

Availability of Serum Samples and Research Support for Validation of Hepatocellular Carcinoma (HCC) Biomarkers

Purpose:

National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), in collaboration with National Cancer Institute (NCI) through its Early Detection Research Network (EDRN), announces the availability of serum samples and research funds for validation of biomarkers for hepatocellular carcinoma among study participants in the Hepatitis C Antiviral Long-Term Treatment against Cirrhosis (HALT-C) trial.

Applications will be accepted for sequential phase II and phase III biomarker validation studies as described below.

Background

HALT-C is an NIH sponsored, multicenter, randomized clinical trial of 1050 patients to determine if long-term treatment with low-dose peginterferon alfa-2a could prevent progression of liver disease among persons with chronic hepatitis C who had not cleared virus on short-term treatment and had histologically advanced liver disease. A full description of the trial has been published (Lee WM, Dienstag JL, Lindsay KL, et al. Evolution of the HALT-C trial: pegylated interferon as maintenance therapy for chronic hepatitis C in previous interferon non-responders. *Control Clin Trials* 2004;19:2919-2926). Additional information, including a catalogue of samples at the HALT-C repository, can be found on the HALT-C web site <http://www.haltctrial.org/>

All patients had a battery of biochemical, serologic and virologic studies performed at baseline, as well as a liver biopsy. Thereafter, all patients (treated and untreated) were evaluated at 3 month intervals by history, physical examination, hematologic, biochemical, serologic and virologic parameters. All patients were requested also to undergo repeat liver biopsies 1.5 years and 3.5 years after beginning treatment. Because HCC was one of the primary outcomes, serum alfa-fetoprotein (AFP) was measured at each visit and an ultrasound examination was performed at week 24 and at months 12, 24, 36, 48, 60 and every 6 months thereafter, seeking evidence of incipient HCC. At each visit, serum samples were sequestered and placed in a repository for future research purposes. Currently, there are approximately 225,000 samples in the repository, consisting primarily of sera, but also including plasma and whole blood, PBMCs, DNA, and liver tissue. Specimen collection began upon initiation of trial enrollment in 2000 and has continued to this date. At each patient visit, serum was collected and aliquoted and subsequently frozen at -80 degrees centigrade for long-term storage at a central repository. Specimens will be provided in 200 µl aliquots that have undergone one freeze thaw cycle.

The fact of development of HCC, together with the extensive collection of serum samples stored in the HALT-C tissue repository, offers the opportunity to define the value of currently known biomarkers for surveillance of HCC, with the hope of establishing indicators for its early diagnosis. Biomarkers evaluated included total AFP, AFP-L3; and des-carboxy prothrombin (DCP). The aim of these studies was to define the precision of these three biomarkers in establishing the diagnosis of HCC, to determine the utility of AFP-L3 and DCP in ruling out HCC among those with an elevated total AFP and no

HCC, and to establish whether interferon treatment affects the levels of these markers. These studies are currently in progress, the results of which may be of value. However, new, effective biomarkers capable of identifying early HCC that are equivalent to or better than those already evaluated are clearly needed. For this purpose, a reference set of sera from patients who had a definite diagnosis of HCC, together with sera from individuals without evidence of HCC, are available for research to establish the value of newer biomarkers of HCC.

The use of HALT-C samples for the evaluation of biomarkers for HCC fits within the current framework for the establishment and testing of biomarkers. Thus, evaluation of cancer biomarkers may proceed through five phases to establish clinical utility (Pepe MS, Etzioni R, Feng Z, Potter JD, Thompson ML, Thornquist M, Winget M, Yasui Y. Phases of biomarker development for early detection of cancer. *J Natl Cancer Inst* 2001;93:1054-106). A phase I study is a pre-clinical exploratory study to characterize a potential biomarker. A phase II study assesses the ability of the biomarker to distinguish subjects with cancer from at risk subjects without cancer. It typically uses a case-control study design with analyses to estimate true positive rates (TPR) and false positive rates (FPR) or creates a receiver operating characteristic (ROC) curve. Phase III is a retrospective longitudinal study whose primary aim is to evaluate the capacity of the biomarker to detect preclinical disease. It applies to a study such as HALT-C in which a cohort at risk of HCC is followed at regular intervals with serial collections of serum samples. Analysis could be performed using time dependent ROC curves. Phase IV and V studies are designed to demonstrate the clinical efficacy of a screening or surveillance program in reducing cancer morbidity and mortality. This notice is restricted to phase II and III studies.

Among the unique characteristics of the present collection are the precision of the diagnosis, the routine conduct of ultrasonography, the extensive numbers of samples sequestered (dating back in time in most instances to well before the diagnosis of HCC), and the sequential liver biopsies performed. Because of the great value of this collection and the precious nature of this limited resource, it is expected that research applicants will provide compelling evidence that their biomarker of interest has real potential for success, having undergone phase I evaluation. Further, it is anticipated that the potential worth of the biomarker under scrutiny will first need to be validated in a phase II study using the available reference set from HALT-C in order to proceed to the phase III approach, namely the access to sequestered samples that were collected prior to the diagnosis of the HCC.

Source of the HCC Reference Set: At scheduled quarterly or semi-annual intervals, patients underwent clinical evaluation that included systematic testing for the presence of liver cancer. Following diagnosis of HCC at the local site, at least 2 independent reviewers determined whether clinical evidence supported the diagnosis. Cases of HCC were called definite or presumed according to the following criteria:

A. Definite:

Histology showing HCC (from a biopsy, surgery or an autopsy), OR
A new hepatic defect on imaging with an AFP rising to >1,000 ng/ml

B. Presumed:

A new discrete hepatic defect shown on US, where histology is not available, the AFP is <1,000 ng/ml, and one of the following characteristics is present:

1. Two other liver imaging scans (MRI, triphasic CT, angiography, lipiodol scan, liver spleen scan with gallium) indicate malignancy with the following characteristics: hypervascularity, arterial to portal vein shunts, portal vein thrombosis near the defect, tumor in the portal vein;
2. A progressively enlarging lesion starting as a new defect eventually associated with liver involvement and death;
3. A new hepatic defect with one characteristic scan and one of the following:
 - i. Increase in size over time (doubling in diameter size, or tripling in diameter size if the initial size is <1 cm when first discovered),
 - ii. An increasing AFP (values 3 months before or after the discovery of the defect by scanning) eventually rising to a level of >200 ng/ml and more than tripling the baseline value.

Upon completion of the treatment trial in 2007, results indicated that the treatment with low-dose peginterferon alfa-2a did not reduce the frequency of the primary outcomes, including HCC. Patients continue to be seen at 6-month intervals for evaluation of study outcomes, including HCC. Including cases identified during the trial and afterwards, approximately 50 patients have thus far developed either definite (about 75% of cases) or presumed HCC. About 75% of cases had stage 1 or 2 at diagnosis and the remainder had stage 3 or 4, and about half had cirrhosis at biopsy prior to entry into the study.

Samples from cases and controls will be made available in two steps according to the definitions above for phase II and phase III biomarker studies. In the first step, serum samples assembled at or close to the time of diagnosis from as many as 50 patients with HCC will be provided together with up to 2 serum samples from study participants who were not known to develop HCC but shared clinical characteristics (control samples). The controls would be selected according to histological fibrosis stage (bridging fibrosis or cirrhosis) and treatment assignment (peginterferon or no treatment) in accordance with the design of the HALT-C trial. The samples will be masked as to source (HCC case or control). Results from this analysis will be provided by the investigator who performs the biomarker testing to the data coordinating center, which will determine whether the test was able to discriminate between the samples from HCC patients and those of the non-HCC patients. A limited data set would also be provided to the investigator at the investigator's request.

In order to proceed to the next step, the tested biomarker must perform at least as well as or must complement the standard tests (AFP, AFP-L3, DCP) that have been evaluated in the study. Masked 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 patients who were not known to have developed cancer (controls). All of these results from this second testing step will also be provided to the DCC for analysis with a corresponding limited data set generated for the investigator at the investigator's request.

If an investigator wishes to propose an alternative study design, the application must discuss the rationale for doing so (see application procedures below).

Eligibility Requirements:

Eligibility: US or Canadian non-profit institutions.

Requirements for Application:

Do not send applications to the Center for Scientific Review. Applicants should submit one electronic copy as an e-mail attachment in PDF format and two hard copies (with original signatures of the PI and institutional official) of the application to Mr. Donald Johnsey of NCI EDRN at the address at the end of this announcement.

This is a one-time announcement. Electronic applications must be received on or before: May 12, 2008.

Review Criteria:

Applications will undergo peer-review by a panel of experts in the proposed testing platform and biomarker and experts in hepatitis C related liver disease and HCC. The review panel will include representatives from NCI-EDRN and HALT-C. Final funding decision will be made by the HALT-C Steering Committee by July 18, 2008.

Funds Available: As many as four awards are anticipated with a total budget for each award limited to \$75,000. Funding will be provided by sub-contract from the HALT-C data coordinating center. Approximately half the funding would be provided for the first step and half for the second step. Selection of samples, shipping of samples to and from the repository, and data analysis will be the responsibility of the HALT-C data coordinating center.

Reports and Data

A written report detailing the study methods and results is required within 3 months of conclusion of testing. Test results will be added to the HALT-C database. Peer review publication of the results of the testing is expected. The site investigators will have the opportunity to lead the publication effort.

Application Procedures:

Use the PHS 398 Form Pages (not the SF-424). See [Office of Extramural Research](#) web site for forms. Applications should be single spaced, preferably use 12 pt. or 11 pt., but no smaller than 10 pt. font based on PHS Form 398

PHS Form 398 Page 1 - Face Page

PHS Form Page 2 - Description (Abstract), Performance Sites, Key Personnel

PHS Form Page 6 -Biographical Sketch Format Page

PHS Form Page 4 and 5 – Budget.

Total budget for each award is limited to \$75,000, which will be funded by sub-contract from the data coordinating center. Adequate budget justification for direct costs is required. Facilities and Administrative costs (indirect costs) are permitted at the grantee institution's current negotiated rate. These must be clearly annotated.

Research Plan [strictly limited to **5 typed pages**] Using the standard PHS 398

Continuation Page: (<http://grants.nih.gov/grants/funding/phs398/continuation.doc>).

Include:

Scientific Rationale – Clearly state the scientific rationale of the proposal for using the specimens. Describe your biomarker(s) and how it was discovered or developed for application in cancer detection.

Methods and Preliminary Data – Provide sufficient information describing how experiments were performed, details on samples used, and presentation of data in terms of specificity, sensitivity, and variance of your measurements. Explicit description of methods and studies will facilitate review considerations. Figures and other supporting documentation can be appended to the proposal. Specimens will be provided in 200 µl aliquots having undergone one freeze thaw cycle. Justification for more than one aliquot must be provided.

Patient sample size - For phase 1, samples from a maximum of xx patients who developed cancer will be potentially available. Samples from as many as 2 controls per patient with cancer would be available. Justification must be provided for inclusion of additional controls. Provide a description and justification of any matching criteria for controls in addition to fibrosis stage and treatment assignment. If an investigator wishes to propose an alternative study design, the application must discuss the rationale for doing so.

Suggested data analysis – Provide adequate detail on how you believe statistical analysis of your data coming from these samples should be performed and justification for the number of samples requested.

Literature Cited

Human Subject Approval - The applicant principal investigator must document human subject approval by an Institutional Review Board (IRB or human subjects committee) constituted according to the requirements of the Dept. of Health & Human Services Office for Human Research Protections (OHRP). Applicant principal investigators should note that all data and materials in the repository were stripped of all personal identifiers before they were submitted to the repository, such that no identifying information is associated with the samples. However, applicants should provide documentation of IRB review and either approval of the proposed project or exemption of the proposed project from the need for further IRB review. The IRB approval will not be required at the time the application is submitted. However, no samples can be released until evidence of IRB approval has been received.

Checklist - (final page unless an Appendix section is included) Use checklist form page of PHS Form 398.

Appendix - the offeror must provide its procedures for assuring proper receipt and processing of the samples. Other appendices are optional.

Inquiries:

Direct program/scientific related questions or inquiries to:

James Everhart, M.D., M.P.H.
National Institute of Diabetes and Digestive and Kidney Diseases
2 Democracy Plaza, Room 655
6707 Democracy Boulevard, MSC 5450
Bethesda, MD 20892-5450
Phone: (301) 594-8878
Fax: (301) 480-8300
E-Mail: je17g@nih.gov

Direct submission and review questions or inquiries to:

Donald Johnsey
National Cancer Institute
Early Detection Research Network
Executive Plaza North, Room 3148
6130 Executive Blvd, MSC 7362
Rockville, MD 20892-7362
Phone: (301) 435-5015
E-Mail: dj156d@nih.gov