

**Response Form for the Final Performance Review Report—  
Salus University 2008F\***

1. Name of Grantee: Salus University
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 overall response for the short-term pilot study entitled “Molecular Mechanisms of Leber’s Congenital Amaurosis” was graded as Favorable. We have always complied with the requirements in the past and received overall favorable critiques for all previously submitted projects. There are no reasons to expect that this trend will be affected in the future.

*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:** 0865001  
**Project Title:** Molecular Mechanisms of Leber's Congenital Amaurosis  
**Investigator:** Dizhoor, Alexander M.

**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.

### **Section B. Recommendations**

#### **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

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

##### Reviewer 1:

1. Failure to identify all possible mutations in the two families and thus assess the real mechanism of disease damaged the concept of the entire experiment. The specific mutations in parents and affecteds (and excluded in non-affecteds) would have truly justified the selection of the two isolated mutations in this program

**Response:** While we could not agree more with the reviewer's comments, no such detailed pedigree is available. We would like to stress that both mutations completely destroy RetGC activation in a direct biochemical assay *in vitro*. In other words, even though the genetic basis for the disease is not completely clear (and we emphasized that in our cited publication), the *biochemistry* of the mutations indicates their involvement. The logic dictates that, after observing a striking biochemical effect of a mutation found in an LCA patient, we should not ignore possible physiological implications despite that more extended genetic study in humans is not possible. Whether or not each mutation triggers the degeneration by itself or require additional components, we will assess by the *in vivo* expression of the mutant protein. Currently, we already have two expressing D639Y+ lines in RetGC1<sup>-/-</sup> background.

2. While the development or acquisition of RETGC-mutant or -deficient (knockout) mice was Aim 1, it is unclear how much other work could have been done to select other mutant pairs, whose individual expression may have influenced the development of the project and permitted the completion of Aim #2.

**Response:** We would just like to reiterate what was stated above – there were not enough patients in either group to perform such analysis. Secondly, our goal is to understand biochemical and physiological implications of those mutations that we already found to be a functional “null” phenotype *in vitro*. Breeding of the several different transgene-positive lines into RetGC1 knockout background was a lengthy and tedious process, but unavoidably needed to (1) accurately evaluate the levels of expression of the transgene, not just the transgene presence, and (2) test dominant versus recessive character of the potential effects. We are happy to report that we have identified two lines that express the mutant RetGC1 in RetGC1<sup>-/-</sup> background, which we are currently breeding into a homozygous (i.e., D639Y<sup>+/+</sup>RetGC1<sup>-/-</sup>) state for the physiological testing.

3. Although the authors devote lip service to “the role of newly found mutation as potentially causing blindness and thus...design future strategies of gene therapy,” nothing in these listed experiments as constructed, is targeting gene therapy for ocular diseases. Paradoxically, the stated objective is the “study of the physiologic implications of two recently identified mutation.”

**Response:** Gene therapy was never stated as the immediate objective for this short-term pilot study (perhaps the Reviewer did not fully realize that this was not a wide program, but rather a short-term pilot format study for two specific mutations found in LCA patients). The objective, as we stated it, was to make the model that could be tested for physiological changes caused by the mutant RetGC1 in a living retina. We need the physiological *in vivo* data, not just genetic and/or biochemical *in vitro* indications, to understand how these mutations can affect photoreceptors. The mutation of interest produces a physiological effect *in vivo*, is not a given pre-determined fact - it needs to be either established or rejected in experiment. Only when/if the mechanism is established, the means by which the corrections can be made could be considered, including gene therapy. A previous study from this PI's group, conducted using similar ideology, has already resulted in a different animal model that was recently successfully employed for gene therapy testing (see Jiang et. al, PNAS 2011). Right now, we have developed two lines expressing D639Y mutant in RetGC1<sup>-/-</sup> background for the study planned in this proposal.

Reviewer 2:

1. The PI may try a more realistic appraisal of the time line when writing the proposal and be prepared to move forward rapidly as soon as a funding decision is announced, but there is a strong tendency to be optimistic, so this is a minor weakness.

**Response:** We accept this as a just and useful criticism. The inherent challenge for short-term pilot proposals like this is to stay within the time frame that we plan it based on an optimistic scenario, yet the transgenics selection may or may not yield the sought-for expression quickly enough. In our case, it clearly took longer than we expected, especially because the selection required us to breed several transgenic lines into RetGC1<sup>-/-</sup> homozygous genotype before we could determine the transgene expression, although we finally did it. The Reviewer's suggestion will be given full consideration in the future applications.

Reviewer 3:

1. The rationale of generating transgenic mice carrying variable copy numbers of RetGC mutant in the RetGC-null background mice is not well justified. The better way to study the proposed goal is to produce knock-in mice instead.

**Response:** This is a reasonable criticism, we agree. However, to make the knock-in in this case would have taken us even longer, and, based on the current costs of developing knock-in models, this was not affordable for the budget of this small project. Since artificially high expression of the mutant protein could (such as via UPR response) compromise photoreceptor survival, it is desirable to have variable levels of expression in different lines. In any case, we finally have developed (and verified) two different expressor lines on which we have started the proposed analysis of the physiological (ERG), morphological (retinal histology), and biochemical changes (RetGC regulation) - in both homozygous and heterozygous variants.

## ***ADDITIONAL COMMENTS***

### Reviewer 2:

This is good work that warrants further support.

**Response:** We appreciate this encouraging opinion. We are currently continuing with this study using the models that we have developed as a result of this project.

### Reviewer 1:

The investigator states that this “ information is expected to benefit future studies....to treat(ing) the inherited blinding diseases.” However, no substantive explanation is offered of how these experiments did or will achieve that goal.

Another goal is to “evaluate the role of newly found mutations as potentially causing blindness and thus ...design future strategies of gene therapy.” However, nothing in the experiments, as constructed, is targeting gene therapy. The stated objective is the “study of the physiologic implications of two recently identified mutations.”

**Response:** The gene therapy experiments were never stated to be an immediate goal. The purpose of the pilot study was to develop a genetic model for testing the mechanisms of degeneration, which could become useful for gene therapy application after (but not before) extensive characterization. We could not plan any gene therapy experiments in the original application if the model itself did not exist at that moment.

### Reviewer 3:

The information provided support the fact that the project did not met any of its objectives or made reasonably acceptable progress.

**Response:** Although we indeed ran beyond the proposed time frame, we have finally developed the expressing D639Y+RetGC1-/- transgenic model and secured additional funding for further in-depth investigation of this mutation (as a small part of a larger extramural grant support). We consider that this demonstrates reasonable progress for a small-scale pilot study, albeit slower than we hoped for.

***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:** We appreciate the thorough and stimulating critiques from all three Reviewers.

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

None.