

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 (1.84)

Project Rating:

Project	Title	Average Score
1085401	Disulfides to Modulate Thiol Homeostasis in Human Colon Cancer Cells	Favorable (2.00)
1085402	Role of TIMP-4 in Breast Cancer Assessment and Treatment	Favorable (1.67)

Project Number: 1085401
Project Title: Disulfides to Modulate Thiol Homeostasis in
Human Colon Cancer Cells
Investigator: Ayene, Iramoudi S.

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 first objective was met, and the second was partially met. The metabolism of the 16 target compounds found to be soluble in aqueous buffer was determined, and the sensitivity of cellular glutathione levels to the three compounds found to be most heavily converted to sulfhydryls was determined. The effect of the other 13 compounds that were suitable for study and more resistant to metabolism on cellular glutathione was not studied.

Weaknesses: There was no explanation for why the 13 other compounds were not simply screened to determine whether they affected glutathione levels. This did not seem to make sense, and it was contrary to the purpose of the study as stated--to select compounds poorly metabolized under oxygenated (or non-oxygenated) conditions and determine whether they would suppress cellular glutathione. It seems they have focused on the compounds that did not meet their criteria and so did not really complete their objectives.

The underlying premise of this study was not very promising. The investigators stated that they could enhance the effects of ionizing radiation against cultured cells but failed to do so against a human tumor xenograft, citing data not shown. They concluded that this was because the lead compound was being highly metabolized by tumor cells, with no evidence that this was, in fact, the case. It would seem more likely that the compound was being metabolized by hepatic or renal tissue, if the root cause of the failure to enhance radiation *in vivo* was metabolism at all. Hence, the premise was not well-founded, and the investigator went on to design experiments *in vitro*, when the relevant questions were all *in vivo*. Having done so, they ignored the 13 compounds that met their somewhat questionable criterion.

Reviewer 2:

Based on the observation that hydroxyethyl disulfide (HEDS) depletes glutathione (GSH) and increases the response of glucose deprived cancer cells to radiation, this project has screened 16 disulfides for better stability and dependence of low glucose availability. In a series of experiments, the investigator has measured the conversion of disulfides into sulfhydryl in the extracellular medium and the effect on intracellular thiol redox levels in HCT116/29 cell cultures in the presence and absence of glucose. Some agents were converted more slowly (only in the

presence of glucose) and therefore are thought to represent possible therapeutic agents. The stated objectives have been achieved, although they are rather limited in scope (few conditions, no hypoxia *in vitro* attempted, any other measurements of cellular redox status, effects of the reduced thiol compounds per se, etc.).

Reviewer 3:

The goals of the original application to determine the ability of 14 disulfides to be metabolized and deplete glutathione in the presence and absence of glucose in colon cancer cells were achieved.

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: None were obvious.

Weaknesses: This project had no foreseeable positive effect on human health, nor does it seem likely that any additional federal research dollars would be obtained from NIH grant applications based on this work.

Reviewer 2:

The authors state that the disulfides under study could become useful as molecular probes or drugs.

Three new disulfides likely have better efficacy than HEDS in killing glucose deprived cancer cells in solid tumors. Activities were observed at 1-5 mM, but the *in vivo* requirements are unknown, so that an extrapolation to therapeutic utility is still uncertain at best. One would have liked to have seen activity of the compounds in the micromolar range, at least in glucose depleted conditions. Given the modest budget, the provided experimental results are reasonable; but, given the emphasis on disulfides in this lab, one would have expected to see some more diverse analyses to explore the potential for new therapeutics.

Reviewer 3:

The results are likely to have a significant impact if they lead to the development of novel compounds that are selectively toxic to cancer vs. normal cells in the glucose restricted condition. The logical next step, which has been proposed by the applicant, is to test this hypothesis using clonogenic cell survival experiments. The only minor weakness is that it would have been helpful to show that when GSH was depleted by drug treatment that glutathione disulfide (GSSG) increased inside the cell and that drug toxicity could be inhibited with the thiol antioxidant, N-acetylcysteine (NAC).

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 leveraging of funds resulted from the pursuit of this project.

The investigator submitted applications for an NIH R03 and an NIH R21. Neither was funded. The investigator stated that a good score was obtained but did not state what that score was. Although the investigators plan to submit grant applications, the data presented in this report would not support a viable NIH R21 or R01.

Reviewer 2:

No co-funding was listed for this small grant; one small NIH grant is pending and two applications were not funded. All three grants are being converted to larger NIH applications; the chances for success are uncertain, while the basic idea of sensitization to radiation by disulfides under low oxygen/glucose conditions is interesting.

Reviewer 3:

Three new applications were submitted to NIH using this work as preliminary data, but none has been funded yet. The applicant is preparing resubmissions of these applications.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

No publications resulted from this work two years after it was finished.

Reviewer 2:

No publications or patents are listed yet.

Reviewer 3:

There were no publications submitted yet, but the applicant plans to submit publications once the differential toxicity issue mentioned above has been addressed.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

This project did not have any positive effect on the infrastructure at the Lankenau Institute.

Reviewer 2:

The grant apparently has helped maintain a drug discovery environment on a limited scale.

Reviewer 3:

The project has stimulated new collaborations at the institution. The project involved one undergraduate student in the research activities.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

No.

Reviewer 2:

No.

Reviewer 3:

The project does not appear to have led to collaborations outside the institution.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

1. The premise that the limited utility of HDES as a radiation sensitizer is due to rapid metabolism should be experimentally tested *in vivo*: does a therapeutically relevant dose of HDES depress GSH in *in vivo* tumors?
2. The premise that the tumor cells are the reason why HDES is metabolized *in vivo* should be tested by directly determining whether tumor bearing mice metabolize HDES any faster than sham-treated animals.
3. The 13 compounds found not to be metabolized appreciably *in vitro* should be tested for suppression of GSH levels.

Reviewer 2:

1. Unless more potent compounds can be identified, clinical application is questionable. All experiments were performed *in vitro*, and even in the absence of glucose, effects were seen only at or above 1 mM.
2. Only a limited number of experimental conditions have been explored; selectivity against cancer cells is unknown for the new compounds. The effect of hypoxia (prominent in their discussion) has not been addressed. These *in vitro* studies should be done on a larger scale, to explore more angles faster and more efficiently.

3. The investigator should include experiments with other means of depleting GSH, for comparison to their approach. Also, effect on cytotoxic potency of common anticancer drugs should be explored. (This could lead to micromolar potency.)

Reviewer 3:

I recommend the applicant move forward with testing in clonogenic cell survival experiments to determine if the new disulfide compounds (identified as causing the greatest increase in GSH depletion and GSSG accumulation in the presence of glucose deprivation or 2-deoxyglucose) are differentially toxic or radiosensitizing or chemosensitizing in cancer vs. normal cells. This should be combined with studies to see if NAC can rescue cancer cells from drug-induced cell killing.

ADDITIONAL COMMENTS

Reviewer 1:

This project was based on a premise that was unproven, namely that the effectiveness of disulfide compounds to enhance radiation effect was limited by the metabolism of these compounds by tumor cells, which did not seem to be the limitation of the approach at this moment.

Having started with that premise, the investigator proposed to find alternative disulfide compounds that were not as avidly metabolized as the initial compound hydroxydiethyldisulfide (HDES) and then test them as suppressors of GSH concentrations in colon cancer cells in culture. They found 13 such compounds and then did not study them as suppressors of GSH.

Project Number: 1085402
Project Title: Role of TIMP-4 in Breast Cancer Assessment and Treatment
Investigator: Wallon, Margaretha

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:

Objective 1: The investigators did make some progress, since they were able to evaluate serial TIMP-4 serum levels in 13 patients with breast cancer.

Strengths: The investigators have demonstrated that they have the ability to detect TIMP-4 levels in the serum from patients with breast cancer, regardless of tumor burden. (The levels were detectable both prior to surgical removal of the tumor and after surgery and chemotherapy.)

Weaknesses: The study performed included a very small sample of patients, and the patients studied were not uniformly treated. As such, it is hard to draw any meaningful conclusions from the study. The investigators report that larger studies are planned.

Objective 2: The investigators were unable to perform the studies outlined, since there was a delay in construction of their animal vivarium. These issues have since been resolved, and the studies have been initiated; however, results were not available at the time of the final progress report.

Strengths: The investigators were able to develop successfully the model to be used for the experiments outlined in the proposal.

Weaknesses: There was a significant delay in initiating the experiments, and no results were available at the time of progress reporting.

Reviewer 2:

The research project is on track to complete the stated objectives. Data were developed to answer research questions and objectives. The target accrual goal of 20 breast cancer patients post-surgery to measure TIMP-4 levels has not yet been reached, but the investigators have full intention to complete accrual (and many more patients via future/alternative funding mechanisms). All the infrastructure is in place, and preliminary analyses and summaries of the trajectories of TIMP-4 levels in thirteen patients have been adequately performed. The 13 patients who have been accrued are continually being followed, and TIMP-4 is being measured.

The additional patients will most likely be accrued through an R21 mechanism from NIH. In this sense, the pilot data achieved thus far will set the foundation for this R21 grant application.

Progress with the second objective was made (animal experiments) but at a somewhat slower pace than what was expected due to construction at the animal facility. Construction has been completed and is no longer an obstacle. Animal experiments were performed with nude mice. Researchers are waiting to compile final data.

Reviewer 3:

The project planned to assess the use of a new therapeutic agent to target the triple-negative breast cancers (TNBC). Previous research showed that elevated levels of tissue inhibitor of metalloproteinases-4 (TIMP-4) in TNBC are associated with poor prognosis for disease-free survival. The project had two aims. Aim 1 was to assess the effect of tumor burden on circulating TIMP-4 level in breast cancer patients, and Aim 2 was to study the effect of PI3K-inhibitors in breast cancer cells with elevated TIMP-4 levels in animal models. Overall the study was preliminary, yet it might have important clinical implications. The study partially met the stated objectives probably due to the preliminary nature of the project. For Aim 1, 13 breast cancer patients were recruited and analyzed, but the sample size was too small to draw any conclusion. Aim 2 was somewhat delayed due to technical reasons, yet the work was continuing. Overall the project partially met its objectives, but more work needs to be done.

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 investigators have previously shown that TIMP-4 elevation is associated with poor prognosis in TNBC. They hypothesize that because TIMP-4 is a marker of poor prognosis, blocking downstream effects of TIMP-4 may be an effective treatment for aggressive TNBCs.

Strengths: There are limited targeted treatment options for patients with TNBC, and newer therapies are needed. Based on data generated prior to the current study, TIMP-4 appears to be a relevant and potentially targetable pathway in the management of TNBC.

Weakness: Unfortunately, it is unclear from the preliminary data of TIMP-4 levels outlined in the progress report if TIMP-4 serum levels are of any significance or utility. The small numbers of patients studied and the variability in their treatment make the results uninterpretable.

Reviewer 2:

This research is very important, since triple negative breast cancer patients have fewer therapeutic options available to them and poorer outcomes. Gaining a deeper understanding of the disease process is critical for proper treatment and drug development. This research agenda attempts to identify and evaluate a potential prognostic marker (TIMP-4). Furthermore, the overall intention of this research is to determine whether this marker is indeed a predictive marker for PI3K inhibitors. This work was preliminary data gathering, and the researchers will

apply for NIH funding to develop this further. This work can impact treatment decisions in the management of triple negative breast cancer.

Reviewer 3:

The project had important clinical implications, since it specifically targeted breast cancer patients who are known to have poor prognoses (so-called triple-negative breast cancer). However, the findings thus far were rather limited due to the short time interval and preliminary nature. More work needs to be done. For Aim 1, a larger patient sample size is needed; and for Aim 2, the animal experiments need to be completed. I am not sure if the preliminary data would be sufficient for an R01 application, but it should be enough for an R21 application.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

The investigators were able to leverage additional funding from the Martha Rogers Charitable Trust for the conduct of related research and are planning on applying for an R21 based on the preliminary results from this work.

Reviewer 2:

A small non-federal grant was submitted to continue the proposed research (Martha Rogers Charitable Trust).

The researchers are planning to apply for an R21 grant to expand the sample of breast cancer patients and collect more data on TIMP-4 levels after surgical resection of triple-negative breast cancer patients.

This research was supported by additional funds from the Sharpe-Strumia Research Foundation of Bryn Mawr College.

Reviewer 3:

The project obtained support from additional small private funds (Martha-Rogers Charitable Trust, etc).

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 project resulted in a poster presentation at the San Antonio Breast Cancer Symposium in December 2011. At the time of the progress report, no publications were yet submitted.

Reviewer 2:

No manuscripts have resulted yet; and no licenses, patents or commercial development resulted as part of this research. However, there are plans to publish results from the pilot studies carried out from this research proposal. Data were presented at the San Antonio Breast Cancer Symposium in December 2011 (one of the most prominent meetings for breast cancer research).

Reviewer 3:

A poster presentation was developed based on findings for Aim 1. It is foreseeable that one publication may be generated once the animal experiment is completed (Aim 2).

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

A hematology/oncology fellow helped to conduct the research and was partially supported by the funds.

Reviewer 2:

No improvements were made to the infrastructure of the grantee's institution. One undergraduate and one post-doctoral student (two in total) participated in project-supported research. No new investigators were brought into the institution to help carry out the research.

Reviewer 3:

It was not specified.

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:

As a result of the poster presentation at the San Antonio Breast Cancer Symposium, collaboration with an outside investigator was established. The collaborator will provide a better mouse model for testing therapy effectiveness.

Reviewer 2:

Data were presented at the San Antonio Breast Cancer Symposium in December 2011, and collaboration with Dr. Lewis (of Baylor Medical School) was established. The collaboration involved sharing his series of stable transplantable human surgical breast cancer xenografts. This will greatly enhance the work on this project.

Reviewer 3:

Research collaborations were established with investigators from Baylor Medical School to expand the research of Aim 2 using xenograft models.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

Unfortunately, it is unclear from the preliminary data of TIMP-4 levels outlined in the progress report if TIMP-4 serum levels are of any significance or utility. The small numbers of patients studied and the variability in their treatment make the results uninterpretable.

Studying a larger and uniformly treated patient population with appropriate controls will help to determine the relevance of serum TIMP-4 levels and determine whether the levels can be used to identify patients at high risk for recurrence.

Reviewer 2:

This is important research that should be published in the near future. These results can help guide clinical research for triple-negative breast cancer patients and lead to clinical trials of novel agents. It is expected that researchers will have or already have results for the therapeutic mouse experiments.

Reviewer 3:

1. Patient sample size should be increased to at least 50 women with TNBC to have a meaningful clinical observation. This means more centers may be needed to be involved. The follow-up time needs to be increased to at least two years post-surgery.
2. The animal experiment is ongoing, and adding xenograft models will be helpful.

ADDITIONAL COMMENTS

Reviewer 3:

Strengths:

1. High clinical impact, especially for women with TNBC
2. TIMP-4 as a potential marker for prognosis

Weaknesses:

1. Small sample size for Aim 1
2. Delayed animal experiments for Aim 1