Genetic Polymorphisms Snap Shot

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

Principal Investigators: Timothy Morgan, MD (University of California-Irvine), Thomas R. O'Brien, MD, MPH (National Cancer Institute)

Co-Investigators: Tania Mara Welzel; Herbert L. Bonkovsky, MD; James Everhart, MD, MPH; Raymond Chung, MD; Stephen Chanock

Study Name: GENETIC POLYMORPHISMS ASSOCIATED WITH NON-RESPONSE TO TREATMENT OF CHRONIC HCV INFECTION

Separate Consent Form: No

Withdrawal Form: Yes (Form# 9)

Eligible Patients: All Lead-in patients who provide consent for genetic testing

Visit Schedule:
Blood samples are obtained at baseline and during follow-up for the preparation of DNA from patients who provide consent for genetic testing. DNA is prepared by the repository and will be sent to National Cancer Institute laboratories for testing.

Genetic Consent:
This consent is part of the main consent form for the HALT-C trial. Genetic consent is recorded on Form #4 and may be changed by the patient at any time, complete Form #9 Genetic Status Change. The current status of genetic consent is checked before DNA is prepared or samples are tested.

HALT-C Subjects:
In the proposed study the host genetic polymorphisms in HALT-C non-responders will be compared to HALT-C participants who achieved a SVR after re-treatment with PEG-IFN and Ribavirin. HALT-C non-responders will be defined as patients with non-response to re-treatment at week 20. Patients without detectable HCV RNA at week 72 (at least 24 weeks after treatment is discontinued) are defined as "sustained virologic responders" (SVR).

Genetic Polymorphisms Associated with Non-Response to Treatment of Chronic HCV Infection

Principal Investigators: Timothy Morgan, MD (University of California-Irvine), Thomas R. O'Brien, MD, MPH (National Cancer Institute)

Co-Investigators: Tania Mara Welzel; Herbert L. Bonkovsky, MD; James Everhart, MD, MPH; Raymond Chung, MD; Stephen Chanock

1. Background

Hepatitis C virus (HCV) is an important cause of cirrhosis and hepatocellular carcinoma. Combination therapy with ribavirin and pegylated (PEG)-interferon-α (IFN-α) is the current "gold standard" for therapy of HCV-infected patients. This regimen leads to a sustained virologic response (SVR) in ~50% of patients who are infected with HCV genotype 1 and in ~80% of patients who are infected with genotype 2 or 3 (Fried et al., 2002). Other factors that are associated with a SVR include a low baseline HCV RNA level, less hepatic fibrosis or inflammation on liver biopsy, younger age, female gender and white race. Little is known, however, about host genetic factors involved in HCV treatment response.

Genes involved in the IFN-α pathway could also alter a patient's response to IFN-α-based therapy. Type I IFN-α is produced endogenously in response to viral infections in fibroblastoid cells and lymphocytes. IFN-α exerts its antiviral properties by mediating both direct (i.e., non-cytopathic) and indirect (e.g., apoptotic) antiviral responses, as well as through immune activation. IFN-α directly targets human hepatocytes (Castet V et al., 2002). It can activate several pathways (particularly the JAK-STAT pathway) and induce the expression of genes with antiviral properties. Furthermore, in patients treated with IFN-α (alone or combined with ribavirin), IFN-α exhibits direct immunomodulatory effects by enhancing HCV-specific CD4+ T-cell responses (Hoffman et al., 1995; Barnes et al., 2002; Kamal et al., 2002). Two recent studies, conducted in Europe and Japan, suggest that a polymorphism in the MxA promoter (SNP G/T, np -88) is associated with better response to IFN-α therapy (Knapp S, 2003; Suzuki, F, 2004).

Genetic polymorphisms in chemokine receptors may also influence therapeutic outcomes for HCV infection, but study results are not consistent (Ahlenstiel et al., 2003; Dorak et al.; 2002, Glas et al., 2003; Promrat et al., 2003).

An understanding of a role of genes involved in the IFN-α pathway and chemokine/chemokine receptor genes in treatment response for patients infected with HCV could identify patients at increased risk of treatment failure and, possibly, lead to the development of new therapeutic strategies. We propose to examine these genes in patients enrolled in the Hepatitis C Antiviral Long-Term Treatment against Cirrhosis (HALT-C) trial.

2. Objective and hypothesis

Our aim is to investigate the impact of host genetic polymorphisms in the IFN-α pathway and in IFN-α induced genes on non- response to IFN-α based therapy in HALT-C patients with chronic HCV infection. We hypothesize that these polymorphisms could cause non-responsiveness to interferon-based therapies in a portion of HCV-infected patients. We also propose to examine the effect of chemokine receptor (CCR2 and CCR5) polymorphisms on treatment response.

3. Candidate genes

Candidate genes we propose to investigate are described below and summarized in Table 1.

3.1 IFN-α receptor and IFN-α (JAK/STAT) pathway

The IFN-α receptor **(**IFNAR**)** consists of two major subunits, **IFNAR1** and **IFNAR2.** Receptor binding of IFN-α activates the "Janus" tyrosine kinases, **JAK1** and **Tyk2**, and subsequent phosphorylation of IFNAR1 on Tyr-466 (Figure 1). Phosphorylated IFNAR1 serves as the docking site for **STAT2. Tyk2** phosphorylates **STAT2** on Tyr-690, leading to the recruitment of **STAT1**, which is phosphorylated by Tyk2 on Tyr-701. Phosphorylated STAT1 and STAT2 form heterodimers that translocate into the nucleus. Binding of the **p48** protein leads to the formation of the heterotrimeric transcription factor complex interferon-stimulated gene factor 3 (**ISGF-3**), which induces the expression of a variety of genes with antiviral properties by binding to IFN-stimulated response elements (**ISRE**). Phosphorylated STAT1 also forms homodimers, which translocate into the nucleus and bind the gamma activation sequence (GAS) element to initiate gene transcription (reviewed by Mogensen et al., 1999; Goodbourn et al., 2000)

3.2 IFN-α induced genes with antiviral properties

Interferon-induced, double-stranded RNA-activated protein kinase **(PKRK)**

This serine/threonine kinase is activated by binding of dsRNA and mediates transcription, translation and apoptosis. PKR can inhibit translation by phosphorylation of **eIF-2α** (eukaryotic protein synthesis initiation factor**-**2α). PKR activates the transcription factor **NF-κB**, which is essential for IFN-ß expression (reviewed in Clemens & Elia, 1997; Tan & Katze, 1999).

2'-5' oligoadenylate synthetase system (**2'-5'-OAS)**

2'-5'-OAS1 mediates the degradation of viral and cellular RNA mainly by activation of RNAse L, which cleaves ssRNA and inhibits protein synthesis (reviewed in Silverman, 1997).

Double-stranded RNA-specific adenosine deaminase (**ADAR)**

This IFN-α induced enzyme exhibits its antiviral properties through dsRNA editing by deamination of adenosine to inosine.

MxA

Human cytoplasmic MxA, a highly conserved GTPase, can inhibit the viral RNA synthesis. The antiviral effect of MxA has been described in several RNA virus families (Rhabdoviridae, Paramyxoviridae, Bunyaviridae, Rhabdoviridae, Togaviridae).

3. 3 IRF-3 (Interferon-Regulatory Factor)

The transcription factor IRF-3 is activated in HCV infected cells and exerts antiviral effects through induction of Type-I IFN and expression of numerous antiviral genes by binding to ISRE (IFN-stimulated response elements). Recently, it was shown HCV (NS3/4A) can block IFR-3 phosphorylation and activation (Foy et al., 2003). Variation in the IRF-3 gene could, therefore, have an impact on viral persistence and reduce response to IFN (Foy et al., 2003, Nakaya et al. 2001).

3.4 CCR5Δ32/CCR2

Studies on polymorphisms in chemokine receptors (CCR5Δ32 and CCR2V64I) and response to IFN based-therapies have yielded conflicting results (Ahlenstil et al., 2003; Glas et al., 2003,

Ruiz-Ferrer, 2004). The HALT-C study population offers another opportunity to investigate these polymorphisms.

4. Study population

HALT-C Subjects

The HALT-C trial is designed to evaluate the safety and efficacy of long-term PEG-IFN-α 2a for treatment of chronic hepatitis C in patients who did not respond to previous interferon therapy (with or without ribavirin) and who have evidence of hepatic fibrosis by biopsy (Ishak 3-6). During a 24 week lead in phase, enrolled patients are treated with PEG-IFN-α2a and Ribavirin (Pegasys®, Roche). HALT-C subjects who are positive for HCV RNA at week 20 (“non-responders”) are randomized to either long-term treatment with PEG-IFN-α 2a (“maintenance phase”) or to no additional therapy. Patients without detectable HCV RNA levels in their serum at week 20 remain on treatment through week 48 and are followed until week 72. Patients without detectable HCV RNA at week 72 (at least 24 weeks after treatment is discontinued) are defined as “sustained virologic responders” (SVR).

In the proposed study we will compare host genetic polymorphisms in HALT-C non-responders to HALT-C participants who achieved a SVR after re-treatment with PEG-IFN and Ribavirin. HALT-C non-responders will be defined as patients with non-response to re-treatment at week 20. A nested analysis will consider persons with non-response at week 12, and thus exclude patients with early response who relapsed before week 20.

5. Study design

An ideal investigation of host genetic factors that predict non-response to PEG-IFN and ribavirin HCV therapy might compare patients who achieve an SVR after a single round of treatment to patients who failed two or more attempts at such therapy. This comparison is not feasible in HALT-C because study eligibility requires prior non-response to interferon-based treatment regimens. HALT-C does, however, provide a group of patients who were **non-responsive** after two courses of IFN-based therapy because only patients who did not respond to a previous regimen with standard IFN or PEG-IFN (with or without ribavirin) were enrolled. Patients may fail who fail to respond to therapy for a variety of reasons, including poor compliance or discontinuation of therapy because of adverse effects. Therefore, patients with non-response after re-treatment with PEG-IFN and ribavirin can be considered as persons with “real” non-responsiveness to treatment and are well suited to investigate host genetic factors predicting treatment response. In the proposed study, we plan to initially examine the MxA gene promoter, which was recently described to be associated with response to treatment with INF-α by two groups (Knapp S, 2003; Suzuki, F, 2004). Confirmation of this finding would strengthen our confidence that the HALT-C study population is well suited to investigate host genetic factors associated with response to treatment of HCV infection with PEG-IFN-α and ribavirin.

HALT-C participants with SVR. Currently, of the 1123 patients enrolled into HALT-C, 179 (16%) achieved a week 60 response (Table 2). Table 3 (from Shiffman et al., 2004) displays factors which were associated with virologic response in the first 604 patients enrolled into HALT-C.

Patients with virologic response at week 20 (virologic response = absence of detectable HCV RNA in serum), but virologic breakthrough during treatment or relapse after therapy discontinuation are not considered in the study design. A comparison of HALT-C non-responders to HALT-C SVR is likely to have more power than an analysis which includes the patients with virologic breakthrough or relapse as non-responders.

6. Selection of Alleles and Genotyping

6.1 Core Genotyping Facility, NCI

Genotyping for this study will be performed at the National Cancer Institute's Core Genotyping Facility (CGF), which is directed by Dr. Stephen Chanock. CGF performs high-throughput genotyping and sequencing in support of the NCI Intramural Research Program. CGF has the capacity to genotype hundreds of previously defined single nucleotide polymorphisms (SNPs) and will develop new assays upon request.

6.2 Selection of alleles and assay development

We will use "SNP" (NCBI; http://www.ncbi.nlm.nih.gov:8080/) to help us select alleles of interest in these candidate genes. Genewindow is a gene viewing tool released by CGF. It allows the user to search for genetic variation in genes of interest and graphically display the location of the allele (i.e., exon, intron, non-coding regions), changes in protein sequence (e.g., non-synonymous SNPs) or structure, as well as available data on allele and genotype frequency. In choosing alleles for investigation, we will give priority to those that are more likely to have functional significance. These include SNPs that are non-synonymous (causing a change in the coded amino acid) or that are located in gene promoter or regulatory regions. Synonymous SNPs, SNPs in introns or untranslated regions will be considered, if other studies suggest a functional role. Most data on genetic variation in **NCBI SNP** has not been validated, and population specific genotype frequencies are frequently missing. To validate the public data, CGF sequences samples from 102 individuals (Coriell Biorepository), which include representatives from various racial/ethnic groups. Decisions regarding the development of specific genotyping assays will be based on these verified sequences and genotype frequencies. Priority will be given to alleles for which the minor variant has sufficient frequency to achieve good statistical power in epidemiologic studies.

6.3 DNA

CGF requests 4 μg of cellular DNA/patient.

6.4 Quality Control (QC)

For QC reasons, batches for genetic testing will contain 10% duplicate and 5-10% replicate samples. These QC samples will be spread across plates or batches. Profiling data will be checked to confirm duplicates and replicates, and the percent-agreement will be calculated to control for random or systematic error.

7. Statistical analysis

We will calculate the odds ratios (OR) and 95% confidence intervals for comparisons of genotype frequency in non-responders and patients with a sustained virological response. Logistic regression analysis will be used to control for potential confounding by race and other factors. Type of previous treatment, HCV genotype, and baseline HCV RNA levels will also be considered in the multivariate analysis.

To assess the likelihood of a false positive result, which has previously been estimated to be as high as 0.95 in genetic association studies (Colhoun et al., 2003), we will use the Bayesian "false positive report probability" (FPRP) approach described by Wacholder et al. (2004). The FRRP is calculated by considering the prior probability of a hypothesis, the statistical power of the study, and the observed p-value.

8. Statistical power calculation

Power calculations are presented in table 4.

9. Budget

Costs are $1/SNP for genotyping and $5.50 for sample handling (per sample). The proposed study will involve ~ 920 samples.

Genotyping cost (e.g. hypothetically assuming 18 SNPs)	$ 16,560
Sample handling ($5.50/sample)	$ 5,060
BBI Repository ($2/sample + $25, carrier cost)	$ 1,865
TOTAL	**$ 23,685**

9. Literature

Almarra A. Interferon-alpha therapy in HCV patients (1998). HLA phenotype and cirrhosis are idependent predictors of clinical outcome. Hum Immunol 59: 239-42.

Ahlenstiel G et al. (2003) Effects of the CCR5-Delta32 mutation on antiviral treatment in chronic hepatitis C. J Hepatol. 39(2):245-52.

Alric L et al (1999). Study of an association between major histocompatibility complex class II genes and the response to Interferon Alpha in patients with chronic hepatitis C infection. Hum Immunol 60: 516-23

Barnes E, Harcourt G, Brown D et al. (2002). The dynamics of T-lymphocyte responses during combination therapy for chronic Hepatitis C virus infection. Hepatology 21: 632-638.

Clemens MJ & Elia A (1997). The double-stranded RNA-dependent protein kinase PKR: structure and function. Journal of Interferon and Cytokine Research 17: 503-524.

Castet V., Fournier, C, Soulier A et al. (2002) Alpha interferon inhibits hepatitis C virus replication in human hepatocytes infected *in vitro.* J Virol 76: 8189-8199

Dorak MT et al. (2002). C-C chemokine receptor 2 and C-C chemokine receptor 5 genotypes in patients treated for chronic hepatitis C virus infection. Immunol Res. 26(1-3):167-75

Fried MW, Shiffman ML, Reddy R et al (2002). Peginterferon alfo-2a plus ribavirin for chronic hepatitis c virus infection. NEJM 347:13. 975-982.

Foy, E.; Li, K.; Wang, C.; Sumpter, R. (2003). Regulation of Interferon Regulatory factor-3 by the Hepatitis C virus serine protease. Science 300: 1145-1147.

Glas J et al. (2003). The Delta 32 mutation of the chemokine-receptor 5 gene neither is correlated with chronic hepatitis C nor does it predict response to therapy with interferon-alpha and ribavirin. Clin Immunol. 108(1):46-50.

Goodbourn S et al (2000). Cell signaling, immune modulation, antiviral response and virus countermeasures. J Gen Virol (81): 2341-2364.

Hiscott H et al, (2001). Hostile takeovers: viral appropriation of the NFkB pathway. J Clin Invest 107(2): 143-151.

Hoffmann RM et al. (1995). Mapping of immunodominant CD4+ T-Lymphocyte epitopes of hepatitis C virus antigens and their relevance during the course of chronic hepatitis C virus infection. Hepatology 21: 632-638.

Kamal SM, Fehr J, Roesler B (2002). Peginterferon alone or with ribavirin enhances HCV-specific CD4 T-helper 1 responses in patiens with chronic hepatitis C. Gastroenterology 123: 1070-1083.

Knapp S et al. (2003). Interleukin-10 promoter polymorphisms and the outcome of hepatitis C virus infection. Immunogenetics. 55(6):362-9.

Lee LJ et al. (2001). Interleukin 10 polymorphisms as predictors of sustained response in antiviral therapy for chronic hepatitis C patients. Hepatology 33: 708-712

Mogensen KE et al. (1999). The type I interferon receptor: structure, function, and evolution of a family business. Journal of Interferon & cytokine research 19: 1069-1098.

Nagakaya, T et al. (2001). Gene induction pathways mediated by distinct IRFs during viral infection. Biochem and Biophys Reseach Comm. 283, 1150-1156.

Promrat et al., (2003), Associations of chemokine system polymorphisms with clinical outcomes and treatment responses of chronic Hepatitis C. Gastroenterology, 124: 352-360

Romero-Gomez M et al. (2003). HLA ClassI B44 is associated with sustained response to Interferon and Ribavirin therapy in patients with chronic hepatitis C. Am J Gastoenterol 98:7, 1621-1626.

Ruiz-Ferrer M et al., (2004). Analysis of CCR5eslta32 and CCR2-V64I polymorphisms in a cohort of Spanish HCV patients using real-time polymerase chain reaction and fluorescence resonance energy transfer technologies. J Viral Hep 11, 319-323.

Shiffman ML et al. (2004). Peginterferon alfa-2a and Ribavirin in Patients with chronic Hepatitis C who have failed prior treatment. Gastroenterology 126: 1015-1023.

Sim H et al. (1998). Response to interferon therapy: Influence of human leucocyte antigen alleles in patients with chronic hepatitis C. J Viral Hepat 5: 249-59.

Silverman, RH & Cirino, NM (1997). RNA decay by the interferon-regulated 2-5A system as a host defense against viruses. In mRNS Metabolism and Post-transcriptional Gene Regulation, pp 295-309. Wiley-Liss Inc., NY

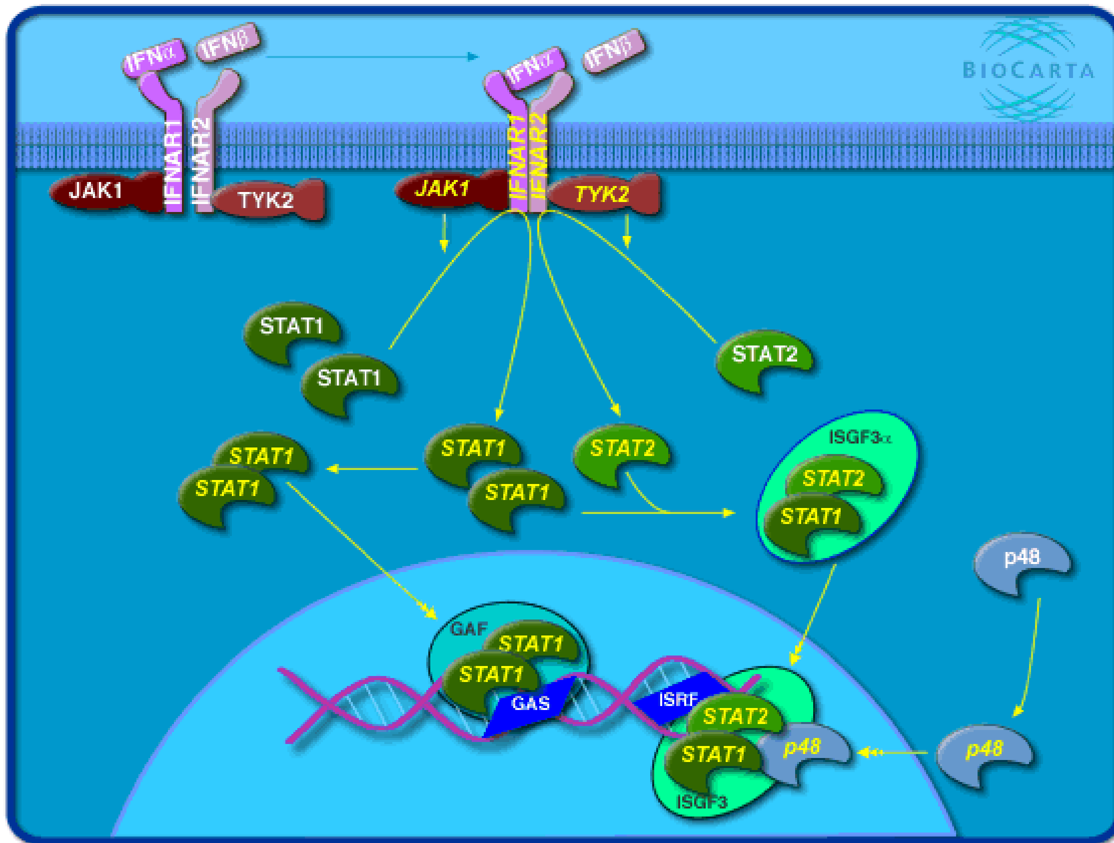
Tan SL and Katze MG (1999). The emerging role of the interferon-induced PKR protein kinase s an apoptotic effector: a new face of death? Journal of Interferon and Cytokine research 19: 543-554.

Vidigal PG et al. (2002) Polymorphisms in the interleukin-10, TNF-alpha and transforming growth factor beta-1 genes in chronic hepatitis C patients treated with interferon and ribavirin. J Hepatol 2002; 36: 271-7/

Yee LJ et al., (2003). Association of CTLA4 polymorphisms with sustained response to interferon and ribavirin therapy for chronic hepatitis C virus infection. J Infect Dis. 15;187(8):1264-71

Yee LJ et al. (2001). Interleukin 10 polymorphisms as predictors of sustained response in antiviral therapy for chronic hepatitis C virus infection. Hepatology 33: 708-712

Figure 1. Interferon-α pathway



http://www.biocarta.com/pathfiles/h_ifnaPathway.asp

Table 1. Candidate genes for the study of interferon-α pathway and chemokine receoptors in patients treated with PEG-IFN-α2a and Ribavirin.

Gene	Name/Function	OMIM	Known variations (NCBI/SNP) ?	Literature (Review)
1. Interferon-α Receptor				
IFNAR1	Interferon-α receptor, subunit 1	* 107450	Yes	Mogenson et al, 1999
IFNAR2 (IFNAR 2b/2c)	Interferon- α receptor, subunit 1	* 602376	Yes	
2. Signal transduction in response to Interferon-α **a) JAK-STAT- Pathway**				
Tyk 2	Protein-Tyrosine Kinase 2	* 176941	Yes	Clemens&Elia, 1997, Goodborn, 2000
JAK1	Janus kinase 1	* 147795	Yes	
STAT1	Signal Transducer and Activator of Transcription1	* 600555	Yes	
STAT2	Signal Transducer and Activator of Transcription2	* 600556	Yes	
p48/ISGF-3	Interferon-stimulated transcription factor	* 147574	Yes	
3. Interferon-α induced genes with antiviral properties				
PRKR (PKR)	ds RNA activated kinase inhibits translational initiation through the phosphorylation of eIF-2α, inhibits protein synthesis	*176971	Yes	Clemens&Elia, 1997, Tan & Katze, 1999
eIF-2α	Involved in protein synthesis through binding of tRNA to the ribosomes	*603907	Yes	Goodborn, 2000
NF-κB	Nuclear factor kappa B. Induces IFN-production. Antiapoptotic.	*164011 *164012	Yes	Hiscott, 2000
OAS1	2'-5' oligoadenylate synthase 1. Activates RNAseL, degrades viral RNA	*164350	Yes	Silverman, 1997
Mx1 (MxA)	Family of Mx-Protein GTPase, with intrinsic antiviral activity	*147150	Yes	Goodborn, 2000

ADAR	RNA-specific adenosine deaminase (ADAR). Destabilizes dsRNA by deamination of adenosine to inosine	*601059	Yes	Goodborn, 2000
4. Interferon regulatory factor (IRF)				
IRF-3	Interferon regulatory factor-3	*603734	Yes	Foy et al., 2003
5. Chemokine Receptors				
CCR5	Chemokine Receptor-5 Ligand of MIP-1α, MIP-1β and RANTES.	*601373	Yes	Ahlenstil et al., 2003; Glas
CCR2	Chemokine Receptor-2	*601267	Yes	Ruiz-Ferrer et al., 2004

Table 2. Treatment response at weeks 20 and 60 among HALT-C participants, by prior treatment.

Prior Treatment	N	Week 20 response		Week 60 response	
Std. IFN-α	282	123	44%	71	25%
PEG-IFN-α	39	18	46%	13	33%
Std. IFN-α + RBV	737	210	28%	89	12%
PEG-IFN-α + RBV	65	11	17%	6	9%
Total	1123	362	32%	179	16%

Table 3. Characteristics associated with virologic response in the HALT-C trial (Shiffman et al. 2004)

	N	Week 20 response[a] (%)	Week 48 response[a] (%)	Week 72 response[a] (%)
Overall population	604	35	32	18
Prior treatment		$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Interferon alone	219	47	44	28
Interferon and ribavirin	385	28	25	12
Race and Ethnicity[b]		$P < 0.0001$	$P < 0.0001$	$P = 0.0019$
White	466	38	35	20
Black	84	11	10	6
Hispanic	39	49	41	18
Other	15	40	40	33
Age		$P = 0.0003$	$P = 0.0002$	$P = 0.0107$
<60 years	541	37	34	19
≥60 years	63	14	11	6
HCV genotype[c]		$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
1	539	30	27	14
2	31	81	81	65
3	26	81	77	54
Others	6	33	33	17
Baseline HCV RNA (*IU/mL*)		$P = 0.11$	$P = 0.12$	$P < 0.0009$
$\geq 1.5 \times 10^6$	452	33	30	15
$< 1.5 \times 10^6$	152	40	37	27
Cirrhosis		$P = 0.0004$	$P = 0.0002$	$P = 0.0005$
Yes	233	26	23	11
No	371	40	37	23
AST/ALT		$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
>1.0	122	19	17	6
≤1.0	482	39	35	21
Body mass index (kg/m^2)		$P = 0.17$	$P = 0.14$	$P = 0.46$
<25	109	38	35	22
25–29	239	37	34	18
30+	254	32	28	17
Early virologic response[d]		$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Yes	309	66	61	34
No	295	2	1	1

[a]Response is defined as HCV RNA undetectable at treatment weeks 20, 48, or 72.

[b]*P* value is for a comparison of black vs. others.

[c]*P* value is for a comparison of genotype 2 or 3 vs. others.

[d]Early virologic response was defined as a 2 $\log_{10}$ decline in HCV RNA from baseline or HCV RNA undetectable at treatment week 12

Table 4. Statistical power to detect differences in the odds ratio (OR) for genotype frequency comparisons of 700 non-responders and 170 sustained virologic responders enrolled in HALT-C, assuming $\alpha = 0.05$.

OR	Genotype frequency	Power
5.0	2.0	80%
	2.5	90%
	4.0	99%
4.0	2.1	80%
	2.9	85%
	3.2	90%
	4.0	95%
	5.0	99%
3.0	4	80%
	4.8	85%
	5.6	90%
	6.7	95%
	9.0	99%
2.0	12.2	80%
	14.1	85%
	17.0	90%
	20.0	93%