

**Response Form for the Final Performance Review Report—
Lankenau Institute for Medical Research 2008F***

1. Name of Grantee: Lankenau Institute for Medical Research
2. Year of Grant: 2008 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.

For each research project contained in the grant, please provide a response to items B-D as listed on the following page(s). When submitting your response please include the responses for all projects in one document. The report cannot be submitted as a ZIP file, because the Department's exchange server will remove it from the email. If the report exceeds 2MB, please contact the Health Research Program for transmittal procedures: 717-783-2548.

* 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: 0863701

Project Title: Arsenic Drugs and Hydroxyethyl Disulfide in the Control of Cancer Cell Growth and Response to Therapy

Investigator: Ayene, Iramoudi S.

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

Reviewer 1:

1. The impact of HEDS needs to be verified in animal models as quickly and completely as possible. A continuing investigation of HEDS on other therapeutic agents, or of related dimercaptans in depletion of GSH without animal studies that also address mechanism of any effects or toxicities, would not be recommended.

Response: We have already initiated studies to determine the impact of HEDS in animal tumor models. Initial studies have demonstrated that HEDS, in combination with topoisomerase II inhibitor, showed a moderate but significant effect on the growth of tumor. Further studies will be conducted to determine the combined effects of arsenic trioxide, which is currently used for leukemia patient, and HEDS in animal tumor models.

Reviewer 2:

1. The PI has a good idea and should pursue it. However, it would be very helpful if he could design experiments to elucidate the mechanism of action of HEDS + arsenite.

Response: We have initiated studies to elucidate the mechanisms of action of HEDS and arsenite. Our major focus is on the pentose cycle control of DNA repair and signaling by HEDS and arsenite. Using some of the preliminary data generated during the funding period, we have submitted an R21 NIH grant application in 2011 to study the mechanisms in vitro and in vivo. Although it was not funded, we received encouraging comments from the study section. We are currently working with our collaborator, Dr. Susan Gilmour, on generating additional preliminary data for resubmission of this grant for funding.

2. Other oxidants, particularly ones that might reach solid tumors, could be tested.

Response: NIH reviewers of another NIH grant application also suggested that other oxidants should be tested as an alternative to HEDS. We have generated additional preliminary data for other disulfide compounds that will be used for resubmission of this grant to NIH.

3. Experiments should have statistical analysis.

Response: We will carry out statistical analysis before submission for publication.

4. The ability of arsenite and HEDS to affect solid tumors should be examined.

Response: We have already initiated studies to determine the impact of HEDS in animal tumor models. Initial studies have demonstrated that HEDS, in combination with topoisomerase II inhibitor, showed a moderate but significant effect on the growth of tumor. Further studies will be conducted to determine the combined effects of arsenic trioxide, which is currently used for leukemia patient and HEDS in animal tumor models.

5. The results should be submitted for publication to demonstrate their strength upon peer review.

Response: Some of the data generated from this project is now part of a manuscript in press. We are currently working on the rest of the data for another publication.

We have also presented our work in two national meetings.

1. 56th Annual Meeting, Radiation Research Society, 2010. Regulation of the metabolic network improves the response of human colon cancer cells to radiation. Iramoudi S. Ayene, Jie Li, Donglan Zhang, Lankenau Institute for Medical Research, Wynnewood, PA

2. AACR 101ST Annual Meeting 2010, HEDS selectively radiosensitizes human cancer cells in glucose-deprived environments via inhibition of Ku protein function. Ward, KM, Li J, Zhang D, Dayanandam E, DeNittis AS, Prendergast GC, Ayene, IS. Lankenau Institute for Medical Research, Wynnewood, PA.

Reviewer 3:

1. Weakness: The major weakness of the project is based on the fact that it was over-ambitious. It is indicated in the Strategic Plan that the effects of various arsenic drugs and HEDS will be tested on various cancer cells including lung (Calu-6, A549), colon (HCT116, HT29), prostate (LnCap, DU145) and acute promyelocytic leukemia (HL-60, HL-60/Mx2) cancer cells. This was not fully accomplished; given the project timeframe (only HCT116 and HT 29 colon cancer cells were tested).

Recommendation: Given the timeframe, the research scope should have been refined and more focused on arsenic trioxide, HEDS, and leukemia cancer cells.

Response: As recommended, we will carry out experiments to demonstrate the effects of HEDS and arsenic trioxide in leukemia cancer cells.

2. Weakness: Another major weakness relates to the statistical analysis of data. From the information provided in the research design section, it appears that the experimental design failed to consider an appropriate number of replicates needed to address the statistical analysis of data.

Recommendation: The design should consider an adequate number of replicates to allow for proper statistical comparisons of treatment groups. Hence, standard deviations of means should be shown on the graphs and (*) should be used to indicate values that are different to the controls using the t-test as stated in the Strategic Plan.

Response: We will carry out statistical analysis of all data before submission for publication.

3. Weakness: No presentations were made from the research.

Recommendations: Although there is a plan for manuscript publication, at least two presentations should have been made at scientific meetings to share information on research data, and more importantly to provide opportunities to the undergraduate student involved to present his research.

Response: Some of the data generated from this project is now part of a manuscript in press. We are currently working on the rest of the data for another publication.

We have also presented our work in two national meetings.

1. 56th Annual Meeting, Radiation Research Society, 2010. Regulation of the metabolic network improves the response of human colon cancer cells to radiation. Iramoudi S. Ayene, Jie Li, Donglan Zhang, Lankenau Institute for Medical Research, Wynnewood, PA

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- 4 Weakness: The future research plan lacks a consideration for preclinical studies.

Recommendation: The PI should leverage collaboration with the radiation oncologist at Lankenau hospital to perform clinical trials studies.

Response: We are currently initiating collaboration with Lankenau hospital clinicians (Dr. Albert DeNittis, Dr. Paul Gilman) and clinical research center director (Dr. John Schrogie) to initiate clinical trials for this and other projects.

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: N/A

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

Response:

We are thankful for the reviewers’ encouraging remarks and positive feed back. As suggested by the reviewers, we will extend these projects to test the efficacy of this approach in tumor models. Along the lines suggested by the reviewers, we have already initiated several projects and submitted grants applications to federal government funding agencies. We

submitted three grant applications to NIH using some of the data generated during this funding period as preliminary results in support of the hypothesis.

The first grant application received a good score from the NIH study section. Although grants with such scores were funded in previous cycles by NIH, we have yet to receive the award notification for this grant application. Due to the highly encouraging comments from the study section, we are converting this RO3 grant into an RO1 with additional preliminary data from other projects for submission to NIH.

The second grant application also received very promising comments from the study section. We are now converting this R21 grant into an RO1 in collaboration with Dr. Susan Gilmour, a leading expert in carcinogenesis and senior Professor at LIMR. Data generated as part of other projects since the submission of this final report will be used as preliminary results in the new NIH grant application.

The third grant will be resubmitted with the focus on autism patients instead of cancer patients since our approach proposed in this grant is readily applicable in autism patients. We will use the results obtained from this and other projects for a new NIH grant application.

Project Number: 0863702

Project Title: Role of IDO in B Cell-mediated Immunity and Autoimmunity

Investigator: Mandik-Nayak, Laura

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

Reviewer 1:

1. The data suggest that IDO may be involved in T-d antibody responses but not T-i response; therefore, it is important that the investigators examine the functions of T cells. Both the arthritis and T-d responses affected by IDO pathway may be mediated through affecting T cells. This notion is further supported by the results that IDO does not play a direct role in B cell activation in vitro.

Reviewer 2:

1. No experiments were performed using any IDO-deficient mice. These experiments are important and should be done.

Reviewer 3:

1. The major strength of this project was the well-established in vitro and in vivo assays to provide useful and definitive information for the questions posted. The major weakness, however, was that there was no back-up plan or alternatives proposed in the original research plan when the results generated do not support their original hypotheses. Also, the most significant part of the project, which is to generate IDO/IDO2 mutant mice, has yet to be achieved.

Response:

Reviewer 1: We agree with the reviewer that our data point to a B cell extrinsic role for IDO in driving B cell-mediated immune responses. This is suggested by the immunization models and supported by the in vitro stimulation data summarized in our report. We are currently addressing the role IDO may play in T cells, as well as other cell types, using several complementary approaches. We have immunized IDO deficient mice using different adjuvant systems to begin to define the conditions under which IDO deficiency affects B cell responses. In the past, this has been a successful approach to define critical components of pathways required for T and B cell immune responses by overcoming activation defects in knockout mouse strains. A second ongoing effort in the lab is to use mixed bone marrow chimeras to define the IDO-expressing cell type required for the anti-arthritic phenotype. Finally, we are using flow cytometry and cytometric bead arrays to define the cell populations and cytokines altered by inhibition of the IDO pathway.

Reviewer 2: Experiments using IDO deficient mice are absolutely critical to this project. Unfortunately, during the short time period of this grant, we were unable to finish the breeding of

our arthritic mice onto the IDO knockout background. Since that time; however, we have completed breeding the arthritic model onto both the IDO1 and IDO2 deficient backgrounds. We are currently analyzing these mice for the time of onset and severity of arthritis, presence of autoantibodies, autoantibody secreting cells, and inflammatory cytokines.

Reviewer 3: The reviewer points out that our original proposal lacked a sufficient back-up plan if the generated results did not support the original hypothesis. In aim 1, alternative experiments were suggested, which included using IDO1/2 double deficient mice if the single deficient mice did not phenocopy the pharmacological inhibitor data. Specific aim 2 was structured differently. The focus of aim 2 was to determine if IDO was playing a B cell intrinsic or extrinsic role. The experiments were designed to give a meaningful result regardless of whether the result was positive or negative. In the future, I will be clearer in the writing of alternative plans.

A second weakness pointed out by reviewer 3 is that we had not achieved our goal of generating the IDO deficient arthritic mice. As stated above in response to reviewer 2, we agree that these experiments are absolutely critical to this project and have now completed the extensive breeding required to cross the arthritic model onto both the IDO1 and IDO2 deficient backgrounds and are in the process of analyzing the mice.

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: N/A

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

Response: N/A.