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HALT-C Ancillary Study Miniproposal:
Suitability of OCT liver samples for Gene Expression Analysis

Purpose/Goal:
To determine whether the RNA derived from HALT-C liver biopsy samples collected in OCT are of sufficient quality for use in gene expression microarray experiments to investigate correlates of liver fibrosis progression and hepatic decompensation.

Background:
From HALT-C study inception, many investigators agreed that a gene expression study of the HALT-C liver biopsies would be desirable. An ancillary study to examine gene expression in HALT-C liver biopsy samples was previously approved (M. Katze and C. Morishima proposal). However, due primarily to the lack of funds to perform preliminary experiments, an R01 application was never submitted. Funds have now become available to generate preliminary data for such a proposal.

Dr. Roger Bumgarner, a colleague here at the University of Washington, has significant expertise in the gene expression microarray field, and is director of the UW Center for Array Technologies (http://www.expression.washington.edu/index.jsp). He was also one of the founding faculty for Michael Katze's Center for Functional Genomics. Dr. Bumgarner's center provides a microarray service for the UW scientific community) and has an Agilent RNA Bioanalyzer 2100, which can determine the integrity of RNA using capillary electrophoresis to determine RNA size.

Request and Plan:
I propose to isolate RNA from 36 OCT liver biopsies (12 from baseline 12 from year 2, and 12 from year 4, where 3 biopsies will have originated from each of the 4 Immunology/Virology clinical sites) using Qiagen kits and analyze the RNA quality at the UW Center for Array Technologies. We will test for RNA integrity in 3 rounds: serial samples from 1 patient from each site will be tested first (4 patients), and the data considered before testing the second set of 4 patients. If the data from the first 2 rounds of testing (total of 8 patients) are definitive, then it may not be necessary to perform the testing for the last 4 patients. Testing the quality of RNA from OCT liver biopsies is essential in order to determine whether a grant submission for gene expression analysis of HALT-C liver samples is viable.

Assuming that the RNA is of sufficient quality, I further propose that we perform six microarray experiments to demonstrate proficiency with our RNA samples. I propose testing 3 longitudinal samples from 2 patients with good RNA quality using 6 Affymetrix Exon 1.0 ST arrays. For the purposes of this preliminary experiment, we would select for HCV RNA testing 6 patients with virologic nonresponse to pegIFN therapy during lead-in and randomized phase, and 6 who were relapsers to lead-in with significant viral suppression during the randomized phase, so that 1 of each could be analyzed using the exon arrays. We anticipate that once all necessary committee approvals are obtained and the samples are in the lab, that the RNA analysis could be completed within 3 months or less. Dr. Bumgarner and his associate Ka-yee Yeung have agreed to assist us with the data analysis of the microarray results, if these assays can be performed.

In the event that the RNA quality is not sufficient for microarray analysis, the remaining RNA samples generated in our laboratory would revert to the repository and would be available for other applications (and the microarray experiments would not be performed).

The liver biopsy specimens collected in OCT for the Replication Study are currently available for this proposal. The unblinding of the HALT-C study results will also now permit specific sample selection and redefinition of the Specific Aims for an R01 proposal. Thus, the timing appears to be optimal for pushing this project forward.

A separate request for access to additional liver biopsy samples for an R01 application will be made when results of these preliminary tests are known.

Cost to HALT-C:
The proposed experiments will be carried out by funds available internally at University of Washington. I do not need additional support from HALT-C.

I will need some help from NERI to select the most appropriate samples for this study, which I understand may require final approval from the HALT-C Ancillary Studies or Steering Committee.