APPENDIX A: HALT-C Ancillary Study PROPOSAL

Part I (1 page)
Proposal Name: **Interferon-alfa Effects on HALT-C Liver Gene Expression**

At the request of the Ancillary Studies Committee, this proposal has been modified to a 2-phase protocol. In Phase I we will evaluate liver gene expression at baseline and at M24 in 26 treated patients, 14 of whom remained viremic at M24 and 12 of whom had undetectable HCV RNA in serum. If these data demonstrate statistically significant associations between specific gene expression profiles and persistent viremia compared to complete viral suppression, we will request approval from the AS Committee and NIDDK to proceed to Phase II of the study. Both phases are described below.

Note: Our request to use OCT specimens was previously approved in April 2009 (through April 2010) by the AS Committee for C. Morishima's NIH applications. We are now requesting to study these specimens using existing funds. Other samples approved in April 2009 (formalin-fixed slides) may still be used if funding is obtained upon resubmission of her R01 application in March 2010, but use of the samples are not requested in the current proposal.

Proposal PI: Chihiro Morishima MD

Co-Investigators: Roger Bumgarner PhD, UW
Tim Morgan MD, UCI

HALT-C PI: Chihiro Morishima MD

Funding Agency and Review Body (e.g., NIDDK; my university/GAC): We will use existing funds at UCI and UW to support this study.

I agree to follow HALT-C Policies and Procedures when conducting this study. I acknowledge that the data obtained from this study will belong to the NIH and will be placed in the HALT-C database for use by other investigators. I understand that I cannot begin experiments using HALT-C specimens/data until I receive approval from the HALT-C Ancillary Studies Committee and funding from the Scientific Review Body for my proposal. I also understand that the data analysis for this proposal will be performed by NERI (unless otherwise approved by the HALT-C study) and that Protocols approved by the HALT-C Ancillary Studies Committee will be placed on the HALT-C Restricted Website.

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Proposal Principal Investigator Date

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HALT-C Principal Investigator Date

1. Aims/Hypotheses

Phase I will identify the hepatic gene expression profiles associated with resistance to peginterferon treatment, defined as <1.0 $\log_{10}$ decline in HCV RNA between baseline and Month 24. This study will analyze, for the first time, hepatic gene expression before and *during steady-state pegIFN treatment in patients who are resistant and sensitive to peginterferon treatment.* **The overall goal of the Phase I and II studies is to identify gene expression patterns that are associated with IFN-resistant chronic HCV infection that could contribute to liver disease progression.**

PHASE I:

Specific Aim 1: To determine the hepatic gene expression profile associated with pegIFN treatment and persistent viremia (interferon resistance) compared to pegIFN treatment and viral suppression (interferon sensitive). Serial HALT-C liver biopsy samples from 26 patients with baseline and Month 24 biopsies frozen in OCT will be tested for gene expression using Affymetrix Exon 1.0 ST arrays. This methodology was selected in order to maximize the amount of information that could be obtained from these precious samples: both overall gene expression levels and effects on alternative splicing will be determined. Alternative splicing has been increasingly shown to play a role in a number of diseases (see for example the following two reviews(1, 2)).

Baseline (prior to peginterferon) samples will serve as controls for each treated patient. First, patients will be selected for HCV genotype 1 infection and *undetectable HCV RNA levels at Month 24*, since a limited number of these patients are available from this cohort. Viremic patients will be matched as closely as possible for race, gender, and baseline fibrosis stage. Patients with HCC will be excluded from both Phase I and Phase II of this study.

We anticipate that the Phase I results will identify a set of genes that is predictive of viral persistence (interferon resistance) in patients with advanced hepatic fibrosis. Assuming that this result is obtained, we will ask the AS Committee to approve Phase II. Phase II will address the following 3 areas:

1. Confirm changes in hepatic gene expression associated with complete viral suppression (interferon sensitivity) by testing patients in the treated arm of HALT-C who had undetectable HCV RNA at Month 48
2. Evaluate the changes in hepatic gene expression over time by examining liver tissue from baseline and Month 24 from patients in the untreated (control) group, and
3. Evaluate changes in hepatic gene expression in patients who have an improvement in HAI at Month 24 as compared with those who have a worsening in HAI at Month 24.

PHASE II:

Specific Aim 1: To determine (in a separate subset of HALT-C patients) the hepatic gene expression profile associated with pegIFN treatment and persistent viremia (interferon resistance) relative to viral suppression by pegIFN (interferon sensitivity) and no treatment. Serial HALT-C liver biopsy samples from baseline and Month 24 or Month 48 will be tested for gene expression using Affymetrix Exon 1.0 ST arrays. Baseline untreated samples will serve as controls for each treated patient. Serial biopsies from untreated patients will also serve as time-dependent controls. Differences between samples of treated and untreated patients will confirm the effects of exogenous IFN on hepatic gene expression. The additional samples proposed for Phase II should increase the power of the analyses and permit confirmation of the findings in Phase I.

Specific Aim 2: To determine the hepatic gene expression profiles associated with IFN treatment and improved hepatic inflammation, compared to worsening hepatic inflammation

during treatment in the randomized phase at Month 24. We reported that long-term treatment with pegIFN did not affect liver disease progression in this cohort. However, post hoc subanalyses (C. Morishima/E.C. Wright, Inflammation manuscript in preparation) have indicated that pegIFN-treated patients with improved hepatic inflammation (HAI) at M24 had less fibrosis progression and fewer clinical outcomes than pegIFN-treated patients with worse HAI. We will evaluate the hepatic gene expression profiles associated with improved vs. worse HAI, in order to identify mechanistic links between increased inflammation and liver disease progression.

2. Background/rationale

Although the results of the HALT-C Trial demonstrated that IFN-α should not be used as maintenance therapy in prior interferon nonresponders with advanced liver disease, treatment with IFN-α remains the backbone of current therapeutic strategies against HCV infection. A number of studies have investigated gene expression profiles from HCV-infected liver biopsy samples obtained pre-treatment (3), and before and after a single dose of IFN-α in different patients (4), or in the same patients (5). The studies with pre-treatment biopsy data all agree that patients who are nonresponders to standard pegIFN-α/ribavirin therapy have elevated levels of interferon-stimulated genes (ISGs) prior to starting treatment. Moreover, ISG expression did not change significantly 4 hours after a single dose of pegIFN-α in 6 patients with slow virological response (defined as <1.5 $\log_{10}$ decrease in HCV RNA levels from baseline to 4 weeks)(5). In addition, expression levels of most ISGs at 4 hours post pegIFN did not discriminate between the rapid virologic responders (RVR) and non-RVR. The 16 patients with serial liver biopsies studied by Sarasin-Filipowicz et al were heterogeneous in terms of liver disease stage and HCV genotype infection. In the proposed study we will test genotype 1-infected HALT-C patients with advanced hepatic fibrosis. The characteristics of HALT-C patients are significantly different from those previously studied; HALT-C patients are known IFN-nonresponders with advanced liver disease, who were treated for ~18 months prior to their second biopsy and who were known to have actively suppressed HCV replication or not at the time of their second biopsy. Therefore, we believe that differences in the patient cohort and timing of the second biopsy relative to duration of treatment should allow the data obtained from our proposal to add substantially to existing knowledge.

The pathogenesis of liver fibrosis progression during chronic hepatitis C is poorly understood, but is thought to involve the immune response, because of the noncytopathic nature of HCV infection as well as the characteristic lymphocytic infiltrate adjacent to areas of hepatocyte cell death seen in HCV-infected liver biopsies. Clinical studies have demonstrated a clear association between greater hepatic inflammation and subsequent fibrosis progression (6, 7). IFN−α is known to have direct anti-viral as well as pleiotropic immunomodulatory effects (8). While it is clear that IFN-α significantly affects both innate and adaptive immune responses, the context of its secretion appears to modify its overall effect. For example, type I interferons are known to have both beneficial and detrimental effects in different autoimmune diseases such as multiple sclerosis and SLE, respectively. Interestingly, our subanalysis of HALT-C patients (C. Morishima/E.C. Wright, Inflammation manuscript in preparation) has indicated that a subset of pegIFN-treated patients with improved hepatic inflammation (HAI) had less fibrosis progression and fewer clinical outcomes, while another subset of pegIFN-treated patients with worse HAI had a greater frequency of clinical outcomes. This leads us to the novel hypothesis that IFN treatment can have deleterious effects on liver disease progression by affecting innate and/or adaptive immunity. However, the mechanism by which IFN could have this effect is unknown. In Phase II, we will evaluate the gene expression profiles associated with worse vs. improved HAI in treated HALT-C patients to identify factors associated with these different clinical states.

3. Relations to aims of HALT-C study

The aim of the HALT-C Trial was to determine if treatment with long-term low dose pegIFN-α could alter the course of liver disease progression in patients with prior non-response to interferon therapy. In this proposal, we will attempt to identify the genes that are associated with interferon sensitivity (viral clearance) and interferon resistance (viral persistence). We will also identify gene expression profiles associated with increased hepatic inflammation compared to decreased hepatic inflammation. These results could reveal important information to aid in developing new therapies for patients targeted in the HALT-C Trial: those with advanced hepatic fibrosis and chronic hepatitis C refractory to IFN therapy. Overall these studies are highly aligned with the main goal of the HALT-C Trial.

These studies will dovetail with future studies (proposed in a March 2010 R01 application) that will investigate whether cirrhosis and pegIFN treatment are associated with higher frequencies and/or increased suppressive function of T-regulatory cells.

4. Study design/Methods

For the proposed experiments, we will analyze gene expression profiles from liver samples collected in OCT for the Immunology/Virology Ancillary Study using Affymetrix Exon 1.0 ST arrays. This microarray analysis will be performed by R. Bumgarner, UW co-investigator. Dr. Bumgarner has a PhD in chemical physics and trained under Dr. Leroy Hood, developing methodologies for gene sequencing and microarray gene expression analyses. His current research focus is on the development of software and methods to generate, analyze and store genomics data, including new methods to infer gene regulation and gene networks from integration of large scale genomics data. His laboratory has performed experiments with nearly every type of microarray ever created, and he has published 20 papers on computational genomics methods and 10 papers on liver or viral functional genomics. He recently published a manuscript with Nelson Fausto on transcriptional programs initiated after partial hepatectomy (9), and a manuscript on antiviral actions of IFN-beta and IFN-gamma against HSV infection in mice (10). He is therefore ideally suited to collaborate with Drs. Morishima and Morgan on this project.

The feasibility of using HALT-C liver biopsy samples collected in OCT for microarray experiments has been assessed. The quality of the RNA extracted was determined using an Agilent Bioanalyzer. Nine of 12 samples had an “RNA Integrity Number” (RIN) >6.6. A total of three patients with 3 serial biopsies per each were profiled using Affymetrix Exon 1.0 ST arrays at the University of Washington Center for Array Technologies (UW-CAT, R. Bumgarner, Director). Using standard metrics to assess the quality of the array results, all samples produced data within acceptable limits (e.g. typical average intensities, similar scaling factors and no outlier arrays as judged by several metrics). **Figure 1** shows a sampling of the data. In brief, the array data was loaded into MultiExperimentViewer (www.TM4.org) and subjected to hierarchical clustering. One cluster of genes showed a statistically significant enrichment for IFN-stimulated genes (see zoom on right side of **Figure 1**). Interestingly, these genes show a decrease in expression in the untreated patients (average fold change of -1.7+/- 0.6) and an increase in expression in the IFN-treated patient (average fold change of +1.5+/-0.2) over time. Since only a few samples were tested, no solid statistical conclusions can be drawn from the current dataset. However, the preliminary data suggests that we may be able to determine the effects of IFN treatment on global gene expression levels. Hence, it is anticipated that data pooled from HALT-C patient gene expression analyses will permit a statistically significant evaluation of changes in gene expression related to time, to IFN treatment, fibrosis stage and virological suppression.

To extract RNA from the OCT samples, we will use a Trizol-based extraction method utilized to generate the data shown in **Figure 1**. As confirmed by Dr. Nury Steuerwald, a collaborator of Dr. Herb Bonkovsky at Carolinas Medical Center, this technique should be adequate for isolating both mRNA for our gene expression studies and microRNAs for potential future studies. Our analysis of the yield of RNA obtained from past HALT-C specimens indicates that at least 2/3 of samples had sufficient RNA content (>3 micrograms per sample) to permit both gene expression and microRNA analyses to

be performed on the same samples, and the samples that are of insufficient integrity for gene expression studies may still be adequate for microarray studies (~25% of samples). We are happy to provide the RNA not required for our study directly to collaborators or alternatively to the NIDDK repository for future microRNA analyses and would be happy to contribute to joint analyses of the data generated.

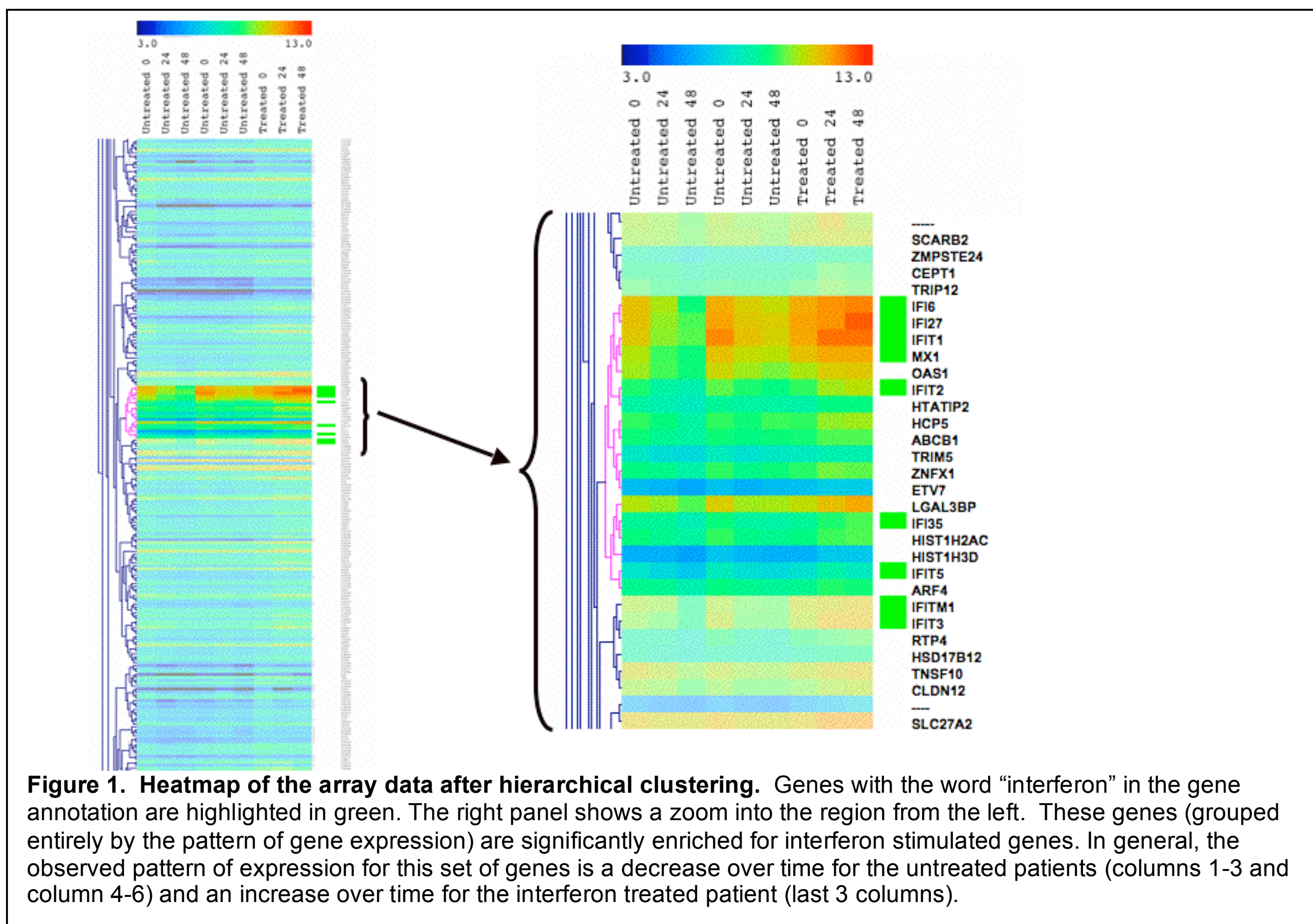

Figure 1. Heatmap of the array data after hierarchical clustering. Genes with the word "interferon" in the gene annotation are highlighted in green. The right panel shows a zoom into the region from the left. These genes (grouped entirely by the pattern of gene expression) are significantly enriched for interferon stimulated genes. In general, the observed pattern of expression for this set of genes is a decrease over time for the untreated patients (columns 1-3 and column 4-6) and an increase over time for the interferon treated patient (last 3 columns).

For Phase II, we will extract RNA from approximately 65 additional patients at baseline, Month 24/Month 48. RNA extracted from liver biopsy samples will be tested for RNA integrity. Those with an RNA Integrity Number > 6 will be considered for microarray analysis; this threshold is subject to change and a higher threshold may be necessary to obtain high quality data.

Results from these samples will be evaluated using both cross-sectional and longitudinal analyses. Initially, we will perform exploratory analyses on this data using hierarchical clustering as implemented in the software program MeV (multi-experiment viewer (11, 12)). This will allow us to identify genes and samples that behave similarly and potentially allow us to identify correlates between gene behavior and clinical parameters (such as fibrosis state, response to therapy, etc). These analyses will be aided by the algorithm "TEASE" or Tree-EASE, which Dr. Bumgarner's group contributed to the MeV software package. In brief, TEASE allows one to label genes or samples with given labels (GO terms, clinical parameters, etc), and then it searches the clustering tree to identify branches that are statistically enriched (using Fisher's exact test) for a given label.

With microarray data from Phase I and Phase II, we will be able to perform the following comparisons.

Longitudinal changes in gene expression (with or without peginterferon):

1. Virological nonresponder in the control group (S00, M24)
2. Virological nonresponder (interferon resistant) in the treated group (S00, M24)

3. Virological responder (interferon sensitive) in the treated group (S00, M24)

Cross sectional analyses will compare hepatic gene expression between the following groups:

1. Virological nonresponder in the control group (M24)
2. Virological nonresponder (interferon resistant) in the treated group M24
3. Virological responder (interferon sensitive) in the treated group M24 (and M48)

Baseline (pretreatment) samples will serve as controls for each treated patient. Serial biopsies from untreated patients will serve as time-dependent controls. Analyses of baseline liver biopsies (all untreated) may reveal gene expression profiles associated with discrete liver disease stages (Ishak 3-6). These liver disease stage data will be considered in the analysis of IFN-α effects on liver gene expression, as described below. From this dataset, we expect to primarily identify a gene expression profile associated with treatment that does *not* result in viral clearance, since most patients continued to be viremic, and that also takes into account the presence of liver fibrosis/cirrhosis.

The pair-wise analyses will be performed using t-tests. The Benjamini–Hochberg method (13, 14) will be used to control the false discovery rate (FDR) which will be set to <= 10%. In addition to the pair-wise analyses, we will perform a joint analysis of the data using a 3-factor ANOVA with treatment being one factor, time being another and viral response being the 3rd factor. This will identify genes that change over time independent of treatment, genes that change only due to treatment and genes that vary over time in a treatment dependent manner. A 3-factor ANOVA will allow all the data to be compared simultaneously and will give the best statistical power. Again, the Benjamini–Hochberg method (13, 14) will be used to control the false discovery rate (FDR) which will be set to <= 10%. To account for changes due to liver disease state, we will take the following two approaches. First, the above analyses will be performed using the entire data set and the dataset split into non-cirrhotic and cirrhotic samples with independent analyses performed in each group. Second, we will also perform a 4 factor mixed model approach in which the cirrhotic state is explicitly modeled. Genes identified from all of the above analyses will be further analyzed to identify functions and pathways that are statistically enriched. After the above analyses, a subset of genes from each enriched pathway or related functional subgroup will be confirmed by RT-PCR using TaqMan assays.

Recent studies have indicated that cell-specific gene expression data may be critical to understanding the key factors associated with virological response/non-response to IFN (15). Two ways to approach this issue include the laser capture microdissection (LCMD) technique and follow-up immunohistochemical analysis. LCMD has the advantage that distinct cell types can be isolated and profiled independently. Hence gene expression changes that are present in only one cell type will not be masked by a lack of change in another cell type. However, in our experience, LCMD has a disadvantage in that the quality of RNA obtained from any LCMD method is generally lower than RNA obtained from the same bulk tissue by traditional isolation methods. This is especially the case with samples that have been subjected to long-term storage such as those from the HALT-C Trial. In such cases, the noise introduced in the array data by LCMD may, in fact, negate the advantages of LCMD. Immunohistochemical (IHC) staining post array analysis provides an alternative way to determine which cell type(s) are responsible for the observed mRNA expression changes. Moreover IHC serves an additional purpose in that it confirms that the protein levels are changing in concert with the mRNA levels.

We have found that RNA of sufficient quality and quantity for gene expression analyses is obtained from only about 75% of the HALT-C samples when the RNA is isolated from the bulk biopsies. In addition, the RNA quality is lower that what we typically obtain from fresh tissue samples or from cells grown in culture. Our past experience indicates that performing LCMD on the HALT-C samples will likely result in a much lower percentage of samples producing useable RNA. Given the potential technical difficulties associated with the LCMD, we have opted to proceed with Phase I using the gene expression analyses described above, as we believe that this will more likely yield useable results. We will consider performing the more labor-intensive and detailed analyses of gene expression at the

cellular level during Phase II of the study, when more samples are available for this procedure, and preliminary data from Phase I are available. Alternatively, we may request HALT-C liver biopsy sections for immunohistochemical analyses.

5. Statistical support

All bioinformatics support for the processing of the array data will be provided by Dr. Bumgarner (as detailed above). Clinical data will be obtained through a data use agreement with NERI or through the NIDDK Repository. All statistical analyses for the proposal will be performed by University of Washington personnel as needed (S. Holte PhD). We will consult with Dr. Libby Wright before completing our analyses to ensure that we have adequately considered all relevant clinical factors that could affect the statistical analyses of our findings.

6. HALT-C samples to be used in the study (complete Part III: Sample Requirements)

Table 1. Phase I Liver Biopsy Samples in OCT

	Baseline	M24
Treatment (Rx), Nonresponder	14	14
Treatment (Rx), M24 HCV-neg	7	12

For Phase I of the study, we request the use of OCT liver biopsy samples from a total of 26 patients, as listed above in **Table 1**.

1. OCT samples from 14 treated patients with S00 and M24 serial biopsies with <1 $\log_{10}$ decrease in HCV RNA levels from baseline, matched for gender, race and baseline fibrosis score with patients in the HCV RNA-negative group at M24.

2. S00 and M24 samples from 12 treated patients who were HCV RNA-negative at M24.

Note that we do not expect that all samples will yield useable RNA for microarray analysis. We predict that we will be able to perform microarray analyses on ~35 out of the 47 samples processed (**Table 1**). All useable data will be included in our final analyses (of Phase I and Phase II together).

For Phase II of the study, we will isolate RNA from additional samples to:

1) Independently corroborate differences found between treated patients with viremia and those with virological suppression using additional samples (from M48).
2) Assess changes in hepatic gene expression over time between baseline and Month 24 from untreated patients, and
3) Identify gene expression profiles associated with worse inflammation and improved inflammation.

Table 2. PhaseI and II Target Number of Arrays

	Baseline	M24	M48
Control (No Rx), Nonresponder	15	15	0
Treatment (Rx), Nonresponder	40	40	0
Treatment (Rx), M24 or M48 HCV-neg	5	10	10

Table 2 shows the target number of arrays we hope to test, which differs from Table 1. Table 1 showed the number of OCT samples we planned to process. The number of samples that will be adequate for microarray analysis can only be estimated.

Overall, our goal is to perform microarray analyses on 135 samples (total, including arrays conducted in Phase I). To achieve this goal, we are requesting a total of 133 additional samples to process under Phase II, as we believe this is the number that will be necessary to achieve the desired target of 135

arrays. Ultimately, however, the number of samples used could be less. Any RNA that is insufficient in quality or quantity for microarray analysis will be saved for future use, and returned to the NIDDK repository.

For Phase II of the study, we will request OCT liver biopsy samples from an additional ~65 patients.

1. OCT samples from 15 untreated patients with S00 and M24 serial biopsies.
2. OCT samples from 40 treated patients with S00 and M24 serial biopsies (with worse or improved inflammation).
3. All samples from treated patients who were HCV RNA-negative at M48 (10 samples) and available baseline samples from the same patients.

7. References

1. Cooper TA, Wan L, Dreyfuss G. RNA and disease. Cell 2009;136:777-793.
2. Tazi J, Bakkour N, Stamm S. Alternative splicing and disease. Biochim Biophys Acta 2009;1792:14-26.
3. Chen L, Borozan I, Feld J, Sun J, Tannis LL, Coltescu C, Heathcote J, et al. Hepatic gene expression discriminates responders and nonresponders in treatment of chronic hepatitis C viral infection. Gastroenterology 2005;128:1437-1444.
4. Feld JJ, Nanda S, Huang Y, Chen W, Cam M, Pusek SN, Schweigler LM, et al. Hepatic gene expression during treatment with peginterferon and ribavirin: Identifying molecular pathways for treatment response. Hepatology 2007;46:1548-1563.
5. Sarasin-Filipowicz M, Oakeley EJ, Duong FH, Christen V, Terracciano L, Filipowicz W, Heim MH. Interferon signaling and treatment outcome in chronic hepatitis C. Proc Natl Acad Sci U S A 2008;105:7034-7039.
6. Yano M, Kumada H, Kage M, Ikeda K, Shimamatsu K, Inoue O, Hashimoto E, et al. The long-term pathological evolution of chronic hepatitis C. Hepatology 1996;23:1334-1340.
7. Ghany MG, Kleiner DE, Alter H, Doo E, Khokar F, Promrat K, Herion D, et al. Progression of fibrosis in chronic hepatitis C. Gastroenterology 2003;124:97-104.
8. Theofilopoulos AN, Baccala R, Beutler B, Kono DH. Type I interferons (alpha/beta) in immunity and autoimmunity. Annu Rev Immunol 2005;23:307-336.
9. Li J, Campbell JS, Mitchell C, McMahan RS, Yu X, Riehle KJ, Bumgarner RE, et al. Relationships between deficits in tissue mass and transcriptional programs after partial hepatectomy in mice. Am J Pathol 2009;175:947-957.
10. Peng T, Zhu J, Hwangbo Y, Corey L, Bumgarner RE. Independent and cooperative antiviral actions of beta interferon and gamma interferon against herpes simplex virus replication in primary human fibroblasts. J Virol 2008;82:1934-1945.
11. Saeed AI, Bhagabati NK, Braisted JC, Liang W, Sharov V, Howe EA, Li J, et al. TM4 microarray software suite. Methods Enzymol 2006;411:134-193.
12. Saeed AI, Sharov V, White J, Li J, Liang W, Bhagabati N, Braisted J, et al. TM4: a free, open-source system for microarray data management and analysis. Biotechniques 2003;34:374-378.
13. Benjamini Y, Hochberg Y. On the adaptive control of the false discovery fate in multiple testing with independent statistics. Journal Of Educational And Behavioral Statistics 2000;25:60-83.
14. Benjamini Y, Hochberg Y. Controlling The False Discovery Rate - A Practical And Powerful Approach To Multiple Testing. Journal Of The Royal Statistical Society Series B-Methodological 1995;57:289-300.
15. Chen L, Borozan I, Sun J, Guindi M, Fischer S, Feld J, Anand N, et al. Cell-Type Specific Gene Expression Signature in Liver Underlies Response to Interferon Therapy in Chronic Hepatitis C Infection. Gastroenterology 2009.

Protocol Part III: Sample Requirements. (link to web site with actual sample availability)

TOTAL SAMPLES FOR PHASE I AND II:

Visit	Liver # patients, mm*	Blood # patients, ml	DNA # patients, ug	Liver Biopsy Slides # patients, slides/patient	Other (describe) # pts, amount
Screen 1					
Screen 2	92 OCT				
Baseline					
Lead in Week 4					
Week 8					
Week 12					
W16					
Week 20					
Week 24					
Randomized Month 9					
Month 12					
Month 15					
Month 18					
Month 21					
Month 24	78 OCT				
Month 27					
Month 30					
Month 33					
Month 36					
Month 39					
Month 42					
Month 45					
Month 48	10 OCT				
Post-treatment					
Responders W30					
W36					
W42					
W48					
W60					
W72					

* Assume 1 mm tissue weighs about 0.75 mg (= $0.5\ mm^2 \times \Pi \times$ density of tissue)

Data needed (please specify):
Comments (if any):

180 total OCT samples from: treated and untreated patients with genotype 1 and 2 serial biopsies (baseline and Month 24); includes patients who were HCV RNA negative at M24 (and M48) and their available baseline biopsies to be used for gene expression microarray analysis.

Samples from patients who later developed HCC or presumed HCC will be excluded.