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 (2.17)

Project Rating:

Project	Title	Average Score
0863701	Arsenic Drugs and Hydroxyethyl Disulfide in the Control of Cancer Cell Growth and Response to Therapy	Favorable (2.33)
0863702	Role of IDO in B Cell-mediated Immunity and Autoimmunity	Favorable (2.00)

Project Number: 0863701
Project Title: Arsenic Drugs and Hydroxyethyl Disulfide in the Control of Cancer Cell Growth and Response to Therapy
Investigator: Ayene, Iraimoudi 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 project met its original stated objectives. Three specific aims were proposed, each of which led from the preceding objective, and each stated experiments to be performed and suggested outcomes expected. There were no surprises, and the experiments proposed were performed exactly as planned.

Strengths: This project addresses the pharmacological interventions to manipulate the reduction state of human tumor cells with the compound hydroxyethyl disulfide (HEDS) in an effort to decrease cellular glutathione. The GSH content of tumor cells is well known to limit the cytotoxicity of alkylating agents, Arsenic trioxide (AsO3), and radiation to tumor cells. Hence, the underlying concept of this project would be expected to enhance the effect of AsO3, alkylating agents or radiation to human tumors. This is a valuable goal.

Weakness: Although the original goals proposed were met and the experiments proposed were performed exactly as planned, the innovativeness of the original objectives of the project were limited.

Reviewer 2:
The PI made some progress on the stated objectives such as whether arsenite affected reduction of HEDS, but this is a trivial objective since, like most GSH oxidants, its reduction (which the PI calls detoxification but is strictly chemical reduction) depends on the levels of GSH. Since arsenite is a potent inhibitor of glutathione reductase, it would be surprising if the levels of GSH and hence the reduction of HEDS were not affected.

This analysis appears superficial. For example, arsenite is known to increase production of ROS and apoptosis. Here, there were no experiments designed to determine how arsenic and/or HEDS affected cancer cell growth. Is it through ROS production, or do they affect DNA repair without changing the redox potential inside the cell?

The data were not very well controlled (for example, no arsenite alone control in Fig. 2). There were no statistical analyses completed, so it is not clear whether the differences are statistically significant.

The strength of the proposal is that its goal – to improve the use of chemotherapeutic drugs and treatments for cancer – is very important. The central hypothesis – that arsenite can be used in combination with other agents such as glutathione oxidants – is worth testing. The major weaknesses are that the specific aims are superficial and lack sufficient approaches to determine a mechanism of action. In addition, it is difficult to see how this could be applied clinically.

Reviewer 3:
This research focused on the evaluation of the sensitivity of selected cancer cells in response to arsenic drugs and hydroxyethyl disulfide (HEDS)treatment. It was hypothesized that a combination of arsenic drugs and HEDS will inhibit the function of DNA repair protein (Ku) and glutathione in cancer cells and therefore improve their sensitivity and responses to radiation and chemotherapeutic drugs. To test this hypothesis, three specific aims were defined and included the following: 1)to determine whether arsenic trioxide is effective in inhibiting the detoxification of HEDS by human cancer cells; 2) to determine whether arsenic compounds are effective in increasing HEDS-mediated oxidation of glutathione and loss of function of DNA repair protein (Ku); and 3) to determine whether arsenic drugs, in combination with HEDS, improve the responses of cancer cells to cisplatin and radiation.

Strengths: The major strength of this project relied on the fact that the research was based on a well defined and testable hypothesis that has been derived from preliminary experiments. Additional strengths included a logical and innovative research approach based on the application's adequate analytical techniques such as HPLC, electrochemical detection and biochemical assays, as well as the experience of project investigators. Also, the data were fairly developed to support the stated objectives and research questions.

Weaknesses: 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 cancers 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). Another major weakness relates to the statistical analysis of data. Standard deviations of means should be shown on the graphs and (*) should be used to indicate values that are different from the controls using the t-test as stated in the Strategic 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:
The impact of the research supported by allocation of formula funds to this project was rather low.

Strength: The investigator has advanced the understanding of the effectiveness of HEDS in enhancing the activity of cisplatin and AsO3.

Weaknesses: The concept of manipulation of the redox potential of tumor cells for the purpose of enhancing the effectiveness of alkylating agents and radiation against tumor cells is far from unique to this application, and has been the topic of numerous literature publications. While the use of HEDS is a relatively new twist on the theme, the PI has already published several papers on the use of this compound for this type of purpose. Hence, the novelty of the originally proposed aims was low.

The major objection to this type of experiment is that the enhancement of the cytotoxicity of DNA damaging conditions in tissue culture, while of interest, is immaterial without knowledge of whether the conditions also enhance the toxicity of the drug to normal dose-limiting tissues. This determination can only be made from studies in vivo.

Reviewer 2:
It would be very beneficial if arsenite could be applied to treatment of solid tumors. It is doubtful that HEDS plus arsenite could be used in the treatment of solid tumors. It is one thing to show that HEDS can affect cells in culture, but another, to show that it reaches solid tumors.

No future plans were delineated, and it is difficult to see how the PI would extend these studies.

The proposal addresses a serious clinical problem – cancer chemotherapy. The major weakness is that very little results of clinical relevance were obtained.

Reviewer 3:
Strengths: Data generated from this research demonstrated that co-exposure with hydroxyethyl disulfide (HEDS) increases the cytotoxicity of arsenic trioxide to human colon cancer cells, suggesting the potential application of HEDS in improving arsenic trioxide-based chemotherapy. Also, HEDS and arsenic trioxide combination enhances the response of colon cancer cells to conventional therapies such as radiation and cisplatin.

Weakness: Arsenic trioxide has been approved by the FDA for the treatment of APL. Hence, it would have been more reasonable to use leukemia cancer cells instead of colon cancer cells to evaluate its pharmacological potentiation by HEDS.

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:
Weakness: There were no funds leveraged and no grant applications submitted. The investigator commented that grants would be submitted, but that has not happened to date.

Reviewer 2:
The principal investigator plans to apply for additional funding, but there is no evidence that any grant proposals will result from these studies.

Reviewer 3:
Strength: The PI plans to write two new NIH R01 grant applications using the preliminary data generated from this research project.

Weakness: No information is provided on the hypotheses that would drive the new NIH R01 grant applications. Future research plans should also consider pre-clinical trials with cancer patients.

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:
Weakness: There were no papers submitted as a result of this work. There was a plan to submit two papers, but the impact of the data and the mechanistic insights coming from the results would seem unlikely to support publications in major journals.

Reviewer 2:
There is no evidence that any publications will result from these studies.

Reviewer 3:
Strength: The PI plans to write and submit two manuscripts for publication.

Weakness: No timeline is provided regarding manuscript completion and submission.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:
Weakness: There were no substantive additions made in the infrastructure of the Lankenau

Institute for Medical Research. The only student involvement was a single undergraduate. One colleague was brought into the project who is a clinical scientist, however, their impact on the project is not apparent.

Reviewer 2:
The use of the funds was not described.

Reviewer 3:
Strengths: The project has helped the PI develop his laboratory. It has, therefore, enhanced the research infrastructure at his institution. It also provided an opportunity for research training of one undergraduate student.

Weakness: No graduate students were recruited to participate in the project.

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:
There was no collaboration with research partners outside of the institution or new involvement with the community.

Reviewer 2:
Outside collaboration was not discussed in the progress reports.

Reviewer 3:
Strength: Collaboration was established with a clinical radiologist at Lankenau Hospital.

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

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

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.

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.

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

3. Experiments should have statistical analysis.

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

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

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.

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.

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.

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.

Generic Recommendations for Lankenau Institute for Medical Research

Reviewer 1:
Perhaps the institute should establish a committee structure to prioritize allocation of formula research funds to individual projects.

Reviewer 3:
Overall, this is a very good project. The preliminary data generated from the research provide new insights into the molecular mechanisms of HEDS potentiation of arsenic trioxide pharmacology, and the potential beneficial effect of their combination on radiation and cisplastin-based therapy. However, the project was over-ambitious, proposing to study the effects of HEDS and arsenic drugs on the responses of several cancer cell lines to conventional therapies. Although the focus on only one cancer cell line is justified by the short timeframe of the project (1 year), it would have been more realistic to narrow the scope of the project to avoid the thought that the project objectives were not completely met. Also, a major flaw of the study design relates to the lack of replications, leading to data that may not be supported by a thorough statistical analysis. Simple statements of percent reduction in HEDS detoxification, GSH, protein Ku, or cell viability in treatment versus control groups (without appropriate replicates and statistical analysis) may lead to inappropriate inferences. It is recommended that the experiments be repeated to ensure appropriate interpretation of data.

ADDITIONAL COMMENTS

Reviewer 2:
Dr. Iraimoudi Ayene, who is an associate professor at the Lankenau Institute for Medical Research, has limited productivity with approximately one publication per year in reasonable journals. His major interests center on the Ku protein, which has a role in DNA recombination and double strand break repair. Glucose deprivation is common in hypoxic cells of several solid tumors because of poor vascularization and higher metabolic activity. One of his hypotheses is that hypoxic cancer cells are susceptible to hydroxyethyl disulfide (HEDS, the disulfide of mercaptoethanol), an oxidant that affects protein cysteines and GSH. He is presently a principal investigator on NIH R01 "Oxidative Pentose Cycle in Hypoxic Cancer Cell Response." The current project is an off-shoot of his current research. Dr. Ayene hypothesizes that arsenic, which is currently used as a chemotherapeutic agent for treatment of forms of leukemia, might work with solid tumors if used in combination with HEDS. The rationale is that HEDS, like other GSH oxidants diamide, are toxic because they prevent DNA repair by enzymes such as Ku, especially in cells that are more dependent on anaerobic metabolism such as solid tumors. They propose that the combination of arsenite and HEDS acts synergistically in sensitizing tumor cells to other treatments such as radiation.

The specific aims are 1) does arsenite affect oxidation of HEDS, 2) does the combination act synergistically on GSH metabolism and DNA repair, and 3) does this affect treatment with radiation and other chemotherapeutic drugs? The PI has made limited progress on these aims. Some data are shown, but no publications, no grants, and no patents have resulted.

The two progress reports show that human colon cancer cells have somewhat less oxidation of HEDS in the presence of arsenite than in its absence. The effect is not dramatic (about 30% at 6 μM arsenite) and no error bars in this, or other experiments are shown; so it is not clear how significant the effect is (likewise true for all of the data presented). This is not a novel observation since it has been shown that arsenite inhibits glutathione reductase and increases the redox potential inside of cells, which would be expected to prevent reduction of HEDS. Would other oxidants have the same effects?

Arsenite also appears to reduce the survival of colon cancer cells synergistically with HEDS. More importantly, it increases sensitivity to cisplatin and radiation. These are the most interesting results. However, these are all done with cells in culture and not solid tumors, so it is not clear whether either HEDS or arsenite could reach solid tumors.

Project Number: 0863702
Project Title: Role of IDO in B Cell-mediated Immunity and Autoimmunity
Investigator: Mandik-Nayak, Laura

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 has met the stated objectives. The research design and methods were appropriate for the objectives. The data answered the posed questions.

Reviewer 2:
The stated overall goals of this project were to "define the mechanisms by which indoleamine 2,3-deoxygenate (IDO) modulates the immune response leading to arthritis" and "provide new insights into strategies that can be used to manipulate this pathway to reduce or prevent debilitating disease." These objectives stemmed from the applicant's earlier observation that the spontaneous rheumatoid arthritis-like disease of K/BxN mice is ameliorated in animals wherein IDO is inhibited. The hypothesis that was tested was that IDO is not a global inhibitor of immune responses, but rather a modifier of cytokine and antibody profiles of the response. To test the hypothesis (in mice), two aims were pursued: 1) to determine "which inflammatory features" of arthritis in K/BxN mice are lessened by targeting IDO (and/or a similar enzyme, IDO2), and 2) to investigate whether IDO directly affects B cell activation. To achieve these aims, two experimental approaches were proposed: the use of small molecule IDO inhibitors, and the use of IDO, IDO2, and IDO+IDO2-deficient mice.

Given the stated goals, the design is adequate. The project met some of its stated objectives. The results obtained established that in the K/BxN mouse an IDO inhibitor called "1MT" exerts its inhibitory action early in the development of arthritis, and that IDO does not play a direct role in B cell activation in vitro. Also found was that IDO2 drives arthritis in this mouse model, inhibition of IDO or IDO2 does not affect cytokine responses, and that IDO is required for efficient T cell responsiveness. A major weakness is that none of the proposed experiments using any of the IDO knockout mice were performed.

Reviewer 3:
This proposal seeks to explore two immune-modulatory enzymes, IDO and IDO2, as a disease modifier of rheumatoid arthritis pathogenesis. This project proposes to target IDO/IDO2 and expects to lead to new approaches for the prevention and treatment of rheumatoid arthritis. The investigators will use both pharmacological and genetic inhibition of IDO/IDO2 activity in a

mouse model of RA to evaluate the role of IDO/IDO2 in shaping autoimmune response resulting in autoimmune arthritis. Thus, this project is of great basic and clinical significance.

Two specific aims were proposed in the original research plan. Specific Aim 1 was to determine which inflammatory features of autoimmune arthritis induced in mouse model can be ameliorated by targeting IDO/IDO2 activity. Specific Aim 2 set out to investigate whether IDO directly affects B-cell activation or acts indirectly through modulating effects on the microenvironment. The research design and methods were adequate in light of the project objectives. The investigators have largely met their original research goals. The data generated in the funding period were sufficient to answer the research questions originally posted in Aims 1 and 2.

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 in the event that the results generated do not support their original hypotheses.

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 beneficial impact for improving health from this project is relatively small. There is a potential for eventual benefits.

Reviewer 2:
Rheumatoid arthritis is a major health concern and IDO inhibition could potentially be used as a therapeutic approach in the disease. This project sought to determine the validity of this therapeutic modality by pursuing studies in a mouse model of spontaneous arthritis. The results are a small step towards clinical reality, but it is difficult to judge the beneficial impact of this project to human health. In light of the dollars budgeted, this is reasonable. Future plans were not well described; these entail an investigation of IDO's impact on B cell memory, but it is unclear if these plans extended beyond the grant's actual funding period.

Reviewer 3:
The results from this project have provided certain valuable information about the role of IDO/IDO2 in modulating autoimmune response and arthritis pathogenesis. The knowledge obtained is likely to be beneficial for the improvement of prevention and treatment of autoimmune disorders such as rheumatoid arthritis. At this stage of research development, it's unlikely that major discoveries regarding new drugs and new approaches for prevention, diagnosis, and treatment can be attributable to the current project. The most important data are yet to come, depending on the generation of mice that are genetically deficient for IDO/IDO2.

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 project did not leverage additional funds, but additional applications have been submitted.

Reviewer 2:
Two grants were applied for based on the data obtained from this project - one to the NIH, and the other to the Arthritis Foundation. I confirmed that the NIH grant was recently awarded for $1,856,250.

Reviewer 3:
The investigators have made an effort in leveraging funding from NIH and a nonfederal funding source. They have submitted two grants, one to NIH and another to Arthritis Foundation. The results are pending.

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 did not result in any peer-reviewed publications, licenses, patents, or commercial development opportunities.

Reviewer 2:
It is stated that a review article was being written that incorporates the data generated, but no submitted papers are reported. There was no information listed about a paper published by the PI of this application regarding this project's results.

Reviewer 3:
The project has not produced any peer-reviewed research publications, although the investigators planned to submit their manuscript to an immunology journal.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:
There were no improvements made to infrastructure at the institution. The funds were used to pay for research performed by a pre-doctoral student.

Reviewer 2:
The project was of a one-year duration and cost was $111,906.67. The funds were used to pay for part of the PI's salary, a research assistant, as well as one graduate student. It is reported that new collaborations with investigators at the applicant's institution were formed.

Reviewer 3:
The project has made some contributions to arthritis research, in general, at the grantees' institution. This funding has allowed the researchers to train one master's student.

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 any collaborations outside of the institution.

Reviewer 2:
No collaborations outside the institution, and no new community involvement were reported.

Reviewer 3:
No collaboration with research partners outside of the institution was indicated. No out-of-state researchers were recruited by this project.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

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.