Response Form for the Final Performance Review Report*

1. Name of Grantee: Lankenau Institute for Medical Research
2. Year of Grant: 2010 Formula Grant

A. For the overall grant, briefly describe your grant oversight process. How will you ensure that future health research grants and projects are completed and required reports (Annual Reports, Final Progress Reports, Audit Reports, etc.) are submitted to the Department in accordance with Grant Agreements? If any of the research projects contained in the grant received an “unfavorable” rating, please describe how you will ensure the Principal Investigator is more closely monitored (or not funded) when conducting future formula funded health research.

The Lankenau Institute for Medical Research has a central Editorial Department which in coordination with the Institute's Finance Department tracks the necessary dates for submission of progress reports and other reports as necessary, alerting principal investigators whose projects are supported by the grant several weeks before deadlines of what kind of report is required, what information must be included, and when the required report is due to allow preparation and submission of documents in a timely manner. Expenditures on grants are routinely reviewed by the Director of Finance and/or her subordinates at the time of purchase request before approval. Detailed records are kept of all grant revenues and expenditures. Principle investigators receive annual reports for review and signature (including effort reports for salary support, if relevant). Random internal audits are performed to monitor the fidelity and completeness of record keeping. Before return to the granting agency, all reports are reviewed and signed by the Director of Finance and the President/CEO of the Institute, as appropriate.

* Please note that for grants ending on or after July 1, 2007, grantees’ Final Performance Review Reports, Response Forms, and Final Progress Reports will be made publicly available on the CURE Program’s Web site.
**Project Number:** 1085401  
**Project Title:** Disulfides to Modulate Thiol Homeostasis in Human Colon Cancer Cells  
**Investigator:** Ayene, Iraimoudi S

**B. Briefly describe your plans to address each specific weakness and recommendation in Section B of the Final Performance Summary Report using the following format.** As you prepare your response please be aware that the Final Performance Review Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program’s Web site.

Reviewer Comment on Specific Weakness and Recommendation (Copy and paste from the report the reviewers’ comments listed under Section B - Specific Weaknesses and Recommendations):

Response (Describe your plan to address each specific weakness and recommendation to ensure the feedback provided is utilized to improve ongoing or future research efforts):

**Reviewer 1:**

1. The premise that the limited utility of HDES as a radiation sensitizier is due to rapid metabolism should be experimentally tested *in vivo* does a therapeutically relevant dose of HDES depress GSH in *in vivo* tumors?

**Response:**

The reviewer refers to HEDS, the initial lead compound used to explore the possible utility of thiol oxidation modifiers to improve radio/chemotherapeutic responses in cancer. In *in vivo* experiments have shown that HEDS can improve antitumor responses to cytotoxic chemotherapy. We did not document its ability to depress GSH levels but have examined how protein thiols are reduced generally or at specific targets such as Ku, a DNA repair factor modulated functionally by thiol redox, implying in *vivo* lowering of GSH levels. These effects occur in glucose-starved tumors selectively, where GSH levels are under particular stress due to the combined effect of lowered oxidative pentose phosphate cycle activity along with HEDS treatment.

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.

**Response:**

Systemic levels are not different given that differences in HEDS metabolism occur only in glucose-starved tissues found as a subset of the tumor tissue in a tumor-bearing animal. We hypothesize, but have yet to show, a local difference of HEDS metabolism in this tumor tissue subset compared the well-oxygenated tissues in the animal as a whole.
3. The 13 compounds found not to be metabolized appreciably *in vitro* should be tested for suppression of GSH levels.

**Response:**
We have identified the dithiol compound 2-mercaptpropionyl glycine disulfide ("thioxin") as a second generation agent. Thioxin is similar to HEDS in its cytotoxic specificity for glucose-deprived cells to HEDS but superior to HEDS in its in vivo properties as an antitumor compound. Thioxin is a dimer of tiopronin, an approved clinical agent used to treat cystinuria. Reduction of thioxin to its monomers creates tiopronin, the clinical safety and pharmacological profiles of which are well established, improving the possible feasibility of developing thioxin as an antitumor agent.

**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.

**Response:**
HEDS offers some proof of concept but it is clearly not a clinical candidate. In contrast, the second generation compound thioxin described above may offer such potential, given its superior in vivo properties, its patent protection (needed for realistic clinical development), and is a thiol dimer of the existing clinical drug tiopronin (the metabolic product of thioxin, of known pharmacology and toxicology).

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.

**Response:**
As a result of preliminary results afforded by the PA award, we were able to compete successfully for an NIH R03 grant that has addressed this desire.

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.)

**Response:**
We believe other approaches to deplete GSH would dilute the productive effort of the new direction we are pursuing. With regard to combinational utility, under the NIH R03 grant mentioned above we have obtained evidence that thioxin exerts single-agent antitumor efficacy but also powerfully leverages the efficacy of several widely used DNA-damaging drugs such as cisplatin, converting tumor growth inhibitions into tumor regressions.
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.

Response:
The second generation compound studied, thioxin, has been found to phenocopy the effects of HEDS in cell survival experiments which our group published recently (Li et al. Toxicol In Vitro 27, 367-377 [2013]).

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.

Response:
Thioxin has been studied in vitro and in vivo as noted above and the findings are planned for preparation for submission later this year.

C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.

Response:

D. Additional comments in response to the Final Performance Review Report (OPTIONAL):

Response:
B. Briefly describe your plans to address each specific weakness and recommendation in Section B of the Final Performance Summary Report using the following format. As you prepare your response please be aware that the Final Performance Review Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program’s Web site.

Reviewer Comment on Specific Weakness and Recommendation (Copy and paste from the report the reviewers’ comments listed under Section B - Specific Weaknesses and Recommendations):

Response (Describe your plan to address each specific weakness and recommendation to ensure the feedback provided is utilized to improve ongoing or future research efforts):

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.

Response:
The provided funding was for one year towards a newly started project that is still enrolling patients. To date we have exceeded the enrollment suggested by Reviewer 3 and can see clear trends in effective/ineffective treatment for patients with elevated TIMP-4 levels in their plasma. By allowing the clinicians to assign treatments, we now also have data indicating that some of the currently standard treatment combinations result in increased TIMP-4 levels. We now also have TIMP-4 positive patients that had continuous elevated levels of TIMP-4 throughout their treatment period returning with local recurrence or metastatic disease. We will continue to follow our cohort for another two years to allow for better statistical analysis of our results.

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.
Response:
We agree with the reviewer regarding the importance of this research but would like to continue the follow-up of our patients for better statistical analysis. Of current standard treatments, one combination is effective in reducing circulating levels of TIMP-4 while others result in increased levels of circulating TIMP-4. Patients with continuous elevated levels of TIMP-4 have suffered relapse or progressed to metastatic disease. With longer follow-up for our cohort, that now is five-fold larger than when the report was submitted, we hope to submit a report with convincing data for an adjustment in treatment regimens for TNBC patients, especially those with elevated TIMP-4.

We have recently finished our animal study comparing tumor growth and spread in normal or elevated TIMP-4 conditions. The animal study was repeated once and confirmed the results from the first study. In both studies presence of elevated TIMP-4 increased the tumor growth rate and metastatic growths were found in 25% of animals (N=16). Tumors in animals treated with a TIMP-4 targeting therapy decreased the tumor size by approximately 20% after the first treatment and were stable throughout the treatment period of two weeks. No signs of metastatic spread could be found in lungs or liver. Two weeks post-treatment, tumors were once again growing but were still smaller than pre-treatment. We are currently finishing up molecular and histological analysis of the tumors and metastatic growths, which we have demonstrated consist of human breast cancer cells. Manuscript preparations are ongoing.

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 need to be involved. The follow-up time needs to be increased to at least two years post-surgery.

Response:
This year we have exceeded the suggested cohort size and do see a clear trend for one chemotherapy combination. Our biostatistician has been monitoring our results throughout the project and will perform the final analyses of the final results. As per his suggestion we are following the patients for three years after surgery with assessment of TIMP-4 levels at regular follow-up visits.

2. The animal experiment is ongoing, and adding xenograft models will be helpful.

Response:
We have the results from two independent studies that generated similar results demonstrating that elevated TIMP-4 levels, accomplished by implanting slow-release pellets into the mammary fat pad, increased the tumor growth rate by 150%. Targeted therapy for TIMP-4 resulted in an early and sustained decrease in tumor size and no signs spread to other organs, while 25% of animals receiving control treatment had metastatic growths in lung or liver, as confirmed by IHC for human specific antigens. The results from this study helped us better design the patient-derived xenograft study that now is on-going with our collaborator at Baylor Medical College.
ADDITIONAL COMMENTS

Reviewer 3:

Weaknesses:
1. Small sample size for Aim 1

Response:
The IRB approved sample size for Aim 1 is 244. The previously submitted report is for the first years of the study. To date we have enrolled over sixty patients and that demonstrates a clear and consistent trend favoring one particular chemotherapy combination as effective in reducing TIMP-4 levels that to date has resulted in DFS, while patients receiving other combinations have continuous elevated TIMP-4 accompanied by relapse and/or progression to metastatic disease.

2. Delayed animal experiments for Aim 1

Response:
We had unexpected problems with the construction and move into our new vivarium. However, we have now finished the animal study that was repeated with good concordance between the two experiments. These experiments provided us with interesting and new knowledge regarding the role of TIMP-4 in tumor growth and metastasis that were used when planning the now ongoing animal clinical trial with patient-derived xenografts.

C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.

Response:

D. Additional comments in response to the Final Performance Review Report (OPTIONAL):

Response: