

## Response Form for the Final Performance Review Report\*

1. Name of Grantee:           Institute for Hepatitis and Virus 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 IHVR has a documented oversight process for all health research grants and projects. Routine key personnel meetings ensure that goals and objectives are met in a timely manner, that barriers to project success are overcome, and that all reports are submitted on deadline. Key personnel are monitored by the Principal Investigator, and the IHVR President provides oversight to ensure that all Principal Investigators are monitored, as well. To date, IHVR has met or exceeded all goals of projects funded through formula fund health research.

\* 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:** 1085301  
**Project Title:** Selective and Therapeutic Elimination of  
Cells that Produce Hepatitis B Virus  
**Investigator:** Block, Timothy

***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. It is not clear why the PI gave up those specific studies proposed in the original application. It would be much appreciated if the PI could provide a reasonable explanation on the modification and alteration of the original research plans.
2. Oubain and ABT-737 did not result in significant selectivity in killing HBV-replicating cells. Their selective killing activity should be corroborated by additional experimentation.
3. Future studies should follow through with those lead compounds described in the original application using more reliable cell culture systems other than the HepG2.2.15 cell clone.
4. The PI should be more cautious to further pursue the approach proposed in the original application. It may not be feasible to selectively kill HBV-producing cells. The rationale to selectively kill HBV-producing cells was not clearly stated in the original application.
5. Alternative approaches should be considered for searching effective therapies to treat hepatitis B.

Response:

- 1) Our team discovered that much more reproducible results were obtained when a cell line which had been engineered to inducibly express and replicate the HBV genome was used, rather than transient transfection of the established Huh7 cell line. This new cell line (termed RGE51) is cultured in the presence of tetracycline (+Tet) to repress HBV expression, and is withdrawn to induce expression and replication of HBV (-)Tet. Using this system, our ongoing screens identified oubain and ABT-737 as more potent and selective agents than those in the original proposal (ketides and statins). While we have

not given up on the original agents, over the limited time span of the funding period we decided to pursue what appeared to be more promising leads.

- 2) With all due respect, we disagree with the reviewer's assessment of the selectivity and potency of these compounds. Oubain showed selectivity for HBV-expressing cells of at least seven fold with a CC50 of below 0.016 micromolar, while ABT-737 had selectivity over 3000, again with a CC50 of 0.015 micromolar. These results are superior to what was observed with the ketides or statins.
- 3) Per the reviewer's suggestion, the original compounds are continuing to be included in our studies with the new inducible cell line.
- 4) This comment is well-taken, and we would like to state that our current studies are focusing on the potential for selective induction of apoptosis in HBV-replicating cells based on our observations that these cells are sensitized to apoptosis over non HBV-replicating cells, as determined with the inducible cell line system. We hypothesize that in this condition, infected cells may only need an apoptotic stimulus to be eliminated, which should be tolerable to non-sensitized (non-HBV-replicating) cells. Our preliminary data, including with the Bcl-2 mimetic ABT-737, indicate that this is the case.
- 5) We agree, and would like to point out that the selective elimination approach is only one of several programs that is being developed at the IHVR and its collaborators at the Center. We are also working on drug candidates that inhibit HBV capsid assembly, HBV surface antigen and virus secretion, and establishment of persistent HBV covalently closed circular DNA in the nucleus.

Reviewer 2:

It is not clear why the research proposed in the original plan was not pursued, but rather, different classes of drugs instead were analyzed. An explanation for this change should have been provided. Furthermore, why does it appear as though so little was accomplished? Was this because the work was primarily carried out by undergraduate research interns? If this is the case, then this weakness is understandable.

Response:

As we pointed out above in response to Reviewer 1, the implementation of a more reliable assay system led to the identification of what we believe are superior candidates, which were studied and reported; however, we did not have sufficient time to extend the proposed studies to the new compounds. While we have not given up on the ketides and statins, our judgment was that the better compounds more worthy of effort. As the review guessed, the work was indeed done by undergraduate and summer students, which frankly limited progress but provided an immeasurable learning experience for several young people who are now pursuing life sciences as a profession. At least on one the people involved are still working at the Center, and we hope to publish the complete results of this project in the next year or so.

Reviewer 3:

It is highly unlikely that “targeted” cytotoxic regimens will be developed. Furthermore, it will be nearly impossible to generate drugs with controlled cell killing without risk of killing the host.

Therefore, this approach most likely will not result in new therapies for hepatitis B but may provide some interesting information which may be utilized for future drug development.

Response:

We don’t disagree with the reviewers as to the very risky nature of this approach, which is an inescapable aspect of any drug discovery project. However, the idea is not completely unlike the prospect of targeting cancer cells for selective killing, as HBV replication induces metabolic differences in cells that may be exploited therapeutically, and which may be better elucidated in the pursuit of this work. These metabolic differences are not very well characterized, but our preliminary results indicate that (for instance) HBV replication disrupts the cell cycle and sensitizes cells to apoptotic stimuli, including experimental cancer drugs like ABT-737. While a long way from a new therapy, this observation may lead to better understanding of specific well known phenomena ( i.e., how does the immune system function in clearing acute HBV infection with a “flare up” in serum levels of liver enzymes?), and may someday suggest a new and feasible therapeutic approach.

***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: None required.

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

Response: We appreciate the opportunity to further address the reviewer’s comments. Our team strongly believes that the “selective elimination” idea is worthy of pursuit despite its difficulty, and would constitute a revolutionary approach if it were successfully developed. For this we thank the Commonwealth for the funding provided to support this exploratory work, and to train young scientists.