

**Response Form for the Final Performance Review Report—
Lankenau Institute for Medical Research 2009F***

1. Name of Grantee: Lankenau Institute for Medical Research
2. Year of Grant: 2009 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: 0989801
Project Title: Tirapazamine and a Novel Drug in the Control of Hypoxic
Human Colon Cancer Cells
Investigator: Ayene, Iramoudi

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. Productivity from the funding was marginal, with a single manuscript and a single small-scale grant submitted. Moreover, the subject of the submitted manuscript (radiation + HEDS) does not seem to match with the funded project (tirapazamine + HEDS), and it is not clear from the title whether the submitted grant truly resulted from the funded project. This discrepancy should be clarified.

Response:

Although the combinatorial therapy with tirapazamine and HEDS did not result into a manuscript due to the absence of synergistic effect, we have included some of these data in the HEDS paper cited in the progress report. Some of the data from HEDS experiments alone, carried out during the funding period, was also included in a manuscript that is currently in press. We have already initiated several projects and submitted grants applications on HEDS and related compounds to federal government funding agencies.

The first grant application (submitted in 2011) 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 R03 grant into an R01 with additional preliminary data from other projects for submission to NIH.

The second grant application (submitted in 2011) also received very promising comments from the study section. We are now converting this R21 grant into an R01 in collaboration with Dr. Susan Gilmour, a leading expert in carcinogenesis and senior Professor at LIMR. The third grant application (submitted in 2011) on the use of HEDS as a biomarker will be submitted to validate its use in autism patients.

2. The data do not support the original concept that tirapazamine and HEDS would synergize in hypoxic tumor cells. The PI should clarify whether the data disprove the concept, or whether the experimental systems are limited in their ability to model a hypoxic tumor. How will these results be followed up? The PI indicates that tirapazamine will be tested at higher doses; however this will engender concerns about toxicity.

Response:

The failure to demonstrate the synergistic effect by HEDS and tirapazamine raises several possibilities. The results did not exhibit a highly synergistic effect with HEDS and TPZ combination even at high concentration of TPZ. Although this may explain the failures associated with the clinical application of TPZ, it raises the possibility that HEDS and TPZ may act by the same mechanism i.e. TPZ may also target DNA repair proteins similar to HEDS and hence may not exhibit synergistic effect. It raises another possibility that TPZ may not be effective in the absence of glucose since the free radical production by TPZ under hypoxia may not occur due to loss of bio-reduction of TPZ in low glucose medium. Although several hypoxic sensitizers have been tested during the last four decades, whose success in cancer therapy is not evident, the current results suggest the possibility that the glucose condition in tumor microenvironment may determine the outcome of TPZ. The data highlighted that further development of hypoxic sensitizers should be carried out at low glucose since it is now known to be present in solid tumors. However, this approach (HEDS + tirapazamine) may still work in combination with radiation and chemotherapeutic agents, and may be followed up in future projects.

3. The work does not appear to have enhanced external collaboration, since the only listed new collaborator is internal to the Lankenau Institute for Medical Research. The PI should expand collaboration beyond the local institution.

Response:

We are currently initiating collaboration with Lankenau Medical Center clinicians (Dr. Albert DeNittis, Dr. Paul Gilman) and clinical research center director (Dr. John Schrogie) to initiate clinical trials for this and other projects. Additionally, we will initiate collaboration with investigators at University of Pennsylvania, Jefferson University and Drexel University all located within 5 to 7 miles from LIMR.

Reviewer 3:

1. This project would benefit from a more mechanism-oriented hypothesis or model for designing appropriate experiments to determine the interactions between the HEDS- and TPZ-regulated pathways that affect cell survival or response to therapy.

Response:

We will consider mechanism-oriented hypothesis including cell signaling, hypoxia inducible factors and thiol homeostasis in tirapazamine and HEDS combinatorial therapy.

2. The design of hypoxia experiments should take into consideration that half maximum OER occurs at approximately 3 mmHg or 0.5% O₂. Other controls should be included to confirm hypoxia responses in treated cells.

Response:

We will include positive controls to confirm hypoxia responses in treated cells in future experiments.

3. Since HEDS does not appear to synergize with TPZ for cell killing, it may be worth considering using HEDS and TPZ together with IR or chemotherapy under normoxia or hypoxia.

Response:

We will use HEDS and TPZ together with IR or chemotherapy under normoxia or hypoxia in vitro and tumor models in vivo.

Generic Recommendations for Lankenau Institute for Medical Research

Reviewer 1:

Overall, the PI performed most of the studies as originally proposed. The productivity was marginal although perhaps not out of line with what is to be expected from a pilot funding mechanism. The results are not sufficiently promising to warrant further funding.

Response:

Although the results with tirapazamine are not promising, the data generated with HEDS alone confirmed its potential use for therapy. Ongoing projects with HEDS and related compounds supported by this and other grants suggest that redox modulation by these compounds is a novel approach to increase the cancer cells response to chemotherapeutic agents and radiation. As suggested by one of the reviewers, the HEDS and TPZ combination may still work in combination with radiation or chemotherapy.

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:

Although the results were not consistent with our original hypothesis, it explained the importance of redox modulation in cancer cell death. Additionally, it also raises several questions and potential mechanisms for the lack of success of tirapazamine as a hypoxic toxin in preclinical and clinical studies. Most importantly, our results indicated that screening of hypoxic sensitizers in vitro should include low glucose conditions to mimic tumor microenvironment for better evaluation of hypoxic sensitizers.

Project Number: 0989802

Project Title: The Role of IDO in B Cell Activation and Memory

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:

The PI should re-think the hypothesis. It may be difficult to seek evidence to support the hypothesis. The experimental design may not be appropriate, and she should guard against over-interpretation. Although the NIH application was successful, she should think carefully about the direction of the research so that she may be able to renew her R01 competitively.

Response:

The studies covered in this 1-year proposal are part of a larger ongoing effort in the laboratory (funded primarily by our RO1 grant) to understand the role of IDO in driving B cell-mediated autoimmunity. Two of the aims of our RO1 proposal are to determine the mechanism by which IDO drives B cell activation and if this IDO-driven B cell activation is unique to the K/BxN system or if it is a universal phenomenon. To address this, we are using both in vitro and in vivo models, including B and T cell receptor transgenics, IDO1 and IDO knockout mice, different models of B cell-mediated immunity, and bone marrow chimeras. The studies detailed in the Pennsylvania Department of Health proposal being reviewed here were meant to complement this larger ongoing effort in the lab. Due to the restrictions given in generating our progress reports, I was only able to report on studies funded directly by this grant and not on the overall project. Even given this restriction, I feel that the results presented, together with studies funded by our RO1, will yield important information that will be included in a future publication (see response to reviewer 3).

Reviewer 2:

1. The project lacked mechanistic data regarding the role of IDO in B cell memory formation. A likely mechanism affecting B cell memory could have to do with activation of T follicular helper cells. Analyze parameters related to T_{FH} cells (histological staining, flow cytometry for CXCR5+ T cells, IL-21 production, etc.). Additionally, consider setting up culture systems of purified B cells from primary immunized mice with anti-CD40 antibodies and/or cytokines in the presence or absence of IDO to show direct effects on B cell activation, antibody production and maturation.
2. The differences between alum and Titermax are not addressed. It seems likely that NP-KLH Titermax overcame the effect of IDO that was seen with NP-KLH alum treatment by being a stronger adjuvant. This could be investigated in side-by-side comparisons, with an analysis not only of B cell activation but also of T cell and cytokine involvement. This could have important implications for patients being treated with IDO blockers who are in need of immunizations against pathogenic microorganisms.

3. The project demonstrates the effects of IDO blockade on establishing a memory B cell response but does not address maintenance of the memory response. This would be important for determining whether IDO blockade could be useful in patients with established auto-antibody-mediated diseases. Modify the treatment regimen to begin instillation of IDO blocker after memory B cell formation, and follow maintenance of antibody titers.
4. The project does not address B cell responses to naturally occurring antigens. A potentially important contribution of this work could be an understanding of the side effects of anti-IDO treatment on community acquired infections. To address this, experiments could be done using viral, bacterial, and/or fungal infections of mice treated or untreated with IDO blockers.
5. The research project does not appear to emphasize collaboration. Many of the recommendations made above could be aided by establishing collaborations within and outside of the institution. It is expected that these would broaden the scope of the research and give new perspectives on the planning and interpretation of results.

Response:

1. The experiments suggested by the reviewer are part of an ongoing effort in the lab to determine the mechanism by which IDO directs both primary and secondary B cell responses. This, in fact, is a major focus of our RO1 grant. We agree that a likely candidate, given our preliminary data and that in the literature, is the T_{FH} cell. We are in the process of comparing the effect of IDO inhibition (both 1MT and genetic deficiency) on the development and function of T_{FH} cells. To do this, we are looking by flow cytometry using the markers CXCR5, ICOS, and bcl-6 and measuring the cytokine IL-21. We are also in the process of breeding our T and B cell receptor transgenic mice onto the IDO1 and IDO2 deficient backgrounds to determine if IDO1/2 is necessary on the T cell, B cell, or both for cognate T cell help. We will also include other non-antigen specific methods of activation, including LPS and anti-CD40 to test whether IDO has a direct effect on B cell activation.
2. We agree with the reviewer that Titermax did seem to overcome the effect of IDO inhibition seen with NP-KLH in alum. This is an interesting result that merits further study. In the studies funded by this proposal, we compared B cell immune responses in mice where IDO was inhibited pharmacologically with 1MT or in IDO1 genetically deficient mice. In addition to inhibiting IDO1, 1MT also inhibits the related gene IDO2. We have recently received IDO2 deficient mice from our collaborators, Dr. George Prendergast and Dr. Richard Metz, here at Lankenau, and are in the process of completing immunization studies in this strain. We will include further parameters looking at T cell responses, in addition to the previously shown B cell responses, in these future studies.
3. The experiment suggested by the reviewer is a good one that we have not tried yet in the immunization model. We have, however, treated arthritic mice with 1MT after the onset of arthritis (Scott, et al. 2009 J. Immunol. 182:7509). When administered at this timepoint, 1MT does not have any effect on auto-antibody titers or arthritis development. Interestingly, we have found that 1MT can be combined with other therapeutics (e.g. B cell depletion therapy) to lower autoantibody titers and reverse the onset of arthritis. This finding, funded

by our RO1, is part of a recently accepted publication (Pigott, et al. 2012, Arth. Rheum.). It would be interesting to see if a similar co-therapeutic strategy would lower antibody titers in secondary responses to immunizations.

4. The reviewer is correct that the studies in this 1-year grant proposal addressed only responses to model antigens in an immunization setting. The role of IDO blockers in response to viral and bacterial infections has been the subject of several recent publications, including one from our collaborators (Divanovic, et al. J. Infect. Dis. 2012, 205:152).
5. In the past few years since this grant was completed, we have established collaborations with several investigators here at Lankenau. These include Dr. George Prendergast, Dr. Richard Metz, and Dr. Alexander Muller (IDO1/IDO2 and immune responses) and Dr. Lisa Laury-Kleintop (IDO and mitral valve disease).

Reviewer 3:

1. The weakness of this study is primarily in the characterization of the K/BxN arthritis model. Since the KRN gene is expressed on only one allele (homozygotes cannot survive), gene typing of the mice should be confirmed to eliminate the possibility of homozygous negative mice being included in the study (this could severely skew the results and was not discussed). In addition, use of only three-week-old mice that were just off weaning was shown. Do older mice (i.e., six to eight weeks) show reduced arthritis development to 1MT treatment? If so, reversal of the arthritic phenotype may be a powerful finding and of significant interest, since most, if not all, RA patients initially present at the later stages of RA development. Also, measuring mouse joint homogenates for pro-inflammatory cytokine expression would strengthen the findings. Finally, having the K/BxN tissues scored by histology for leukocyte infiltration, as well as vascularity and/or bone destruction, would be helpful and would validate the model and the effects observed by inhibiting IDO both pharmacologically and by gene deletion.
2. The PI may be aided by additional collaborators; none are listed either internally or externally.

Response:

1. We do actually maintain the KRN tg mice as homozygotes in our colony. Homozygosity has been confirmed by many generations of progeny testing, in addition to PCR. KRN tg mice are viable and fertile as homozygotes. Thus, in our breeding scheme to generate K/BxN mice, where KRN homozygous mice are crossed to NOD mice, 100% of the progeny express the KRN tg. This excludes the possibility of including tg negative mice in the study.

The preliminary data shown in the grant proposal showed inhibition of arthritis when 1MT was administered prior to the onset of arthritis. We have also done the experiment suggested by the reviewer, starting 1MT treatment after the onset of arthritis (Scott, et al. 2009 J. Immunol. 182:7509). When administered at this timepoint, 1MT does not have any effect on arthritis development. However, we have found that 1MT can be combined with other therapeutics (e.g. B cell depletion therapy) to reverse the onset of arthritis. We agree with the reviewer that reversal of the arthritic phenotype is a finding of significant interest and points to the feasibility

of using 1MT as a therapeutic. This finding, funded by our RO1, is part of a recently accepted publication (Pigott, et al. 2012, Arth. Rheum.).

The preliminary data included in the grant proposal showed cytokine levels in the joint draining lymph nodes. In several of our experiments, we have also measured cytokines in joint homogenates using the same cytometric bead array. Similar to the results shown for the draining lymph nodes, we detected high levels of TNF α , IFN γ , MCP-1, IL-6, and IL-10 in the joint homogenates from control-treated K/BxN mice, whereas levels of MCP-1, IL-6, and IL-10, but not TNF α or IFN γ , were reduced in the 1MT-treated mice.

We agree with the reviewer that having the K/BxN joints scored by histology for leukocyte infiltration, vascularity and bone destruction is important and to that effect have enlisted the help of our collaborator, Dr. Alessandro Soler, a pathologist, to help us score the slides.

2. In the past few years since this grant was completed, we have established collaborations with several investigators here at Lankenau. These include Dr. George Prendergast, Dr. Richard Metz, and Dr. Alexander Muller (IDO1/IDO2 and immune responses) and Dr. Lisa Laury-Kleintop (IDO and mitral valve disease).

Generic Recommendations for Lankenau Institute for Medical Research

Reviewer 3:

All in all, this was a very interesting study using sophisticated animal models to study B cell activity and IDO inhibition in RA models. Only minor weaknesses were noted. Lastly, it is highly recommended that the PI publish the results, since none were listed pertaining to this application.

Response:

We agree with the reviewer that the results should be published. The studies funded by this proposal included comparing B cell immune responses in mice where IDO was inhibited pharmacologically with 1MT or in IDO1 genetically deficient mice. In addition to inhibiting IDO1, 1MT also inhibits the related gene IDO2. We have recently received IDO2 deficient mice from our collaborators, Dr. George Prendergast and Dr. Richard Metz, here at Lankenau, and are in the process of completing immunization studies in this strain. Once these studies are complete, we will publish the work together.

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: None.