Early Detection of Hepatocellular Carcinoma by Biomarkers Using Serum from the Hepatitis C Anti-viral Long-term Treatment Against Cirrhosis (HALT-C) Trial

This document details the process for obtaining and testing a set of samples intended for the study of serum biomarkers for the early detection of hepatocellular carcinoma (HCC) among patients with hepatitis C related advanced liver disease. Because these samples constitute a unique and limited resource, they are to be used for validation of promising biomarkers and not for biomarker discovery.

Introduction

A link to all HALT-C numbered manuscripts and other information about the study is at: http://archives.niddk.nih.gov/haltctrial/displaypage.aspx?pagename=haltctrial/index.htm

Serum samples were saved as part of the HALT-C trial, conducted between 1999 and 2009. HALT-C had two major treatment phases (clinical trials.gov identifier NCT00006164) and an observational phase.[1-3] A lead-in treatment phase used full dose peginterferon alfa-2a (Pegasys, Hoffmann-La-Roche) and ribavirin to attempt to achieve sustained virological response (SVR) among patients with advanced liver disease characterized by bridging fibrosis or cirrhosis on liver biopsy (Ishak fibrosis score of 3 or greater) who had previously been treated with standard interferon. Patients who did not achieve SVR were eligible for the randomized treatment phase, a controlled clinical trial of peginterferon alfa-2a at half dose of 90μg per week for 3.5 years, as compared with no treatment. The primary end point was progression of liver disease, as indicated by death from any cause, hepatocellular carcinoma (HCC), hepatic decompensation, or, for those with bridging fibrosis at baseline (Ishak fibrosis score of 3 or 4), an increase in the fibrosis score of 2 or more points. Most patients entered the randomized trial through the lead-in phase as non-responders after 20 weeks of therapy (based on detectable HCV RNA) or after subsequent breakthrough or relapse. Other patients entered the randomized phase as “express” patients by having failed to clear virus outside of the HALT-C lead-in. All patients had liver biopsies scheduled at 18 months after randomization and at the end of treatment, 42 months after randomization. Patients continued to be followed in the observational phase for clinical outcomes off therapy for as long as 5 additional years. The median duration of participation in the trial (time from randomization to first outcome or last time known to be outcome-free) was 6.0 years (range, 0-8.7 years).

Patient visits were scheduled every 3 months during the 3.5 years of the randomized trial and every 6 months thereafter in the observation phase. Those patients who stopped treatment continued to be followed in the study unless consent was withdrawn or the patient underwent liver transplantation. Alpha fetoprotein (AFP) level was obtained at the local clinical center at each visit. Ultrasound was repeated at the time of randomization, 6 months after randomization, and every 6-12 months thereafter. Patients with an elevated or rising AFP and those with new lesions on ultrasound were evaluated further with a CT or MRI. Diagnostic liver biopsy and treatment for HCC were performed at the discretion of the investigators at each of the ten sites.

Two definitions of HCC were adopted, one for “definite” HCC and one for “presumed” HCC. Definite HCC was defined by histologic confirmation or a new mass lesion on imaging with AFP levels increasing to >1,000 ng/mL. Presumed HCC was defined as a new mass lesion on ultrasound in the absence of histology and AFP <1,000 ng/mL in conjunction with one of the following characteristics: a) 2 liver imaging studies showing a mass lesion with characteristics of

HCC (vascular enhancement with or without wash out), b) progressively enlarging lesion on ultrasound leading to death of the patient, or c) 1 additional imaging study showing a mass lesion with characteristics of HCC that either increased in size over time or was accompanied by increasing AFP levels. All cases of HCC (presumed and definite) were reviewed by an outcomes review panel comprised of rotating panels of three clinical investigators.

A total of 92 patients were diagnosed with definite HCC (n=71) or presumed HCC (n=21). All 92 patients had serum stored for future use. This is one more case than was reported in the published case-control study of the association of occult hepatitis B with HCC in HALT-C.[4]

Selection of Cases and Controls

A case was defined as any enrolled HALT-C patient with Presumed HCC or Definite HCC that was confirmed by the outcome review board. Two controls were matched to each case. Controls were selected from among HALT-C patients who were not known to develop HCC but shared clinical characteristics. Specifically these controls were matched to cases based on 1) histological fibrosis stage (bridging fibrosis or cirrhosis), 2) treatment assignment (peginterferon or no treatment), and visit at which diagnosis was made for the case. Each control was followed for at least a year longer than the matched case to help ensure that the control was not "incubating" HCC. For the few cases that were diagnosed during the Lead-in phase controls were matched on visit and fibrosis/cirrhosis only, irrespective of randomization assignment of the controls.

Each of the 92 cases had 2 matched controls (184 total) selected. The first case was diagnosed in November 2000 and the last case was diagnosed in October 2009. The median date of diagnosis was October 2006. Three patients who had presumed HCC without confirmation were included. Two died within 8 months of diagnosis of the presumed HCC diagnosis. A third patient died 19 months after diagnosis and had HCC listed as cause of death on death certificate.

Steps for Sample Testing

Samples from cases and controls will be made available in two steps according to definitions for phase II and phase III biomarker studies for the early detection of cancer (Phase I is discovery, the initial identification of potential biomarkers).

<u>Step one</u>:

In the first step, serum samples assembled at or close to the time of diagnosis of HCC will be provided together with serum samples from two matched controls. The sample selection window for cases was from within 3 months prior to the HCC diagnosis to any time after diagnosis but before treatment (ablation, chemotherapy, transplant, etc). Samples are available for all control patients at the visit corresponding to the visit closest to that of the case's diagnosis.

The samples are masked as to source (HCC case or control). In order to proceed to the next step, the tested biomarker must perform at least as well as or must complement the standard serum test of AFP that has been evaluated in the study.[5] The investigator will provide the testing results to the NIDDK data repository or other pre-specified independent analysis center that has been provided the relevant data for the patients. In most cases these results are expected to be quantitative or semi-quantitative.

The analysis center will determine the success of the results in discriminating cases from controls. To proceed to testing of earlier samples, the biomarker(s) must perform substantially better than chance and at least as well as AFP. Attached is an example of a statistical analysis of two potential biomarkers tested among 55 cases and 110 controls that failed to improve on AFP and did not proceed to step 2. (powerpoint link).

The data analysis will be shared with the investigator regardless of success in identification of HCC cases.

Step two:

If step 1 is successful, samples from earlier time points will be provided to the investigator.

In the second step, samples obtained between 2 and 6 time points prior to the diagnosis of HCC will be provided together with samples from a suitable number of controls. Samples from some time points prior to diagnosis may not be available at all time points for all cases and all controls. Thus, not all 92 cases and 184 controls have serum available at all time points.

As in step 1, samples will be masked to case/control status and other clinical features. The investigator will provide the testing results to the NIDDK data repository or other pre-specified independent analysis center that has been provided the relevant data for the patients. The data analysis will be shared with the investigator regardless of success in identification of HCC cases.

Aliquoting and Storage

Eight 0.25 mL aliquots were created for all HCC cases and two matched controls from serum samples collected at the study visit closest to the HCC diagnosis. Four 0.25 mL aliquots were created from samples collected at all earlier time points. For step 2 of an early detection study, samples will be provided for the period between 2 years prior to diagnosis up to the time of sample collection prior to the diagnosis. However, 0.25 ml aliquots were created for the entire period of the study, beginning with the samples collected at the screening visit for entry into the trial, and are available for valid study purposes. Following sample selection, at least 1.0 ml of serum remained in the repository at each time point. Any of these remaining samples in the repository may be used for other purposes.

Samples have been stored continuously at -70 degrees centigrade. There was one thaw-freeze cycle, which occurred when the samples were aliquoted.

Application to Obtain Samples

For the evaluation of biomarkers for the early detection of HCC, serum samples from HALT-C patients present a unique opportunity:

- The patients were well characterized as to treatment history, severity of disease, and many other features.
- Cases met uniform diagnostic criteria.
- Cases had samples prior to diagnosis and at diagnosis.
- Controls were similar to cases.
- Samples are masked as to status as case or control.

Because of the unique characteristics and limited supply, these samples are not intended to be used in the discovery stage of potential biomarkers. Rather, the samples are intended to be used for validation of promising biomarkers that have been identified using samples from other studies of serum from patients with HCC. It is not required that those patients would have had HCV.

Request for these non-renewable samples must be made through an X01 application: http://grants.nih.gov/grants/guide/pa-files/PAR-11-306.html

Because of the potential range and test characteristics of potential biomarkers, the exact requirements as to the type and quality of the preliminary data cannot be pre-specified. At a minimum, the X01 application should provide the following:

- A biological rationale for the test as a marker of HCC.
- Evidence that the test(s) can discriminate between patients diagnosed with HCC from persons without HCC. More robust evidence would demonstrate that the biomarker could identify early stage HCC among a group of patients with same underlying cause of and the same severity of liver disease.
- Indication of how the test(s) could be applied generally as a surveillance tool for HCC.

References

1. Di Bisceglie AM, Shiffman ML, Everson GT, Lindsay KL, Everhart JE, Wright EC, Lee WM, et al. Prolonged therapy of advanced chronic hepatitis C with low-dose peginterferon. N Engl J Med 2008;359:2429-2441.

2. Dienstag JL, Ghany MG, Morgan TR, Di Bisceglie AM, Bonkovsky HL, Kim HY, Seeff LB, et al. A prospective study of the rate of progression in compensated, histologically advanced chronic hepatitis C. Hepatology 2011;54:396-405.

3. Lee WM, Dienstag JL, Lindsay KL, Lok AS, Bonkovsky HL, Shiffman ML, Everson GT, et al. Evolution of the HALT-C Trial: pegylated interferon as maintenance therapy for chronic hepatitis C in previous interferon nonresponders. Control Clin Trials 2004;25:472-492.

4. Lok AS, Everhart JE, Di Bisceglie AM, Kim HY, Hussain M, Morgan TR. Occult and previous hepatitis B virus infection are not associated with hepatocellular carcinoma in United States patients with chronic hepatitis C. Hepatology. 2011;54:434-42.

5. Lok AS, Sterling RK, Everhart JE, Wright EC, Hoefs JC, Di Bisceglie AM, Morgan TR, Kim HY, Lee WM, Bonkovsky HL, Dienstag JL, and the HALT-C Trial Group. Des-gamma-Carboxy Prothrombin and alpha-Fetoprotein as Biomarkers for the Early Detection of Hepatocellular Carcinoma. Gastroenterology. 2010;138:493-502.