Bayer TMA Snap Shot

Sites Participating: All sites and all patients in the HALT-C Trial

Principal Investigator: David Gretch, MD (University of Washington)

Co-Investigator: Chihiro Morishima, MD

Study Name: Bayer Versant bDNA 3.0 Quantification and TMA Detection of HCV RNA for the HALT-C Trial

Separate Consent Form: No

Withdrawal Form: No

Eligible Patients: All patients (Lead-in, Express, and Breakthrough/Relapser)

Visit Schedule

Note: "**X**" means all participating sites take part. No additional specimens are collected for this study. Tests are performed on the specimens collected for the Main Trial HCV RNA Roche Monitor testing (seq.# 101-104, seq.# 105-108, seq.# 150-153). Bayer bDNA 3.0 and Bayer TMA results are maintained in a separate data file and are not included in the main HALT-C database.

Lead-In Phase (seq.#101-104)

Visit Number →	S00	W00	W12	W20	W24
Main Trial: Roche Monitor	X	X	X	X	X
AS: Bayer bDNA 3.0 and Bayer TMA		X		X	X

Responder Phase (seq.#150-153)

Visit Number →	W36	W48	W60	W72
Main Trial: Roche Monitor	X	X	X	X
AS: Bayer bDNA 3.0 and Bayer TMA	X	X	X	X

Randomized Phase (seq.#105-108)

Visit Number →	R00	M12	M18	M24	M30	M36	M42	M48	M54
Main Trial: Roche Monitor	X	X	X	X	X	X	X	X	X
AS: Bayer bDNA 3.0 and Bayer TMA	X	X		X		X		X	X

Bayer Versant bDNA 3.0 Quantification and TMA Detection of HCV RNA for the HALT-C Trial

Principal Investigator: David Gretch MD PhD, University of Washington

Co- Principal Investigator: Chihiro Morishima MD, University of Washington

Outside Collaborators: Bayer Diagnostics, Emeryville, CA

Specific Aims/Hypotheses:
The University of Washington proposes to utilize the Bayer bDNA 3.0 assay and Bayer TMA to respectively quantify and detect HCV RNA in all HALT-C patient sera at a limited number of time points.

Our hypotheses are:

1. In untreated hepatitis C–infected nonresponders, HCV viremia at baseline and changes over time, as determined by the Bayer bDNA 3.0 test, may more accurately predict clinical outcomes compared to the Roche Monitor test.
2. In treated hepatitis C–infected nonresponders, the degree to which viral replication can be suppressed over time, as detected by the Bayer bDNA 3.0 test, may better predict outcome after 4 years of antiviral therapy compared to the Roche Monitor test.
3. Increased sensitivity of HCV RNA detection using the TMA assay may better predict which individuals will have subsequent virologic breakthrough or relapse, despite continued combination therapy, compared to the Roche Amplicor test.

Specific aims:

1. To compare the performance of Bayer bDNA 3.0 and Roche Monitor quantitative tests in measuring HCV RNA levels for the HALT-C Clinical Trial and correlate with both histologic and clinical outcomes.
2. To compare the performance of HCV RNA qualitative detection by Bayer TMA and Roche Amplicor at weeks 20 and 24, and correlate with virologic outcome (virologic breakthrough, relapse) in those treated with 48 weeks of combination therapy in the responder arm of the study.

Background, rationale, significance, relation to aims of the HALT-C Trial:
Hepatitis C virus (HCV) infection is a major cause of viral hepatitis leading to chronic liver disease and liver failure in humans. A remarkable feature of this blood-borne pathogen is its ability to persist chronically in approximately 85% of infected individuals. The development of optimal therapeutic strategies to eliminate HCV infection or at a minimum, avert its long-term sequelae, are therefore major health priorities in the United States. Recently, the combination of interferon- and ribavirin has been used successfully to induce sustained viral clearance (6 months after end of therapy) in approximately 30-50% of treated persons (1, 2). However, those 50-70% of individuals who do not achieve sustained virologic response (SVR) after state-of-the-art therapy have had no other recourse than to await the development of new treatments.

The HALT-C study is a randomized multi-center clinical trial to assess the effects of long-term interferon- therapy on the progression of liver fibrosis and development of decompensated liver disease (ascites, liver cancer, etc.) in hepatitis C patients who are nonresponders to prior therapy. The main protocol includes a testing schedule for the qualitative detection and quantification of HCV

RNA using the Roche Amplicor and Roche Monitor assays, respectively. The Roche Amplicor test will be used at the critical week 20 juncture to determine if subjects have cleared HCV after 20 weeks of combination pegylated interferon- 2b and ribavirin therapy. Those subjects who are persistently viremic will be randomized to no treatment or treatment with pegylated interferon alone for 3.5 years.

The Roche Monitor quantitative assay will be used to follow changes in viral levels over time in both treated and untreated subjects for the duration of the Trial. These measurements of HCV RNA are a critical part of the HALT-C Trial, in that significant correlations may be found between these data and other clinical and histologic findings of the study. Both the size of the cohort and breadth of the studies to be performed provide an opportunity for the delineation of important factors which could significantly impact the future treatment of hepatitis C nonresponders. Given the importance of this study, it is imperative to obtain the best virologic data possible with the best technologies available.

Both the Roche Amplicor and Monitor assays are PCR-based (3). Until recently, the Amplicor test has been the most sensitive qualitative test available for the detection of HCV RNA in serum (4). However, a newly developed transcription-mediated amplification (TMA) assay for qualitative detection of HCV RNA has recently become available. In the first report of its use on clinical study specimens, Sarrazin et al. found that 8/22 or 36% of those individuals who had undetectable HCV RNA at the end of treatment by the Roche Amplicor assay were found to have HCV RNA detected by the TMA test (5). The FDA-approved COBAS Roche Amplicor test, which is being used in the HALT-C Trial, is reported to have a sensitivity of 50 IU/mL (106 positive/106 tested) using the internationally accepted WHO standard (4). In contrast, the Bayer TMA assay is reported to have an analytical sensitivity of 10 IU/mL (14 positive/14 tested, Meisel et al., poster presentation at Pan American Society for Clinical Virology Symposium, April 2001). These data raise concerns that the methodology currently in use for differentiating “responders” from “nonresponders” to the initial lead-in therapy may not be the most sensitive measure available.

The Roche Monitor quantitative test has been in widespread use in many clinical laboratories, and has been compared to the branched DNA methodology (version 2.0) in many publications (6-12). Both tests have different advantages and disadvantages. The Roche Monitor assay has been more sensitive (600 IU/mL) (4) than the Quantiplex bDNA 2.0 assay (approximately 80,000 IU/mL) (13), but Monitor is also known to have a limited dynamic range (600 to 500,000 IU/mL) (4). This dynamic range can be extended by diluting the serum of interest. Recent unpublished studies from our laboratory have demonstrated increased variability in the Roche Monitor assay with dilution, raising the concern that values obtained with dilution are less accurate. Other groups, including Lunel and colleagues, have reported increased inter-assay variability of the Monitor assay (CV=9.8%) compared to bDNA 2.0 (CV=2.8%) (7, 8). In contrast, the strengths of the bDNA 2.0 assay have included its broad dynamic range (80,000-19,000,000 IU/mL) and its excellent reproducibility (13). Changes in viral load over time have been found to correlate between the two assays in a small number of patients (24 subjects total studied) (14-16), but one of these subjects had a greatly discrepant result reported (15). Moreover, our laboratory has found the bDNA technology to be superior to PCR-based assays in terms of detecting statistically significant changes in viral load during treatment studies (Gretch, unpublished). Technical differences between the two assays raise the concern that the inherent assay variability within Roche Monitor may be large enough to prevent optimal quantification of viral load changes over time. For example, Reichard et al used the bDNA 2.0, NGI Superquant and Roche Monitor assays to examine the relationship between pre-treatment HCV RNA level and sustained virologic response to interferon monotherapy. They found a statistically significant difference between low and high baseline HCV RNA levels by Superquant (p=.002) and bDNA 2.0 (p=.0005), but not by the Monitor assay (p=.08) (6). Other studies have also reported differences in the performance of these two assays, sometimes leading to disparate conclusions (8, 9). Finally, patterns of change in viral load over time, as detected by bDNA 2.0 in our laboratory, have been found to correlate with long-term histologic outcome in the Alaskan Native American cohort (manuscript in preparation, Sullivan and Gretch).

The new version 3.0 bDNA assay has recently been released and is reported to be similar in format and performance to the 2.0 assay. Although its performance characteristics will need to be validated to confirm the claims by the manufacturer, this updated version of the assay is reported to be as sensitive as the Roche Monitor assay, and yet maintain linearity up to 8.3 million IU/mL. The Bayer TMA also has increased analytical sensitivity compared to the Roche Amplicor qualitative PCR assay. Thus, the Bayer tests should provide important complimentary data for our virological analyses of the HALT-C cohort. As the clinical implications of HCV viral load monitoring are presently unclear, these additional data sets should enhance our ability to detect significant changes in viral burden which may occur over the course of treatment and follow-up in this clinical trial.

Design and methods:
The Bayer bDNA 3.0 assay and Bayer TMA will be used to perform the quantitative and qualitative HCV testing proposed. In-house validation of the bDNA 3.0 and TMA will be performed on University of Washington clinical archive specimens at the outset, to confirm the reported characteristics of the assays in our hands. These studies will include an assessment of the analytical sensitivity of both assays, using the Acrometrix HCV RNA panel (NAP)(17) and possibly the WHO standard (18), if available. Clinical sensitivity and specificity will be tested using clinical archive specimens. In addition, the linearity of the bDNA 3.0 assay will be compared with bDNA 2.0 and Roche Monitor, and confirmed against the Acrometrix panel. All HALT-C serum specimens from a limited number of timepoints (as shown below) will be utilized for the study.

Main Protocol

	S1	W0	W12	W20	W24	M12	M18	M24	M30	M36	M42	M48	M54
Roche	X	X	X	X	X	X	X	X	X	X	X	X	X
Bayer	-	X	-	X	X	X	-	X	-	X	-	X	X

Responders

	W36	W48	W60	W72
Roche	X	X	X	X
Bayer	X	X	X	X

All specimens will be tested first with bDNA 3.0 and re-tested with TMA if negative by bDNA (sensitivity 520 IU/mL). All week 20 specimens will be tested in singlet with the TMA before quantification. **The virology laboratory should have sufficient specimen to perform both the Roche and Bayer assays.** However, in a limited number of difficult specimens, additional serum may be requested from the Repository. All HALT-C data will be transmitted to NERI. The HALT-C Steering Committee has already determined that the results obtained using the Bayer technologies will not be used for clinical decision making.

Preliminary Data:
The only preliminary data available thus far is a comparison of the bDNA 2.0 assay and the Roche Monitor assay using the NAP HCV RNA panel which has been calibrated against the WHO standard. These data demonstrate a closer approximation of the fold increases in HCV RNA by the bDNA2.0 assay compared with Roche Monitor.

Anticipated results:

NAP HCV RNA (IU/mL)		bDNA 2.0 (Eq/mL)		Roche Monitor (IU/mL)	
Value	(fold increase)	Value	(fold increase)	Value	(fold increase)
50		nd		LO	
500	(10)	nd		1,210	
5,000	(10)	nd		6,470	(5.3)
50,000	(10)	350,000		88,000	(13.6)
200,000	(4)	1,340,000	(3.8)	189,000	(2.1)
500,000	(2.5)	3,704,000	(2.8)	681,000	(3.6)
2,000,000	(4)	11,165,000	(3.0)	3,910,000	(5.7)

Qualitative testing:
We anticipate that the TMA will be more sensitive than the Roche Amplicor assay, and thus detect HCV RNA in some subjects who have been deemed responders at the week 20 timepoint; some of these may be subjects who display virologic breakthrough on therapy. Follow-up through the responder protocol will allow a direct assessment of both the Roche Amplicor assay and the Bayer TMA in detecting true non-responders to lead-in therapy. We predict that very few, if any, of the TMA positive Amplicor negative subjects would become SVRs. However, it will be very interesting to evaluate the degree of concordance between the two tests used at this important week 20 timepoint, as well as the week 24 timepoint.

Quantitative testing:
We anticipate that the values obtained using the Bayer bDNA 3.0 assay and the Roche Monitor assay will differ. How these differences will affect the overall outcome of the study is difficult to predict. However, it is possible that one, if not both, of the quantitative assays will allow the delineation of a relationship between changes in viral load on long-term pegylated interferon monotherapy and improved outcome. In addition, it should be possible to establish whether baseline HCV RNA levels can serve as a predictor of clinical outcome or response to long-term pegylated interferon monotherapy.

Collaborators: Bayer Diagnostics
Bayer Diagnostics has offered to provide all test kits and instrumentation free of charge, using the original estimate of 1300 enrolled into the lead-in phase. In addition, they have offered to provide labor costs to perform the additional testing for this ancillary study. No additional funds will be requested from the NIDDK. The recently planned increase in enrollment will need to be negotiated with Bayer if this proposal is approved.

References:

1. McHutchison JG, Gordon SC, Schiff ER, et al. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. *New England Journal of Medicine*. 1998;339(21):1485-1492.
2. Davis GL, Esteban-Mur R, Rustgi V, et al. Interferon alfa-2b alone or in combination with ribavirin for the treatment of relapse of chronic hepatitis C. *New England Journal of Medicine*. 1998;339(21):1493-1499.
3. Morishima C, Gretch DR. Clinical use of hepatitis C virus tests for diagnosis and monitoring during therapy. *Clinics in Liver Disease*. 1999;3(4):717-40.

4. Lee SC, Antony A, Lee N, et al. Improved version 2.0 qualitative and quantitative AMPLICOR reverse transcription-PCR tests for hepatitis C virus RNA: calibration to international units, enhanced genotype reactivity, and performance characteristics. *Journal of Clinical Microbiology*. 2000;38(11):4171-9.
5. Sarrazin C, Teuber G, Kokka R, Rabenau H, Zeuzem S. Detection of residual hepatitis C virus RNA by transcription-mediated amplification in patients with complete virologic response according to polymerase chain reaction-based assays. *Hepatology*. 2000;32(4 Pt 1):818-23.
6. Reichard O, Norkrans G, Fryden A, Braconier JH, Sonnerborg A, Weiland O. Comparison of 3 quantitative HCV RNA assays--accuracy of baseline viral load to predict treatment outcome in chronic hepatitis C. *Scandinavian Journal of Infectious Diseases*. 1998;30(5):441-6.
7. Lunel F, Cresta P, Vitour D, et al. Comparative evaluation of hepatitis C virus RNA quantitation by branched DNA, NASBA, and monitor assays. *Hepatology*. 1999;29(2):528-35.
8. Yu ML, Chuang WL, Chen SC, et al. Clinical application of the Quantiplex HCV RNA 2.0 and Amplicor HCV Monitor assays for quantifying serum hepatitis C virus RNA. *Journal of Clinical Pathology*. 1999;52(11):807-11.
9. Soffredini R, Rumi MG, Del Ninno E, Parravicini ML, Russo A, Colombo M. Serum levels of hepatitis C virus RNA predict non-response to interferon therapy: comparison of two commercial assays. *Journal of Viral Hepatitis*. 1999;6(1):65-71.
10. Mellor J, Hawkins A, Simmonds P. Genotype dependence of hepatitis C virus load measurement in commercially available quantitative assays. *Journal of Clinical Microbiology*. 1999;37(8):2525-32.
11. Chayama K, Suzuki F, Tsubota A, et al. Evaluation of quantitative measurements of hepatitis C virus RNA to predict sustained response to interferon by genotype. *Journal of Virological Methods*. 2001;95(1-2):33-45.
12. Martinot-Peignoux M, Boyer N, Le Breton V, et al. A new step toward standardization of serum hepatitis C virus-RNA quantification in patients with chronic hepatitis C. *Hepatology*. 2000;31(3):726-9.
13. Gretch DR, dela Rosa C, Carithers RL, Willson RA, Williams B, Corey L. Assessment of Hepatitis C Viremia Using Molecular Amplification Technologies: Correlations and Clinical Implications. *Ann. Intern. Med.* 1995;123(5):321-329.
14. Trabaud MA, Bailly F, Si-Ahmed SN, et al. Comparison of HCV RNA assays for the detection and quantification of hepatitis C virus RNA levels in serum of patients with chronic hepatitis C treated with interferon. *Journal of Medical Virology*. 1997;52(1):105-12.
15. Tong CY, Hollingsworth RC, Williams H, Irving WL, Gilmore IT. Effect of genotypes on the quantification of hepatitis C virus (HCV) RNA in clinical samples using the Amplicor HCV Monitor Test and the Quantiplex HCV RNA 2.0 assay (bDNA). *Journal of Medical Virology*. 1998;55(3):191-6.
16. Halfon P, Bourliere M, Halimi G, et al. Assessment of spontaneous fluctuations of viral load in untreated patients with chronic hepatitis C by two standardized quantitation methods: branched DNA and Amplicor Monitor. *Journal of Clinical Microbiology*. 1998;36(7):2073-5.
17. Jorgensen PA, Neuwald PD. Standardized hepatitis C virus RNA panels for nucleic acid testing assays. *Journal of Clinical Virology*. 2001;20(1-2):35-40.
18. Saldanha J, Lelie N, Heath A. Establishment of the first international standard for nucleic acid amplification technology (NAT) assays for HCV RNA. WHO Collaborative Study Group. *Vox Sanguinis*. 1999;76(3):149-58.