available through both the Pennsylvania Department of Health and the National Institutes of Health.

Reviewer 3:

Recommendation (not a weakness): Further development of the drugs is highly warranted, including pharmacokinetic and pharmacodynamic (PK/PD) studies and the possible introduction of the compound in clinical trials.

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**Project Number:** 0863404  
**Project Title:** Anti-Glucose Transporter-1 Antibodies as a  
Novel Treatment against Human Cancers  
**Investigator:** Simon, George

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

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

#### **STRENGTHS AND WEAKNESSES**

##### Reviewer 1:

The original projects had two specific aims that were planned to be executed between January 1, 2009, and December 31, 2011. However, the project ended prematurely on January 31, 2010, apparently in view of the departure of two post-doctoral fellows working on the project, refocusing of the PI's work onto clinical care, and eventual closure of the PI's laboratory at Fox Chase Cancer Center with his departure to another institution (Medical University of South Carolina).

The project only partially met its stated objectives. The PI states that regarding Aim 1 they have worked out mechanisms by which Glut-1 antibody induces cell kill. However, no information is provided regarding correlation of Glut-1 expression with overall survival and disease-free survival. Regarding Aim 2, only some conceptual aspects of the aim were clarified without final production and testing of a viable anti-Glut-1 antibody suitable for humans.

In summary, the project made limited progress toward achieving the two aims without productivity in terms of relevant publications or submission of other grants during or after the project period. Only one relevant publication by the PI is noted on PubMed, but that was published in 2007 prior to funding of this project (Rastogi S, Banerjee S, Chellappan S, Simon GR. Glut-1 antibodies induce growth arrest and apoptosis in human cancer cell lines. *Cancer Letter* 2007). The investigators may like to refer to the following paper that outlines potential strategies for use of glycolysis as target for therapy (Gatenby RA, Gillies RJ. Glycolysis in Cancer: A Potential Target for Therapy. *The International Journal of Biochemistry & Cell Biology* 2007; 39:1358-1366).

##### Reviewer 2:

The project failed to meet any of its stated objectives or milestones. In his strategic plan, Dr. Simon proposed two specific aims: In Aim 1, he proposed to correlate GLUT1 expression in primary human non-small cell lung cancer (NSCLC) specimens with overall survival and disease-free patient survival. In Aim 2, he proposed to develop fully human IgG1 antibodies against the first extracellular aminoterminal loop of GLUT1 that would ostensibly be capable of blocking facilitated glucose uptake via this transporter. There is no evidence presented in the

form of unpublished data, documented preliminary reports (e.g., meeting presentations or published abstracts), or finished research products (e.g., peer-reviewed manuscripts, either published or in process) to suggest that either aim was fully or partially achieved. The final progress report indicates no intention to rectify these deficiencies and affirms this general impression. With this in mind, it bears noting that the awardee has published a single peer-reviewed original manuscript topically relevant to this award. That publication predated receipt of this award (2007) and served as the principal basis for the work proposed in this project. No other productivity relevant to the proposed line of research could be found via the usual online search engines (PubMed, NIH RePORTER, etc.).

- Aim 1 was purely descriptive, was fully independent of Aim 2, and involved existing clinical material and reagents; so it is not clear why this aim was not achievable within the allotted project period. Reported *in vivo* difficulties using commercially available antibodies to kill tumor cells in Aim 2 should have had no impact on their use as immunohistochemical probes in Aim 1.
- Aim 2 proposed to generate and test human anti-GLUT1 using the established core resources at Fox Chase Cancer Center. Since this aim was fully independent of both Aim 1 and the reported functional ineffectiveness of commercially available antisera-- and was specifically proposed to circumvent such difficulties--it is also not clear why this aim was not fully achievable. In other words, the reported technical difficulties encountered by the PI were wholly anticipated and posed no barriers to the conduct of the work as proposed.

Publicly available progress reports for Department of Defense award W81XWH-07-1-0348 detail identical objectives, preliminary findings, and technical issues outlined in the strategic plan and progress reports for the present award. Since this award is not listed as concurrent support, it appears that the applicant was previously funded to perform similar, if not identical, work, raising additional concerns regarding the complete lack of documented productivity related to this general line of inquiry. A 2008 Department of Defense progress report (DTIC Accession No. ADA494504) is almost identical to progress reports subsequently filed for this project between 2009 and 2011. The antibody-generating capabilities of the Fox Chase Cancer Center antibody core facility was uniformly promoted as the answer to all encountered technical difficulties in these reports. These types of contractual arrangements typically yield deliverable products within a predictable time frame. It is unclear, however, whether or not human anti-GLUT1 antibodies were ever generated.

Preliminary data included in the strategic plan were limited and of marginal quality. No substantive additional data were provided in subsequent annual or final progress reports. The experimental design and strategic plan were incompletely developed and unlikely to unambiguously achieve the proposed objectives. Specific issues are outlined below:

Studies correlating NSCLC GLUT1 expression with clinical outcomes were proposed using an existing tumor tissue repository. Specimens to be tested were to be drawn from Fox Chase Cancer Center patients between 1991 and 2001 undergoing thoracotomy for resection of primary lung cancer with intent to cure. Patients receiving prior chemotherapy or radiotherapy were to be

excluded. Specific clinical characteristics, demographics, and other comorbidities to be analyzed were not clearly delineated.

All proposed immunohistochemical analysis of NSCLC GLUT1 expression involved automated methods (AQUA) with division into two broad categories of expression for analysis – high (upper 50% of samples) and low (lower 50% of samples). No normal tissue controls were included, and no corresponding manual analysis of temporospatial expression patterns was referenced, demonstrated, or proposed. The rationale for this specific experimental design was neither detailed nor justified. It is not clear how intratumoral and/or intracellular heterogeneity in expression were to be addressed.

GLUT1 is monolithically considered both conceptually and experimentally without regard to the potential co-expression of other glucose transporting mechanisms. Both normal and pathological tissues frequently express more than one facilitative glucose transporter, and secondary active glucose transport systems (typically seen in glucose-transporting epithelial tissues) are also well described. From a functional perspective, the proposed examination of GLUT1 alone without consideration of other potentially redundant or inducible glucose transport systems seems a non-trivial omission.

As an extension of the concern above, tumor substrate preferences for glucose are well described, but tumors are also capable of utilizing alternative endogenous and exogenous substrates to meet their catabolic and anabolic needs.

The facilitated uptake of glucose is functionally coupled to its intracellular phosphorylation by hexokinases which catalyze the first committed step of glucose utilization and thereby maintain the concentration gradient required for facilitated glucose entry into cells. Since these enzymes have also been implicated as contributors to dysregulated cancer metabolism, tumorigenesis and cancer progression, it is not clear why hexokinases were not also considered in the experimental design.

All testing, both preliminary and proposed, examined tumor cells without examining corresponding effects on their normal cell counterparts. As such, no work was proposed or conducted to establish specificity for tumor cells per se. The consequences of systemic disruption of GLUT1 function were also incompletely considered. These are not trivial considerations insofar as GLUT1 is widely expressed in both adult and developing tissues, with highest expression levels observed in normal erythrocytes and endothelial cells. Although not the predominant isoform in most other normal tissues, it is thought to play an important and ubiquitous role in basal cellular glucose uptake, and increased GLUT1 expression has been reported in a number of both neoplastic and non-neoplastic pathological conditions. GLUT1 is also thought to play an important role in glucose transport across the blood-brain barrier, a disrupted function that would be predicted to have profound effects on normal brain metabolism.

The awardee suggests that tumor GLUT1 differs from wild-type transporter expressed in normal tissues, although no structural or functional evidence is provided to support such a contention. It also bears noting that the limited preliminary data and prior work used to justify the proposed

work all employed commercially available antibodies directed against highly-conserved wild-type GLUT1.

The investigator hypothesized that antibodies directed against GLUT1 utility of erythrocytes as an antibody sink. The functional consequences in either erythrocytes or other GLUT1-expressing cell types are not addressed.

Preliminary cell culture data suggesting an ~30% decrease in total ATP content following anti-GLUT1 treatment is difficult to interpret. No functional consequences were examined, and fundamental considerations of the types and amounts of energy substrates available and corresponding catabolic demands are conspicuously absent.

Cross-reactivity of the GLUT1-directed antibodies that Dr. Simon proposed to generate in Aim 2 is incompletely addressed both conceptually and experimentally.

In reviewing the general lack of productivity, it is clear that pitfalls and alternatives were incompletely considered.

Reviewer 3:

The project did not make reasonable progress and did not meet the stated objectives. The research was not performed due to lack of specified personnel and closure of the principal investigator's research lab. While there is a statement on the identification of specific mechanisms associated with anti-GLUT1 directed therapy and a proposal for development of new inhibitors to alternative targets, this data is not described in the final performance review.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

The project's findings will not have significant immediate impact on human health until the original aims of the research plan are actually fully investigated. It is unclear if the PI is planning to continue along the same line of work at his new location at Medical University of South Carolina.

Reviewer 2:

The likely beneficial impact of this project is deemed to be minimal. The awardee raised an important, albeit not particularly original, idea for consideration in proposing to target GLUT1 and glucose metabolism for therapeutic benefit in cancer. Tumor cells are characterized by fundamental changes in metabolism required to support the catabolic and anabolic demands of rapidly proliferating tumor cells. As such, targeting metabolism to prevent or reverse cancer genesis and progression is an area of great biomedical interest, albeit with a number of practical accompanying challenges that relate to the fact that nonselective maneuvers to alter tumor metabolism may have profound consequences on normal cell metabolism and metabolic homeostasis. Although this project addresses an area of major biological and clinical

significance, the prospects that the proposed work will meaningfully inform our fundamental understanding of tumor metabolism or will translate into viable therapeutic interventions are deemed nominal. As noted above, the limitations of the proposed work are numerous and incompletely considered. The general feasibility of systemic GLUT1 disruption also remains to be established.

Performance measures outlined in the original strategic plan included independent publication of results for both Aims 1 and 2, anti-GLUT1 antibody generation by the Fox Chase Cancer Center antibody core (Aim 2), and an NIH grant submission by 2011. As noted above and elsewhere in this review, none of these measures were apparently achieved, and no measurable products or tangible evidence of scientific progress have resulted from this project despite generous funding from both this award and the Department of Defense dating at least to 2007. In light of the generous funding available, relatively straightforward and readily achievable objectives, and ample time and resources to permit completion of the studies as proposed, the lack of demonstrable products seems unacceptable at best and wasteful at worst.

Reviewer 3:

The beneficial impact of this project is negligible. The research proposed was never completed. The initial experimentation performed (i.e., demonstrating that normal red blood cells express GLUT1 and that two commercial antibodies bind to GLUT1 expressed on red blood cells) undermines the basis of the proposal. It is well known that GLUT1 is ubiquitously expressed which is the current caveat to targeting tumor glucose metabolism by inhibition of GLUT1. The investigator proposes to switch to investigating alternative GLUT1 inhibitors, which is not justified if the agent continues to target GLUT1 on normal cells. While there is continued interest in the area of targeting glucose transport and abnormal glucose metabolism for cancer therapy, this particular project failed to incorporate specific controls – testing anti-GLUT1 antibodies for its impact on glucose uptake and viability in cells that do not express GLUT1, such as the U937 cell line (as described in the PI's *Cancer Letter* 2007 publication) and testing in normal cell types. In addition, strategies to address the impact of these agents on normal GLUT1 function should have been included in the initial submission of this proposal. The PI is continuing work at a different institution on the development of inhibitors to what appears to be alternative targets that are not detailed.

***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 Department of Defense grant is mentioned that was apparently funded prior to this project and served as providing some relevant preliminary data. However, no additional research grants or funding were received.

Reviewer 2:

The original strategic plan anticipated submission of an application for independent NIH support by 2011, ostensibly based on preliminary and published data arising from this project. There is,

however, no demonstrable leveraging of this project or CURE support to obtain additional funding of any kind after the PI's arrival at Fox Chase Cancer Center. Dr. Simon makes several internally inconsistent and conflicting statements regarding long-term plans for this project, but early references to his lab's closure and to refocusing his career in a clinical direction in the 2009-10 annual progress report suggest that the project has been abandoned. Subsequent progress reports do little to alter this impression despite vague allusions to continuing this line of research at the Medical University of South Carolina.

No concurrent funding was listed in any of the CURE progress reports. As noted elsewhere in this review, however, a publicly available 2008 Department of Defense progress report (DTIC Accession No. ADA494504) for an identical project (DoD W81XWH-07-1-0348; Simon and Banerjee, co-PIs at USF) filed immediately before the PI's transfer to Fox Chase Cancer Center suggested that this work would be continued at his new institution. Whether or not the Department of Defense continued to support this work after transfer to Fox Chase Cancer Center is unclear. If so, questions of scientific and budgetary overlap with the present project should be directly addressed. If not, the fact that this work was previously funded by another agency raises additional concerns regarding the lack of demonstrable related research productivity.

Reviewer 3:

The project did not leverage additional funds or provide data for additional grant applications. Negative results have led to the proposal of alternative strategies that are not detailed.

***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:

None resulted.

Reviewer 2:

This project produced no measurable products in the form of either preliminary reports (e.g., abstracts or meeting presentations) or published peer-reviewed original manuscripts.

In the final progress report, there are vague references to the identification of novel GLUT1 inhibitors arising from this work that ostensibly mimic the action of GLUT1 antibodies. No details are given regarding the identity, functional relevance, mode of action, or specific relation to this project. These are followed by brief unclear references to future intended work, not substantiated elsewhere in the review material:

- "Work with these inhibitors confirmed our suspicions that generated ideas for clinical trials that are currently under consideration for funding by private agencies." The nature of the mechanistic "suspicions" and the ideas for clinical trials are not detailed. Do these ideas differ from those initially proposed? If so, how and why?
- The PI goes on to remark, "The confidential nature of these collaborations preclude me from revealing this here but are from private agencies." Insofar as this is a confidential review, and

the review process affords the awardee ample opportunity to redact confidential information from final public reports, it is not clear exactly what cannot be disclosed.

- The vague references above do not represent an acceptable accounting of progress toward the stated objectives, nor acceptable substitutes for the original experimental aims without further qualification. In fact, in view of the general lack of demonstrable related productivity, these claims seem somewhat suspect.

A cursory patent database search revealed an international patent application (PCT/US2008/073361; Oncotherapeutic Application Of Inhibitors of High-Affinity Glucose Transporters) by the awardee and the University of South Florida dated 15 August 2008. An identically named U.S. Patent Application (12/673,677) was filed at the same time. No disclosure of these applications was found in any of the progress reports. Clarification would seem to be in order.

Reviewer 3:

The project did not result in any peer-reviewed publications, licenses or patents; however, it appears to have led to commercial opportunities that have not been disclosed. Future submissions of articles/patents are not likely based on the current data.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

No data is provided to judge if the quality or capacity for research at Fox Chase Cancer Center was enhanced specifically through this project.

Reviewer 2:

All proposed and executed work ostensibly used existing resources and capacities.

An automatic specimen labeling and handling system purchased for the institutional biosample repository does not appear specific to this project. All biorepository samples and accompanying information proposed for use in these studies were preexistent and had been deposited into the biorepository one to two decades prior to the initiation of this project.

There is no evidence that the program established new research capabilities or contributed to the professional development of anyone other than the PI.

Two post-doctoral associates accompanied the PI to Fox Chase Cancer Center from University of South Florida. Notably, both left the project early in the project period (Banerjee, 100% funded for 1 year; Das, 100% funded for 2 years), and these departures are later cited by the PI as a reason for his lack of productivity. No details regarding the circumstances surrounding these departures or any information regarding their subsequent destination(s) or professional development were provided. Dr. Banerjee, listed as one of the post-doctoral trainees on this

award, was also previously listed as co-PI with Dr. Simon on their topically identical Department of Defense award at University of South Florida (W81XWH-07-1-0348).

Reviewer 3:

There do not appear to be improvements to the infrastructure of the institution or hiring of new personnel, since the project was not completed and the PI has left this institution. Training of two students is indicated; however, it is not clear if funds were used in their training.

***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:

There is no evidence or stated intention of engagement with outside research partners in work associated with this project.

Dr. Simon proposed to partner with Drs. Adams and Robinson at Fox Chase Cancer Center in the generation and testing of epitope-specific antibodies directed against the human facilitative glucose transporter, GLUT1 (Aim 2). Dr. Robinson was named as a material collaborator in this project and was charged with the oversight and direction of the antibody engineering component of Aim 2. It is not clear whether antibody generation efforts were initiated and successful or not. If not, why not? If so, what is the present stage of development for this project?

Reviewer 3:

The principal investigator has moved to a new institution and indicates that he has established new collaborations. He also appears to have redirected his research efforts to clinical research.

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

### **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

#### Reviewer 1:

1. The investigators may like to refer to the following paper that outlines potential strategies for use of glycolysis as target for therapy (Gatenby RA, Gillies RJ. Glycolysis in Cancer: A Potential Target for Therapy. *The International Journal of Biochemistry & Cell Biology* 2007; 39:1358-1366).
2. The investigator should state if this line of work will be continued and if not what may be the major scientific obstacles that were identified through his initial attempt. The investigator should state how another investigator can continue this work or whether it should be continued at all by any other investigators.
3. The project ended prematurely. There were no publications and no additional funding. The aims were only partially achieved, but the exact details are missing. Apparently, the proposed line of work will not be continued.

#### Reviewer 2:

1. The most serious concern is that there is little evidence that the work was conducted as proposed. In fact, the application and progress reports provide substantial evidence that the converse may be true. It is therefore difficult to provide specific recommendations for improvement without more specific detail. At a minimum, the work should be completed as proposed and fully presented.
2. In addition to the under productivity or nonproductivity issues above, the experimental design was largely descriptive and incompletely developed. Specific weaknesses included lack of proper controls and corresponding metabolic studies. These should be individually addressed. The feasibility and efficacy of systemic disruption of GLUT1 function have also not been established. The most direct approach might involve the generation and testing of conditional GLUT1-deficient adult mice. If successful, this would validate the viability and potential efficacy of this approach and would remove a number of ambiguities associated with unsuccessful GLUT1-directed antibody or small molecule attempts.
3. There was no demonstrable research productivity that can be directly attributed to this work. No abstracts, publications, or grant applications are listed. The project failed to meet any of its stated objectives, and there is little, if any, evidence that acceptable progress was made in meeting them.
4. Tangible benefits arising from this project are highly unlikely for reasons outlined above.

Reviewer 3:

1. In general, the premise for this particular project was not based on strong preliminary data. The PI had one publication (Cancer Letter, 2007) that suggested the utility of targeting GLUT1 for the treatment of breast cancer. However, this publication lacked several critical controls (i.e., testing the impact of anti-GLUT1 on glucose transport at early time points), testing the impact of anti-GLUT antibodies on glucose transport and cell viability/proliferation in cell lines lacking GLUT1 (such as U937) or normal cells. And it did not utilize alternative RNAi based strategies to validate the role of GLUT1 in facilitating glucose transport and/or viability in the tested cell lines. The absence of a robust system to test target specificity and efficacy therefore undermined the proposed research strategy. This, in addition to minimal preliminary data (impact on pAMPK and pAKT), did not strengthen the rationale for Specific Aim 1. While work from other groups continues to substantiate the utility of targeting GLUT1 for cancer therapy, this project failed to take into account testing the impact of developed agents on normal cell types, and they appear to have realized that after the project was initiated. Subsequent efforts at targeting GLUT1 seem to have been directed towards identification of a tumor specific GLUT1 epitope but were terminated with the closure of the PI's laboratory.
2. While the effort towards targeting GLUT1 is worthy of investigation, the PI did not have the right systems in place to evaluate critically the target specificity of the GLUT1-directed agents or to evaluate efficacy. Critical evaluation of prior publications and preliminary data appears to be lacking prior to funding this proposal.
3. The PI has closed his research lab and moved to a new institution; therefore, there are no recommendations for future improvement of this particular project.

**Recommendations for Fox Chase Cancer Center**

Reviewer 2:

It is not clear whether Dr. Simon's lack of productivity could be attributed, in part, to either competing professional obligations or lack of protected time or support. It is also not clear how rigorously the protocol was reviewed for scientific merit prior to initiation or whether institutional or departmental processes were in place to monitor progress and address barriers to successful completion. Institutional core resources played critical roles in both aims, but their proportionate responsibility and accountability for a lack of demonstrable research products are also not readily apparent. In other words, does Fox Chase Cancer Center share some responsibility with the PI for the lack of acceptable research productivity?

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**Project Number:** 0863405  
**Project Title:** Regulation of Human Somatic Wee1 by Cyclin A/Cdk2 Complexes  
**Investigator:** Enders, Greg

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### *Section A. Project Evaluation Criteria*

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

#### **STRENGTHS AND WEAKNESSES**

##### Reviewer 1:

The investigators set out to test the hypothesis that cyclin A/Cdk2 complexes direct Wee1 nuclear export via phosphorylation of T173 and to elucidate the functional role of Wee1 nuclear export by knock-in mouse technology. The investigators discovered that cyclin A/Cdk2 might not be the sole kinase responsible for this phosphorylation event and have not made much progress toward mouse engineering or characterization. They have not yet produced a publication from this work nor submitted a federal grant application to continue the work.

##### Reviewer 2:

The main focus of this application is on the regulation of Wee1, a nuclear kinase belonging to the Ser/Thr family of protein kinases, which phosphorylates the ATP binding site of Cyclin-dependent kinase 1 (Cdk1) thus inhibiting entry into mitosis in all human cells, a key regulator of cell cycle progression. The overall proposal is based on the hypothesis that cyclin A/Cdk2 complexes direct Wee1 nuclear export via phosphorylation of Thr173 (Aim 1). The PI plans to elucidate further the functional role of Wee1 nuclear export (Aim 2). The approaches are mainly mechanistic.

**Strengths:** The project is relevant to the regulation of cell division, and cell division dysregulation is a hallmark of cancer.

Regulation of Wee1 is largely unknown and needs to be clarified.

Generated the first mouse knock-in mice with mutation in the Wee1 regulatory region (SITE1E) - Modified Aim 2b (see below).

**Weaknesses:** The relevance of Wee1 in cancer is not shown, and it is speculative at this point. For example, functional inactivation of Wee1 by cyclinA/Cdk2 complex is not demonstrated in cancer cells, and cyclinA/Cdk2 may not be the only kinase that targets Wee1 and therefore may be redundant.

The approaches and research design described in this proposal are not innovative.

The project lacks a Wee1 mouse model for understanding the regulation and its impact on cell cycle progression.

Details or data of the knock-in mice (SITE1E) are missing.

Several technical difficulties were faced and appropriate changes were made to the research protocol:

- Aim 1: with the specificity of the antibodies and dissection of the NES/RxL1 regulatory region in mWee1.
- Aim 2: low transfection efficiency; difficulty in getting the mouse knock-in mutant lines, cloning the mWee1 region, etc.
- Therefore, a number of subaims have not been completed during this grant period (Aims 1a, 1c, 1e; and Aim 2a).

#### Reviewer 3:

As even the PI states, the initial proposal was overambitious to begin with, and many of the subaims are incomplete. Only Subaims 1b and 1d appear near completion. The lack of progress on Subaims 1a, 1c, 1e, and 2a is somewhat striking/puzzling. These experiments are not that technically difficult and were likely ignored. The results of Subaim 1d introduced more questions than answers, which is not necessarily a problem, since it introduced an interesting further avenue of research that will likely support future manuscripts or grants. The generation of knock-in mice (Subaim 2b) was ill-suited for this grant; and, not surprisingly, it is going slower than expected. However, it will eventually produce results. Overall, there is some progress.

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

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

#### Reviewer 1:

I consider the results quite modest and somewhat disappointing in light of a \$581K award.

#### Reviewer 2:

Future plans for the project are to apply for a competitive renewal R01 application R01 and to publish a manuscript.

**Strengths:** Identifying a specific molecular pathway through which Cyclin A/Cdk2 complex inhibits Wee1 is of interest in the regulation of cell division and in the development of a new drug inhibitor as a cancer therapy.

**Weaknesses:** Wee1 may or may not be a valuable target for cancer chemotherapy.

Significance of this project for improving health is very limited due to the *in vitro* setting and lack of *in vivo* data. The overall results are still preliminary at this stage, and a number of studies are incomplete.

Reviewer 3:

Like most basic research projects, the short-term impact of this project is small. It will take many years to fully realize the impact of any discoveries from this project. This time frame is not unreasonable. The budget is higher than normal, likely due to animal costs and commercial contracts for certain technologies. The project seems slightly behind schedule for the costs incurred. As even the PI acknowledges, the initial proposal was too ambitious.

***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:

None were leveraged or submitted.

Reviewer 2:

There was no leveraging of funds or any additional grant application submitted.

Reviewer 3:

A future NIH R01 application is planned.

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

None resulted.

Reviewer 2:

There was no published manuscript or patent filed at this stage.

Reviewer 3:

A future publication is planned (but will likely take much longer than the PI suggests).

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

***STRENGTHS AND WEAKNESSES***

Reviewer 1:

Funds were used to support an associate professor and a post-doctoral fellow at the Fox Chase Cancer Center.

Reviewer 2:

Funds were used to pay one post-doctoral fellow (45%), a technician (70%) and 50% of the PI's salary.

Reviewer 3:

One post-doctoral fellow worked on this proposal.

***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:

The proposal did not lead to collaboration.

Reviewer 3:

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

**Section B. Recommendations**

**SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

Reviewer 1:

It appears that the basis for the project may not have been quite as sound as proposed. It is not clear to me how compelling this problem is, going forward.

The project had no publications or grant submissions, limited scientific impact, and no community outreach.

Reviewer 2:

Overall, the project did not meet the goals due to several technical difficulties and lack of back-up plan for both aims.

1. Weakness: Aim 1 should not rely solely on the generation of reagents such as antibodies.

Recommendation: The experimental design should have a "Pitfalls" section, and other techniques/experimental designs need to be implemented rapidly. Better validation using *in vitro* studies and better design of the targeted sequence could have been more successful.

2. Weakness: For Aim 2, generating mice knock-in mutant lines can be long and tenuous.

Recommendation: Validation using either pre-clinical models or a stable expression using cell lines could have been an alternative for studying the effect on cell proliferation.

Reviewer 3:

1. The structure of this grant is flawed in terms of time frame, as the PI acknowledges. Two years is not sufficient time for the generation and characterization of mouse models. However, this reviewer does not have a problem with this type of grant funding the initial establishment of mouse models. (It is difficult to obtain such funding from other sources, but it is important.)
2. There are several specific/technical problems with the proposal. It is unclear why Subaims 1a, 1c, 1e, and 2a were not completed. Subaim 1a requires only simple interaction experiments (co-IPs, GST pulldowns, etc.), which are not difficult. Likewise, Subaim 1c requires only cell cycle synchronizations. Subaim 1e requires only that the experiments used as preliminary data for Wee1 be applied to Cyclin D. The technical impasse described for Subaim 2a should easily be overcome through the use of retrovirus-mediated gene transfer. These experiments are all simple and could have easily been completed within the two-year grant.
3. The outsourcing of certain tasks to commercial vendors is somewhat questionable in terms of expense and time saved.