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Original Research

Clinical Evaluation of Terap C Vaccine in Combined Treatment with Interferon and Ribavirin in Patients with Hepatitis C

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ABSTRACT

Background: An estimated 170 million individuals worldwide are infected with the hepatitis C virus (HCV). Although treatment options using a combination of pegylated interferon and ribavirin (P-IFN/RBV) are available, sustained clearance of the virus is only achieved in approximately 40% of individuals infected with HCV genotype 1. Recent advances in the treatment of HCV using directly acting antiviral agents have been achieved; however, treatment can be very expensive and is associated with substantial side effects. The development of a new treatment modality is needed. One possible modality could be specific immunotherapy. Terap C is a therapeutic vaccine candidate composed of pIDKE2, a plasmid expressing HCV structural antigens, with a recombinant HCV core protein, Co.120.

Objective: To assess the safety and efficacy of concomitant therapy with the candidate vaccine, Terap C, IFN α-2b and ribavirin in untreated individuals with HCV genotype 1 infection.

Methods: This was a Phase II randomized, placebo-controlled, double-blind clinical trial evaluating the safety and efficacy of Terap C concomitant with IFN α-2b/RBV in 92 treatment-naïve patients with HCV genotype 1 infection. The study was conducted at the Gastroenterology Institute in Havana, Cuba. Patients were randomly assigned to 1 of 5 groups. The control group (Group 1) received IFN α-2b/RBV and placebo for 48 weeks. Groups 2 and 3 were administered Terap C 6 and 9 times, respectively, in addition to standard IFN α-2b/RBV treatment. In groups 4 and 5, Terap C was introduced 12 weeks after the initiation of IFN α-2b/RBV and administered 6 and 9 times, respectively, concomitant with IFN α-2b/RBV.

Results: All patients showed some adverse events. Out of 3615 adverse events, only 18.8% were considered to be probably associated with administration of Terap C. Most events (47.4%) were considered to be improbably associated with of administration Terap C. Only 33.8% were considered possibly temporarily associated with Terap C, and can be explained by the use of conventional IFN α-2b + RBV or by HCV itself. The most common adverse events ($\geq$ 65%) observed were pain at the injection site, headache, asthenia, psychiatric disturbances, fever, and gastrointestinal symptoms. Regarding sustained virological response, a 20% superiority was observed in the patients who received concomitant Terap C treatments from the beginning of the study compared with those who started after Week 12.

Conclusions: Vaccination with Terap C in patients with chronic HCV infection was safe and well tolerated. Clinical trial protocol code: IG/VHI/HC/0701; Public Register Code: RPCEC00000074.

Introduction

An estimated 170 million individuals worldwide are chronically infected with hepatitis C virus (HCV),[1] and approximately 3 to 4 million new cases are identified annually.[2] HCV accounted for

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approximately 500,000 deaths worldwide in 2010,[3] and it is a major indication for liver transplantation in the United States[4] and Europe.[5] There are variations in the burden of HCV across the globe, as depicted by prevalence rates of 1.5%, 2.3%, and 3.2% for the Americas, Europe, and Africa, respectively, as reported by the World Health Organization.[1] In Cuba, the seroprevalence of HCV among blood donors ranged between 0.7% and 1.2% during the past 4 years.[6]

In infected individuals, HCV triggers an immune response against virtually all viral antigens; however, viral escape mechanisms can prevent effective HCV clearance.[7] As a result, 85% of HCV infections are persistent. Approximately 25% of chronic carriers may develop cirrhosis within 2 decades of HCV infection, and 1% to 4% develop hepatocellular carcinoma annually.[8,9] Together, these data reveal the need for effective HCV treatment.

The immunological parameters associated with effective protection against HCV infection have not yet been identified[10]; therefore, the therapeutic efficacy of a vaccine candidate should be evaluated by its ability to reduce or eliminate viral load.[11,12] Studies of patients in which HCV is spontaneously eliminated suggest that the early development of both humoral and cellular immune responses and longer response durations against a broad spectrum of viral antigens predict HCV elimination.[13,14]

Although treatment options using a combination of conventional or pegylated interferon (P-IFN) and ribavirin (RBV) are available, a sustained virological response (SVR) is only achieved in approximately 40% of individuals infected with HCV genotype 1. Adverse events (AEs) with this therapy are common and may lead to suspension of treatment in 15% to 20% of cases.[15,16]

The first generation of direct-acting antiviral agents (DAAs), including telaprevir and boceprevir, were approved for clinical use in 2011.[17] The introduction of triple therapy with P-IFN, RBV, and DAA achieved cure rates of 75% in treatment-naïve patients infected with HCV genotype 1 and in nearly 50% of nonresponders.[18] In 2014, triple therapy was removed from the recommendations of the American Association for the Study of Liver Diseases and the American Society for Infectious Diseases due to AEs and drug interactions.[19,20]

The advent of newer DAAs (eg, sofosbuvir, simeprevir, daclatasvir, ledipasvir, ombitasvir/paritaprevir/ritonavir combination, and dasabuvir) have further increased the SVR rates in genotype 1 patients to 80% to 90%, with a shorter course of therapy, fewer monitoring requirements, and without the need to supplement with P-IFN.[21] These HCV drug regimens have dramatically improved treatment efficacy, despite higher drug prices. In Cuba, few patients have been treated with these antivirals. Therefore, novel strategies for determining which patients would benefit most from treatment should be carried out.

Evaluations support the view of therapeutic vaccination against HCV as a promising alternative. In particular, recombinant protein vaccines are very attractive even for those patients who are unresponsive to conventional therapies. They induce potent humoral immune responses and, to a lesser extent, cell-mediated responses. The latter result from specific T cells developed by the direct presentation of the antigen to the T-cell receptor through human leukocyte antigen molecules.[22,23] In addition, the reinfected individuals who had spontaneously cleared their first HCV infection displayed reduced viremia levels and chronicity rates compared with those with primary infections, suggesting the presence of immune memory against HCV infection. The goal of HCV vaccine development should be to find and understand this natural immunity against HCV, and more importantly, to induce this natural immunity via vaccination to a level that renders the maximal protection against HCV infection.[24] There is therefore an immediate need for the development of new, cost-effective treatments, such as therapeutic vaccines, as adjunctive or alternative treatments for chronic HCV infection.[25]

In this study, the safety and efficacy of concomitant therapy with the vaccine candidate TERAP C (CIGB 230, Center for Genetic Engineering and Biotechnology, Havana. Cuba), and the standard treatment in the country at the moment in which the study was performed, interferon (IFN) α-2b and RBV, was assessed in treatment-naïve individuals with HCV genotype 1 infection.

Table I
Demographic characteristics of the sample studied.*

Variable		Value (frequency)
Sex	Male	41 (44.6)
	Female	51 (55.4)
Age	Half (SD)	46.2 (7.8)
	Median (interquartile range)	46.0 (10.8)
	Min, max	24, 59

* Values for sex are presented as n (%).

Materials and Methods

Study population

Terap C is a therapeutic vaccine candidate composed of pIDKE2, a plasmid encoding HCV structural antigens, with a recombinant HCV core protein variant, Co.120. The clinical trial (Protocol code: IG/VHI/HC/0701; Public Register Code: RPCEC00000074) was conducted at the National Institute of Gastroenterology (Havana, Cuba) and was approved by the institutional ethics committee and the Cuban National Regulatory Authority.

Written informed consent was obtained from every patient. All procedures were conducted in accordance with the national ethics guidelines and the Helsinki Declaration of 1975. The study included 92 treatment-naïve patients whose blood serum levels were positive for HCV genotype 1b RNA, were diagnosed with chronic hepatitis by liver biopsy, and had no other documented liver diseases.

The exclusion criteria included pregnancy, nursing, coinfection with HIV or active hepatitis B virus infection, liver cirrhosis, hepatocellular carcinoma, uncontrolled chronic diseases, blood disorders, autoimmune diseases, severe allergy, suspected acute infection, and a history of immunosuppressive/immunomodulatory drug consumption in the previous 6 months. Patient demographic characteristics are shown in **Table I**.

Study design and interventions

The study was a Phase II, randomized, controlled, double-blind clinical trial. All patients received IFN α-2b (3 million IU administered subcutaneously 3 times per week, on alternate days) and RBV (1000 or 1200 mg daily, depending on body weight) for 48 weeks because that was the standard treatment at that moment in the country in which the study was performed. Patients were randomly assigned to 1 of 5 groups according to their Terap C treatment condition, as shown in **Table II**. The control group (Group I; n = 30) received 12 inoculations with the vaccine placebo (in the same buffer as Terap C but lacking the active vaccine component). Two

Table II
Treatment regimens by group. All groups also received Interferon □α-2b (3 million IU administered subcutaneously 3 times per week, on alternate days) and ribavirin (1000 or 1200 mg daily, depending on body weight).

Time, wk	4	8	12	16	20	24	28	32	36	40	44	48
Group I (control)	P	P	P	P	P	P	P	P	P	P	P	P
Group II	TC	TC	TC	TC	TC	TC	P	P	P	P	P	P
Group III	TC	TC	TC	TC	TC	TC	TC	TC	TC	P	P	P
Group IV	P	P	P	TC	TC	TC	TC	TC	TC	P	P	P
Group V	P	P	P	TC	TC	TC	TC	TC	TC	TC	TC	TC

P = placebo; TC = terap C.

groups received 6 inoculations of Terap C. In Group II (n = 16), the inoculations started simultaneously with the antiviral treatment at Week 0, as an early add-on, whereas in Group IV (n = 15), the inoculations instead started at Week 12 of therapy as a late add-on. The remaining 2 groups were inoculated 9 times with Terap C: Group III (n = 16) as early add-on and Group V (n = 15) as a late add-on. Inoculations of the candidate vaccine or placebo were administered once every 4 weeks. To maintain the blinded nature of the study, placebo inoculations were administered once every 4 weeks on weeks not corresponding to vaccine candidate administration. Terap C immunization consisted of intramuscular administration of 0.5 mg pIDKE2 plasmid mixed with 0.05 mg Co.120 recombinant protein in saline solution.

Evaluation of safety

AEs were classified by a blinded physician according to their intensity (mild, moderate, severe, or serious/life-threatening), location (local or systemic), and causality (very likely, probable, possible, unlikely, unrelated, or unclassifiable).[26]

Laboratory parameters

Appearance of laboratory parameters in the pathologic range during the treatment period was considered to be an AE. For transaminases, which fluctuate in patients with HCV,[27] the appearance of ranges 3 times above the normal reference values was considered an AE.[28]

Changes in liver histology

Because DNA immunization stimulates the immune system and can induce autoimmune responses, liver histology was evaluated before and after treatment to assess the safety of Terap C.[29] All patients underwent liver biopsy before and 72 weeks after starting treatment. Liver histology was evaluated by an experienced pathologist using the Knodell-Ishak modified hepatic activity index.[30] Worsening of liver histology was defined as an increase of 2 points from baseline scores for each patient[31–33] in fibrosis and/or necroinflammatory activity. No worsening of liver histology was defined as the maintenance of baseline or a decrease within 2 points of baseline fibrosis and necroinflammatory activity scores.[28]

Evaluation of therapeutic efficacy

The virological response (presence of HCV RNA by reverse transcription polymerase chain reaction [UMELOSA HCV CUALITATIVO, Center for Immunoassay, Havana, Cuba]) was assessed on Weeks 0, 12, 48, and 72. A complete early virological response (EVR) and SVR was defined as having undetectable HCV RNA on Weeks 12 and 72, respectively.

Table IV
Frequency of local adverse events by treatment group.*

Local adverse events	Group I	Group II	Group III	Group IV	Group V	Total
N	30	16	16	15	15	92
Pain	27 (90)	16 (100)	14 (87.5)	14 (93.3)	15 (100)	86 (93.5)
Induration	6 (20)	4 (25)	8 (50)	10 (66.7)	6 (40)	34 (37)
Erythema	3 (10)	1 (6.3)	2 (12.5)	5 (33.3)	4 (26.7)	15 (16.3)
Hot	1 (3.3)	3 (18.8)	2 (12.5)	3 (20)	3 (20)	12 (13)
Pruritus	3 (10)	2 (12.5)	2 (12.5)	1 (6.7)	2 (13.3)	10 (10.9)

* Values are presented as number (%) of patients.

Histology was evaluated at baseline and on Week 72, by liver biopsy and analyzed according to the Ishak score.

Statistical analysis

The distribution of categorical variables between treatment groups was analyzed by χ^2 test. To study the changes that occurred over time in quantitative variables paired analysis, Student *t* tests or Wilcoxon tests were used to analyze parametric and nonparametric data, respectively. The assessment of the influence of the factors studied (scheme and number of applications) in the primary end point was carried out by using regression models to evaluate the compliance with the working hypothesis. Kappa indexes were calculated to evaluate the correlation between viral response and liver histology. The benefit-risk analysis was performed using Bayes factors as a measure of the odds ratio.[34] Analyses were performed with GESS 2006 Trial software (Kaysville, Utah), calculation of samples size was conducted with NCSS PASS 2005 (Kaysville, Utah), and statistical analyses were completed with EPIDAT (Dirección Xeral de Saúde Pública de la Consellería de Sanidade Xunta de Galicia, España) SPSS versions 15.0 (SPSS Inc, Chicago, IL, USA) and 3.0.

To determine the independence, we used a Bayesian test of independence that handles 2 hypotheses: hypothesis H0, which states that the 2 variables are independent, and the complementary hypothesis, which states that the 2 variables are dependent or are somehow related. Under the Bayesian approach, we assessed the relative support given by the data to each of these 2 hypotheses in terms of the Bayes factor, which compares the probabilities of the data observed under the hypotheses.

Results

Of 92 patients enrolled in the study, 11 did not complete the treatment. One patient missed a dose, 8 patients withdrew due to

Table III
Frequency of systemic adverse events by treatment group.*

Adverse events	Group I	Group II	Group III	Group IV	Group V	Total
N	30	16	16	15	15	92
Headache	27 (90.0)	14 (87.5)	14 (87.5)	13 (86.7)	11 (73.3)	79 (85.9)
Gastrointestinal manifestations	26 (86.7)	13 (81.3)	14 (87.5)	14 (93.3)	12 (80.0)	79 (85.9)
Fever	26 (86.7)	13 (81.3)	12 (75.0)	15 (100)	11 (73.3)	77 (83.7)
Asthenia	25 (83.3)	15 (93.8)	11 (68.8)	13 (86.7)	13 (86.7)	77 (83.7)
General discomfort	24 (80.0)	12 (75.0)	11 (68.8)	14 (93.3)	12 (80.0)	73 (79.3)
Neuropsychiatric disorders	24 (80.0)	12 (75.0)	11 (68.8)	12 (80.0)	10 (66.7)	69 (75.0)
Neutropenia	22 (73.3)	11 (68.8)	7 (43.8)	10 (66.7)	8 (53.3)	58 (63.0)
Thrombocytopenia	15 (50.0)	6 (37.5)	6 (37.5)	8 (53.3)	7 (46.7)	42 (45.7)
Anemia	10 (33.3)	12 (75.0)	6 (37.5)	9 (60.0)	3 (20.0)	40 (43.5)
Leucopenia	12 (40.0)	7 (43.8)	5 (31.3)	8 (53.3)	1 (6.70)	33 (35.9)

* Values are presented as number (%) of patients.

Table V
Characterization of adverse events by treatment groups by intensity, behavior, consequences, and causality.*

Adverse events	Group I	Group II	Group III	Group IV	Group V
Intensity					
Mild	961 (82.8)	521 (79.3)	563 (89.6)	543 (82.9)	446 (86.8)
Moderate	194 (16.7)	129 (19.6)	64 (10.2)	111 (16.9)	65 (12.6)
Severe	6 (0.5)	7 (1.1)	1 (0.2)	1 (0.2)	3 (0.6)
Total	1,161 (100)	657 (100)	628 (100)	655 (100)	514 (100)
Consequence					
Not serious	1,159 (99.8)	656 (99.8)	628 (100)	655 (100)	513 (99.8)
Serious	2 (0.2)	1 (0.2)	0 (00.0)	0 (00.0)	1 (0.2)
Causality					
Very likely	165 (14.2)	110 (16.8)	138 (22)	145 (22.1)	121 (23.5)
Potential	382 (32.9)	237 (36.1)	230 (36.6)	191 (29.2)	182 (35.4)
Unlikely	614 (52.9)	310 (47.2)	260 (41.4)	319 (48.7)	211 (41.1)

* Values are presented as number (%) of events.

AEs, 1 patient was excluded due to pregnancy, and another was excluded due to a positive HIV test.

A total of 81 patients (88.04%) completed the study protocol. Of 465 doses of Terap C to be administered to 92 patients according to the study design, only 435 were applied: 28 patients received 9 doses, 29 received 6 doses, 1 received 4 doses, 1 received 3 doses, and 1 received 2 doses.

Safety

The frequency of individuals who experienced systemic AEs within each treatment group is reported in **Table III**. All study participants showed some AEs, and these presented in some patients more than once. There were 1159 AEs among all treatment groups. Of these events, 1002 were systemic and 157 were local. A total of 92 different AEs were reported in 92 patients. The most common ($\geq$ 65%) were headache, gastrointestinal symptoms, fever, fatigue, malaise, and neuropsychiatric symptoms. For the systemic and local adverse events, if the same type of AE occurred more than once in the same patient, it was counted as only 1.

The frequency of patients who experienced local AEs within each treatment group is reported in **Table IV**. A total of 157 local AEs were reported. Overall, pain was the most frequently reported local AE and occurred in 93.5% of patients, followed by induration at a frequency of 37%. Induration was observed at statistically significantly higher rates in the groups receiving Terap C (45.2%) compared with the control group (20%), which was considered a Terap C-related AE.

The clinical characterization of all AEs is summarized in **Table V**. A total of 3615 EAs were classified according to their intensity: 3034 (83.9%) were mild, 563 (15.6%) were moderate, 18 (0.5%) were severe, and 4 (0.1%) were serious. For the intensity classification, if 1 patient had the same type of AE more than once, each time was counted, classified, and separated based on its intensity.

As for causality, most AEs were classified as unlikely to be attributable to the Terap C administration (47.4%). The remaining AEs were classified as possible (33.8%) or very likely (18.8%).

During the treatment period, biochemical parameters showed a tendency to normalize in all groups, with better response profiles in Groups I, III, and IV, which had significantly reduced levels of not only alanine aminotransferase (ALT) and aspartate aminotransferase, but also γ-glutamyl transferase (γ-GGT) (**Table VI**).

Glycemic, creatinine, uric acid, bilirubin, and alkaline phosphatase were additional biochemical variables that were studied. There were no evident differences between groups or between the values of the different variables over time (data not shown).

Treatment response

Table VII shows the results of the analysis by intention to treat (considering the original group assignment of the patients and including those patients who did not complete the treatment). At each time point assessed, the chances of independence were small (< 0.25), suggesting a probability of greater reliance (0.75), which from the descriptive point of view could be explained by the tendency of inferiority of Group V compared with other groups. At Week 12, 60% of patients in the control group (Group I) were negative for HCV RNA. In Groups II and III (the concomitant Terap C scheme), 62.5% and 56.3% of patients, respectively, were negative for HCV RNA, and these rates were similar to those of the control group. The values at Week 12 for Groups IV and V were lower, at 46.7% and 26.7%, respectively. The overall analysis showed an EVR rate of 52.2%, which demonstrates the nonhomogeneity of the sample at treatment initiation. At 24 and 48 weeks, slightly higher

Table VI
Behavior of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyl transpeptidase (γ-GGT) by treatment groups at Weeks 0 and 72.

ALT	Group I	Group II	Group III	Group IV	Group V	
Week 0	N	29	16	16	15	15
	Median (IQR)	57.2 (45.3)	44.7 (28.9)	46.9 (54.3)	48.4 (45.5)	60.9 (40.9)
	Min, max	7.0, 246.5	18.7, 445.8	22, 20	12, 169.9	18, 216
Week 72	N	27	15	13	14	11
	Median (IQR)	22.9 (8.8)*	20.1 (44.3)	23.1 (38)*	33.3 (32.8)*	49.5 (64.5)
	Min, max	7.4, 130	8.1, 76.1	9, 69.5	4.2, 65.1	8.7, 124.6
AST						
Week 0	N	26	12	13	13	13
	Median (IQR)	49 (6.4)	39.6 (19.3)	37.9 (25.8)	45.9 (47.7)	47.8 (64.2)
	Min, max	16, 73.3	21.7, 318.8	25, 164	18, 261.9	21.5, 174.9
Week 72	N	28	15	13	14	11
	Median (IQR)	24.2 (21)*	21.3 (13.8)*	19.4 (20)*	25.3 (26.8)*	33.5 (55.3)*
	Min, max	9.7, 129.4	12.5, 647	13, 61.9	13.5, 77	12.3, 106
γ-GGT						
Week 0	N	29	16	14	15	15
	Median (IQR)	49 (69)	37 (53)	48.5 (77)	62 (174)	71 (139)
	Min, max	4, 620	14, 325	9, 218	11, 1186	15, 288
Week 72	N	27	15	13	13	11
	Median (IQR)	23 (60)	17 (33)	19 (48)*	21 (112)*	42 (202)
	Min, max	15, 415	7, 336	11, 149	8, 408	15, 394

IQR = Interquartile range.

* Wilcoxon test compared with Week 0 ($P < 0.05$).

Table VII
Viral response (hepatitis C virus [HCV]) by treatment group.*

Patients with HCV RNA-PCR negative	Group I	Group II	Group III	Group IV	Group V	Total	P(H_0)
N	30	16	16	15	15	92	
Week 12	18 (60)	10 (62.5)	9 (56.3)	7 (46.7)	4 (26.7)	48 (52.2)	0.1967
Week 24	18 (60)	11 (68.8)	11 (68.8)	8 (53.3)	5 (33.3)	53 (57.6)	0.2029
Week 48	18 (60)	11 (68.8)	10 (62.5)	8 (53.3)	4 (26.7)	51 (55.4)	0.1266
Week 72	14 (46.7)	8 (50)	8 (50)	7(46.7)	2 (13.3)	39 (42.4)	0.1516

P(H_0) = probability of accepting the hypothesis of independence between viral load and treatment received; RNA-PCR = RNA polymerase chain reaction test.
* Values for Week 12, 24, 48, and 72 are presented as n (%).

values of virological response were observed in Groups II and III (concomitant scheme, 6 and 9 applications, respectively) compared with Group I (control). The values for Groups IV and V were still lower in these moments. At Week 72, in Groups I (control), II, III, and IV, SVR behaved similarly. The patients who corresponded to treatment Group V had a still-lower SVR.

The correlation between EVR and SVR is reported in **Table VIII**. Importantly, in groups III and IV, the typical drop-in virological response observed after stopping the administration of P-IFN and RBV was not observed. The SVR in both groups was higher than expected. No relapses were detected in Group IV. Although few patients were included in the study, this virological behavior may have been influenced by the vaccine candidate.

Table IX summarizes the hepatic activity index scores for necroinflammatory activity and architectural changes, including fibrosis and cirrhosis. A statistically significant reduction in necroinflammatory activity was observed in Groups I, II, and III, at a magnitude of approximately 50% compared with baseline. No differences between the treatment groups were detected at the time points assessed (results not shown).

The index-architecture-fibrosis was not significantly changed ($P < 0.05$) in any of the treatment groups relative to pretreatment, although in general, the index was reduced. At Week 72, most patients presented with fewer alterations in their histologic activity index and architecture, fibrosis, and cirrhosis with respect to baseline. It should be noted that in 1 patient in Group III, who initially presented histologic abnormalities, the liver sample examined at Week 72 was within normal ranges.

In **Table X**, the correlation between viral response and liver histology is shown. Significant concordance ($P < 0.005$) between treatment virological response and changes in necroinflammatory activity was observed; however, no relationship was detected between the treatment virological response and changes in architecture, fibrosis, and cirrhosis ($P > 0.005$).

A T logistic regression model was used to study the relationship between treatment factors and response at 72 weeks (**Table XI**). A significant effect of moment for starting treatment with the vaccine candidate was detected, suggesting a higher probability of virological response in patients administered Terap C starting at Week 0 with respect to those starting at Week 12. No interaction effect was detected. The results may be influenced by the fortuitous distribution of patients resistant to therapy, as evidenced by the patients in Group V, who had a negative predisposition to respond to treatment with P-IFN and RBV.

Table VIII
Actual values and forecasts of sustained viral response (SVR) from early viral response (EVR) per group.

		Group I	Group II	Group III	Group IV	Group V
Week 12	N	30	16	16	15	15
	EVR	18	10	9	7	4
	%	60	62.5	56.3	46.7	26.7
Predicted SVR (75% EVR) %		14	8	7	5	3
		46.7	50	43.7	33.3	20
Week 72	SVR real	14	8	8	7	2
	%	46.7	50	50	46.7	13.3

Discussion

Until 2011, the existing standard therapy consisting of the administration of conventional or P-IFN + RBV, reached SVR rates from 30% to 90%, depending on several factors, including the HCV genotype and stage of liver disease.[35] Many patients are IFN-intolerant or have contraindications to P-IFN/RBV therapy that preclude the treatment.[36] New therapies with the DAA have provided a significant breakthrough in the treatment of hepatitis C, at the present time they constitute the first treatment line and have replaced the treatment with P-IFN and Rib.[37] IFN-free combinations were registered in 2014 and 2015 for the treatment of chronic HCV infection.[38] Nevertheless, IFN-based therapy is still a treatment choice in several countries, although IFN α administration at therapeutic doses leads to several AEs,[39] which, together with those related to RBV, provoke frequent dose adjustments, treatment interruptions, or contraindication in certain patients. The immune modulatory properties of IFN α and RBV have been clearly described.[40,41] In fact, RBV's beneficial effects on treatment outcomes in patients with chronic HCV have been demonstrated and may help to develop new treatment approaches.[42]

Terap C (CIGB-230) administration, as was previously described by Canizares et al,[43] was able to increase the neutralizing antibody activity against HCV and induce significantly greater numbers of de novo core-specific lymphoproliferative and IFN γ responses than antiviral therapy alone. However, this response had no influence on the SVR. These facts suggest that more research regarding the optimization of Terap C administration is needed.[43]

Table IX
Changes in liver histology by treatment group.

		Group I	Group II	Group III	Group IV	Group V
Necroinflammatory activity						
Week 0	N	19	13	14	9	8
	Median (IQR)	7 (6)	7 (4)	5.5 (5)	4 (5)	6 (8)
	Min, max	1, 14	1, 12	2, 13	2, 13	1, 14
Week 72	N	19	13	14	9	8
	Median (IQR)	2 (3)*	3 (3)*	2.6 (3)*	5 (6)	6 (6)
	Min, max	1, 10	1, 9	0, 11	1, 9	1, 10
Architecture, fibrosis, and cirrhosis						
Week 0	N	19	13	14	9	8
	Median (IQR)	1 (1)	2 (2)	1 (1)	1 (2)	1 (2)
	Min, max	0, 5	0, 5	0, 5	0, 4	0, 7
Week 72	N	19	13	14	9	8
	Median (IQR)	1 (2)	2 (2)	1 (1)	2 (3)	2.5 (3)
	Min, max	0, 5	0, 5	0, 5	0, 5	0, 5

IQR = interquartile range.
* Wilcoxon test from Week 0 ($P < 0.05$).

Table X
Correlation between hepatitis C virus (HCV) viral response and histology.*

HCV-ARN		Necroinflammatory activity (n = 63)		Kappa (*P* value)
		No worsening	Deterioration	
Week 72	NR	19 (30.2)	11 (17.5)	0. 009
	SVR	30 (47.6)	3 (4.8)	
		Architecture, fibrosis, and cirrhosis (n = 63)		Kappa (*P* value)
		No worsening	Deterioration	
Week 72	NR	22 (34.9)	8 (12.7)	0.750
	SVR	23 (36.5)	10 (15.9)	

ARN = Ribonucleic acid. NR = no response to antiviral therapy; SVR = sustained viral response.
* Values are presented as n (%).

The AEs most commonly associated with IFN therapy and RBV range from mild events, such as fever and symptoms similar to the flu, to serious AEs such as neuropsychiatric symptoms, cerebral hemorrhage, pulmonary thrombosis, arrhythmia, heart failure, and gland dysfunction thyroid.[44]

The systemic AEs reported most frequently in this study were headache, gastrointestinal symptoms, fever, fatigue, malaise, osteomioarticular pain, and neuropsychiatric symptoms.[44] Compared with the previous Phase I Terap C study, the overall proportion of AEs was lower. The most frequently reported AE was pain at the injection site.[45] This was followed by headache, fatigue, and osteomioarticular pain. These AEs may be related to Terap C treatment, or they may be associated with HCV, a factor present in both studies.[45,46] Gastrointestinal symptoms, fever, malaise, and neuropsychiatric symptoms were not present in the Phase I study, and therefore may be associated with the use of IFN α-2b and RBV.[45–47]

The predominant gastrointestinal symptoms were anorexia and nausea. Moreover, diarrhea occurred in 37% of cases, which represents a slightly higher rate than reported in the literature (20%).[48] The predominant neuropsychiatric symptoms were insomnia, dizziness and vertigo, and depressive symptoms. These findings are consistent with those previously reported during IFN treatment of chronic hepatitis.[49,50] However, in other studies, depression was a predominant neuropsychiatric symptom during treatment with IFN, and it has been attributed to proinflammatory cytokines (eg, interleukin 1, interleukin 6, and tumor necrosis factor α) production in the central nervous system, as well as to an inhibition of serotonin synthesis.[31] Moreover, in a study by Kraus et al,[51] depression was reported in HCV patients who did not receive antiviral treatment. This association has been reported by other authors[51,52] who assessed anxiety and depression in patients with chronic HCV using the English Hospital Anxiety and Depression Scale. These studies showed that 45% of patients with chronic HCV, before receiving any antiviral treatment, had symptoms of depression compared with 4% of the control group.[51,52] In the case of Chron Q Vac (Tripep, Sweden) vaccaine candidate-C, depression was observed during a Phase II trial.[53]

Pain and induration were the most frequently reported local AEs. Induration occurred at higher frequencies in the groups receiving Terap C compared with the control group, so this AE is likely associated with Terap C. This AE was presented with great frequency in Phase I trials.[45]

The AEs considered most likely to have a causal relationship with Terap C were local, such as pain, redness, and induration. In a studies of the vaccine candidate IC41 (Intercell, Austria), local erythema (46%) and edema at the site of immunization (37%) were observed more frequently than for Terap C.[54,55] The authors describe a predominance of local manifestations in about half of patients treated with this vaccine candidate, such as pain, induration, edema, and itching. In addition, there is consensus among studies that local manifestations primarily appear within the first 24 hours of immunization.[56]

Most AEs were considered unlikely to be associated with Terap C, or in some cases with only a transient association likely, according to the moment of the vaccine administration. Most AEs detected in the clinical trial were attributed to the use of IFN α-2b and RBV, or the HCV infection itself, taking into account the previous abundant data in the field.[57–59]

During the trial, 4 serious AEs occurred, all of which were attributed to treatment with IFN α-2b and RBV because these serious AEs were observed in 2 patients in the control group (Group I) and in 2 patients in treatment Groups II and IV during the initial stage before starting the administration of Terap C. No serious AEs occurred during the administration of the vaccine, which confirms the results obtained in the Phase I study with the vaccine, where no serious or severe AEs occurred.[58] In fact, in the current study, the administration of Terap C generated a safety profile similar to that reported in the Phase I trial with this vaccine. Severe AEs, including myocardial infarction, stroke, pancytopenia, and vomiting with dehydration were observed; however, it was considered that these events had no direct relationship with the vaccine candidate.[57–59]

During the Phase II study, deterioration in hematologic parameters was observed in all groups. Similar hematologic deterioration was previously described in patients receiving typical IFN + RBV treatment.[57,58,60] In the current study, neutropenia occurred with a greater frequency than previously reported. Moreover, as reported in the literature, this AE was well tolerated, and although infections have been described, they do not correlate with the intensity of neutropenia. Neutropenia was not attributed to Terap C because similar frequencies were observed in the control group.

Generally, in all groups, significant reductions in hemoglobin were observed. The hemoglobin level was below normal in a significant number of patients and was the main cause of modification or discontinuation of therapy. This result agrees with previous reports of anemia in approximately 40% of patients with chronic HCV undergoing antiviral treatment. In these studies, RBV induced hemolysis and IFN suppressed hematopoiesis. The behavior also corresponded to that described by other authors and appeared in the first 4 to 8 weeks of the study.[61–63]

Table XI
Logistic regression of treatment factors and response (at 72 weeks).

Factors*	Model coefficient (β)	Standard error	*P* value	Odds ratio
Time (concurrent)	1.872	0.909	0.040	6.500
Dose (6)	1.738	0.919	0.059	5.687
Dose (6) × time (concurrent)	−1.738	1.160	0.134	0.176
Constant	−1.872	0.760	0.014	0.154

* Time, dose, and dose × time are entries in the first step variables.

In this study, an association between HCV virological response and normalization of transaminases was observed.[64] During Week 12, a tendency toward normalization of ALT and aspartate aminotransferase was observed in all groups, as has been previously described in patients receiving IFN and RBV treatment. The normal ALT values in all Group II patients during Weeks 24 and 48 are worth noting. A similar normalization of liver enzymes after vaccination was described by Batdelger et al[65] during therapeutic immunization with oral vaccine V-5.[65]

Most patients in the study who presented with γ-GGT levels >65 IU/L before treatment had no SVR. This is a parameter that has been described as a predictor of poor response to treatment with INF α-2b + RBV.[66]

Considering the reference values of the γ-GGT enzyme from the descriptive point of view, a greater proportion of cases with high enzyme levels in Groups IV and V was observed. From the quantitative point of view, these groups were disadvantaged from the beginning of the study with higher γ-GGT values. In Groups IV and V, more HCV-infected patients were detected at Week 48 with respect to the other groups, although after this time, rebounds occurred in greater proportions in the other groups.[67] In general, the therapy favored liver enzyme stabilization in all treatment groups.

The samples studied in this trial were all classified as genotype 1. Many authors have reported that this genotype responds poorly to antiviral treatment.[68,69] In our study, the global result shows achievement of SVR in 33% of all patients treated. That was higher than expected for patients with genotype 1 chronic HCV treated with IFN α-2b and RBV alone.[69] The concomitant administration of Terap C with IFN α-2b and RBV did not increase the SVR because the behavior of the members of Group I (control), II, III, and IV was similar.

Several additional predictors of favorable response to antiviral treatment have been previously described and include IL28B CC genotype and pretreatment viral load $<600{,}000$ IU/mL.[70] Because in our work it was not possible to evaluate some of the above-mentioned well-established predictors of virological response such as IL28B CC genotype and pretreatment quantitative viral load, the homogeneous distribution of patients among groups regarding their predisposition to respond to therapy could not be ensured.

Indeed, considering the prediction value of early virological response regarding SVR in this study, the blind distribution of patients into the different groups, according to the presettled random list, generated a fortuitous bias with an influence later in SVR that was clearly observed at Week 12, as evidenced by the differences in EVR observed in Groups I, IV, and V, which received the same treatment at the same time (ie, IFN + RBV). Based on this bias, patients in Group V were at a disadvantage because they showed very low values of EVR before they had even received their first dose of Terap C.

In studies with IFN α-2b, we observed that 75% of patients with RNA were negative at Week 12 (EVR) after reaching SVR.[70,71] Similarly, in studies with P-IFN α-2a it has been observed that 65% of patients with response EVR achieve SVR, whereas 97% of those who failed to show this early response did not reach SVR.[70] In Groups III and IV, SVR levels slightly higher than predicted were observed. Although Terap C treatment was applied in these groups, we cannot be sure that the response was related to the therapy because Group II was also treated with this vaccine candidate and behavior was similar to Group I (control). In Group IV, the course of the virological response was modified because the typical drop in virological response from Week 12 to 72, described for IFN + RBV treatment, was not observed.[72] Comparing the results with other vaccine candidates, in the case of IC-41,[73] which failed to prevent relapse in the study group. and TG4040 (Transgene, France), in a Phase II trial[74] we were able to reduce HCV viral load to approximately 0.5 log IU/mL. Although those results were attributed by the authors to the vaccine candidate, criteria described elsewhere[66] suggest that the resulting changes in viral load should be >1 log IU/mL to be considered as secondary to therapeutic intervention. For this reason, we believe that the observed changes in the viral load were too small to be sure that they were related to that vaccine candidate.[66,75]

Approximately 90% of patients who experience a rapid virological response develop SVR.[76,77] Unfortunately, in this study, no virological response at Week 4 of treatment with IFN α-2b and RBV was evaluated, but it could be a parameter to consider for future studies.

Biopsies are important tools used to determine the grade and stage of liver damage in hepatitis. The extent of liver damage defines the extent of necroinflammatory activity, whereas the stage defines the extent of fibrosis or presence of cirrhosis.[70] The presence of fibrosis is an inherent factor in the progression of hepatitis that negatively influences the virological response to treatment. In patients with cirrhosis, lower SVR rates (43%–52%) were observed.[70,78]

In this study, the evaluation of liver histology showed a decrease in necroinflammatory activity that was statistically significant in Group I (control), II, and III. It has been generally observed that patients who became negative when the virological response occurred showed lower quantitative values of necroinflammatory activity and fibrosis index and cirrhosis than those who did not. Differences in the necroinflammatory activity not related to the index values of fibrosis and cirrhosis among patients who cleared the virus and those who did not were detected. This phenomenon has been reported previously in the literature and suggests the contribution of HCV in the generation of liver damage.[70,79] These results are consistent with those reported by the GI-5005 (GlobeImmune, United States) vaccine candidate in a Phase II study where an improvement in necroinflammatory parameters of liver histology in 39% of patients who took triple therapy was appreciated.[80]

Moreover, the combinations evaluated in Groups III and IV showed a higher benefit-risk profile than those of other groups, mainly because no serious AEs were detected in these groups. The calculation took into account the SVR and the presentation of severe AEs.

Conclusions

The administration of the vaccine candidate Terap C was safe and did not increase adverse events related to therapy with IFN α-2b and RBV. However, the treatment did not achieve a significant influence on chronic HCV.

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Conflicts of Interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

References

1. Lavanchy D. Evolving epidemiology of hepatitis C virus. *Clinical Microbiology and Infection.* 2011;17(2):107–15.
2. Lavanchy D. The global burden of hepatitis C. *Liver International.* 2009;29(s1):74–81.
3. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *The Lancet.* 2013;380(9859):2095–128.
4. Davis GL, Alter MJ, El-Serag H, Poynard T, Jennings LW. Aging of hepatitis C virus (HCV)-infected persons in the United States: a multiple cohort model of HCV prevalence and disease progression. *Gastroenterology.* 2010;138(2):513–21.e6.
5. Mühlberger N, Schwarzer R, Lettmeier B, Sroczynski G, Zeuzem S, Siebert U. HCV-related burden of disease in Europe: a systematic assessment of incidence, prevalence, morbidity, and mortality. *BMC public health.* 2009;9(1):1.
6. Ministry of Health publishes of Cuba (MINSAP). Seroprevalencia de hepatitis en donantes de sangre. Departamento de Epidemiología y en Sistema Nacional de Estadistica del MINSAP, 2013.
7. Fafi-Kremer S, Fauvelle C, Felmlee DJ, Zeisel MB, Lepiller Q, Fofana I, et al. Neutralizing antibodies and pathogenesis of hepatitis C virus infection. *Viruses.* 2012;4(10):2016–30.
8. Westbrook RH, Dusheiko G. Natural history of hepatitis C. *Journal of hepatology.* 2014;61(1):S58–68.
9. Lee M-H, Yang H-I, Yuan Y, L'Italien G, Chen C-J. Epidemiology and natural history of hepatitis C virus infection. *World J Gastroenterol.* 2014;20(28):9270–80.
10. Wang Y, Keck Z-Y, Foung SK. Neutralizing antibody response to hepatitis C virus. *Viruses.* 2011;3(11):2127–45.
11. Takaki A, Wiese M, Maertens G, Depla E, Seifert U, Liebetrau A, et al. Cellular immune responses persist and humoral responses decrease two decades after recovery from a single-source outbreak of hepatitis C. *Nature medicine.* 2000;6(5):578–82.
12. Inchauspé G, Honnet G, Bonnefoy JY, Nicosia A, Strickland GT. Hepatitis C vaccine: supply and demand. *The Lancet Infectious Diseases.* 2008;8(12):739–40.
13. Thimme R, Oldach D, Chang K-M, Steiger C, Ray SC, Chisari FV. Determinants of viral clearance and persistence during acute hepatitis C virus infection. *The Journal of experimental medicine.* 2001;194(10):1395–406.
14. Neumann-Haefelin C, Thimme R. Success and failure of virus-specific T cell responses in hepatitis C virus infection. *Digestive Diseases.* 2011;29(4):416–22.
15. Dorta Guridi Z, Castellanos Fernández M, Nodarse Cuní H, Arús Soler E, Pérez Triana F, González Fabián L. Tolerancia del tratamiento con interferón estándar y ribavirina en pacientes cirróticos por virus de la hepatitis C. *Revista Cubana de Medicina.* 2010;49(2) Available at: www.bvs.sld.cu/revistas/med/vol_49_2_10/med01210.htm.
16. Conteduca V, Sansonno D, Russi S, Pavone F, Dammacco F. Therapy of chronic hepatitis C virus infection in the era of direct-acting and host-targeting antiviral agents. *Journal of Infection.* 2014;68(1):1–20.
17. Zhang X. Direct anti-HCV agents. *Acta Pharmaceutica Sinica B.* 2016;6(1):26–31.
18. Schaefer EA, Chung RT. Anti– Hepatitis C Virus Drugs in Development. *Gastroenterology.* 2012;142(6):1340–50 .e1.
19. Wilby KJ, Greanya ED, Ford J, Yoshida EM, Partovi N. A review of drug interactions with boceprevir and telaprevir: implications for HIV and transplant patients. *Ann Hepatol.* 2012;11(2):179–85.
20. Chung RT, Davis GL, Jensen DM, Masur H, Saag MS, Thomas DL, et al. Hepatitis C guidance. *Hepatology.* 2015;62(3):932–54.
21. Clements KM, Clark RE, Lavitas P, Kunte P, Graham CS, O'Connell E, et al. Access to New Medications for Hepatitis C for Medicaid Members: A Retrospective Cohort Study. *Journal of managed care & specialty pharmacy.* 2016;22(6):714-22b.
22. Dorta-Guridi Z, Dueñas-Carrera S, Arús-Soler ER, Castellanos-Fernández MI, Cinza-Estévez Z. Vacuna terapéutica contra el virus de la hepatitis C en la era de los antivirales de acción directa. *Biotecnología Aplicada.* 2015;32(1):1121–4.
23. Golestan I, Hepatitis C. Virus and Vaccine Development. *Int J Mol Cell Med Autumn.* 2014;3(4):208.
24. Li D, Huang Z, Zhong J. Hepatitis C virus vaccine development: old challenges and new opportunities. *National Science Review.* 2015;2(3):285–95.
25. Chidi AP, Rogal S, Bryce CL, Fine MJ, Good CB, Myaskovsky L, et al. Cost-effectiveness of new antiviral regimens for treatment-naïve US veterans with hepatitis C. *Hepatology.* 2016;63(2):428–36.
26. Guidelines for good clinical practice (GCP) for trials on pharmaceutical products, the use of essential drugs. *Sixth report of the WHO Export Committee, WHO Technical Report Series.* Geneva, Switzerland: World Health Organization; 1995; 850.
27. Roshan B, Guzman G. Histological and clinical characteristics of patients with chronic hepatitis C and persistently normal alanine aminotransferase levels. *Hepatitis research and treatment.* 2014;2014:760943.
28. Nevens F, Roskams T, Van Vlierberghe H, Horsmans Y, Sprengers D, Elewaut A, et al. A pilot study of therapeutic vaccination with envelope protein E1 in 35 patients with chronic hepatitis C. *Hepatology.* 2003;38(5):1289–96.
29. Guidelines for assuring the quality and nonclinical safety evaluation of DNA vaccines. *Adopted by the 56th meeting of the WHO Expert Committee on Biological Standardisation.* Geneva, Switzerland: World Health Organization; 2005.
30. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, et al. Histological grading and staging of chronic hepatitis. *Journal of hepatology.* 1995;22(6):696–9.
31. Caneo C, González M, SOZA A. Trastornos psiquiátricos y cognitivos de la hepatitis C y su tratamiento con interferón. *Revista médica de Chile.* 2010;138(11):1431–40.
32. Ghany MG, Kleiner DE, Alter H, Doo E, Khokar F, Promrat K, et al. Progression of fibrosis in chronic hepatitis C. *Gastroenterology.* 2003;124(1):97–104.
33. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. *The Lancet.* 1997;349(9055):825–32.
34. Kass RE, Raftery AE. Bayes factors. *Journal of the American Statistical Association.* 1995;90(430):773–95.
35. Solbach P, Wedemeyer H. The New Era of Interferon-Free Treatment of Chronic Hepatitis C. *Viszeralmedizin.* 2015;31(4):290–6.
36. McHutchison JG, Lawitz EJ, Shiffman ML, Muir AJ, Galler GW, McCone J, et al. Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. *New England Journal of Medicine.* 2009;361(6):580–93.
37. Dore GJ, Conway B, Luo Y, Janczewska E, Knysz B, Liu Y, et al. Efficacy and safety of ombitasvir/paritaprevir/r and dasabuvir compared to IFN-containing regimens in genotype 1 HCV patients: The MALACHITE-I/II trials. *J Hepatol.* 2016;64(1):19–28.
38. Flisiak R, Pogorzelska J, Flisiak-Jackiewicz M. Hepatitis C: efficacy and safety in real life. *Liver Int.* 2017;1:26–32.
39. Ferguson MC, Bergman S, Santanello C. Interferons as Therapeutic Agents in Infectious Diseases. *Infect Dis Clin North Am.* 2011;25(4):819–34.
40. Furusyo N, Kubo N, Toyoda K, Takeoka H, Nabeshima S, Murata M, et al. Helper T cell cytokine response to ribavirin priming before combined treatment with interferon alpha and ribavirin for patients with chronic hepatitis C. *Antiviral research.* 2005;67(1):46–54.
41. Jain MK, Zoellner C. Role of ribavirin in HCV treatment response: now and in the future. *Expert opinion on pharmacotherapy.* 2010;11(4):673–83.
42. Rigopoulou EI, Abbott WG, Williams R, Naoumov NV. Direct evidence for immunomodulatory properties of ribavirin on T-cell reactivity to hepatitis C virus. *Antiviral Res.* 2007;75(1):36–42.
43. Amador-Cañizares Y, Martínez-Donato G, Álvarez-Lajonchere L, Vasallo C, Dausá M, Aguilar-Noriega D, et al. HCV-specific immune responses induced by CIGB-230 in combination with IFN-α plus ribavirin. *World Journal of Gastroenterology: WJG.* 2014;20(1):148.
44. Hauser G, Awad T, Brok J, Thorlund K, Štimac D, Mabrouk M, et al. Peginterferon plus ribavirin versus interferon plus ribavirin for chronic hepatitis C. *Cochrane Database Syst Rev.* 2014;(2):CD005441.
45. Castellanos M, Cinza Z, Dorta Z, Veliz G, Vega H, Lorenzo I, et al. Immunization with a DNA vaccine candidate in chronic hepatitis C patients is safe, well tolerated and does not impair immune response induction after anti-hepatitis B vaccination. *The journal of gene medicine.* 2010;12(1):107–16.
46. Nodarse-Cuní H, Arús-Soler E, Rivera-Reimón LL, Pérez-Lorenzo M, Samada-Suárez M, García-Ferrera WO, et al. Interferón alfa-2b y ribavirina como tratamiento combinado para la hepatitis C crónica en Cuba: Programa Nacional. *Biotecnología Aplicada.* 2012;29(3):184–8.
47. Tang L, Marcell L, Kottilil S. Systemic manifestations of hepatitis C infection. *Infectious agents and cancer.* 2016;11(1):1.
48. Shiffman M. Side effects of medical therapy for chronic hepatitis C. *Ann Hepatol.* 2004;3(1):5–10.
49. Raison CL, Demetrashvili M, Capuron L, Miller AH. Neuropsychiatric adverse effects of interferon-α. *CNS drugs.* 2005;19(2):105–23.
50. Dan AA, Martin LM, Crone C, Ong JP, Farmer DW, Wise T, et al. Depression, anemia and health-related quality of life in chronic hepatitis C. *Journal of hepatology.* 2006;44(3):491–8.
51. Kraus MR, Schäfer A, Faller H, Csef H, Scheurlen M. Psychiatric symptoms in patients with chronic hepatitis C receiving interferon alfa-2b therapy. *The Journal of clinical psychiatry.* 2003;64(6):708–14.
52. Kraus MR, Schäfer A, Schöttker K, Keicher C, Weissbrich B, Hofbauer I, et al. Therapy of interferon-induced depression in chronic hepatitis C with citalopram: a randomised, double-blind, placebo-controlled study. *Gut.* 2008;57(4):531–6.
53. Weiland O, Ahlén G, Diepolder H, Jung M-C, Levander S, Fons M, et al. Therapeutic DNA vaccination using in vivo electroporation followed by standard of care therapy in patients with genotype 1 chronic hepatitis C. *Molecular Therapy.* 2013;21(9):1796–805.
54. Wedemeyer H, Schuller E, Schlaphoff V, Stauber RE, Wiegand J, Schiefke I, et al. Therapeutic vaccine IC41 as late add-on to standard treatment in patients with chronic hepatitis C. *Vaccine.* 2009;27(37):5142–51.
55. Klade C, Firbas C, Bürger V, Zauner W, Lingnau K, Tauber E, et al. 588 Optimization of schedule, route and adjuvant of therapeutic peptide vaccines against HCV. *Journal of hepatology.* 2006;44:S218.

56. Firbas C, Jilma B, Tauber E, Buerger V, Jelovcan S, Lingnau K, et al. Immunogenicity and safety of a novel therapeutic hepatitis C virus (HCV) peptide vaccine: a randomized, placebo controlled trial for dose optimization in 128 healthy subjects. *Vaccine.* 2006;24(20):4343–53.
57. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *The Lancet.* 2001;358(9286):958–65.
58. Fried MW. Side effects of therapy of hepatitis C and their management. *Hepatology.* 2002;36(S1):S237–44.
59. Druyts E, Thorlund K, Wu P, Kanters S, Yaya S, Cooper CL, et al. Efficacy and safety of pegylated interferon alfa-2a or alfa-2b plus ribavirin for the treatment of chronic hepatitis C in children and adolescents: a systematic review and meta-analysis. *Clinical infectious diseases.* 2013;56(7):961–7.
60. Jadoon SA, Ahmed A, Ahmad N. EFFECT OF STANDARD INTERFERON AND RIBAVIRIN ON LEUKOCYTE COUNT. *Journal of Ayub Medical College Abbottabad.* 2015;27(2):364–6.
61. Peck–Radosavljevic M, Wichlas M, Homoncik–Kraml M, Kreil A, Hofer H, Jessner W, et al. Rapid suppression of hematopoiesis by standard or pegylated interferon-α. *Gastroenterology.* 2002;123(1):141–51.
62. Sulkowski M, Wasserman R, Brooks L, Ball L, Gish R. Changes in haemoglobin during interferon alpha-2b plus ribavirin combination therapy for chronic hepatitis C virus infection. *Journal of Viral Hepatitis.* 2004;11(3):243–50.
63. Wu L, Jimmerson L, MacBrayne C, Kiser J, D'Argenio D. Modeling Ribavirin-Induced Anemia in Patients with Chronic Hepatitis C Virus. *CPT: pharmacometrics & systems pharmacology.* 2016;5(2):65–73.
64. Hernández A, Domper F, León A, Lorente R, López B, De la Santa E, et al. Viral kinetics during the first month of treatment in patients with genotype l chronic hepatitis C. *Revista Española de Enfermedades Digestivas.* 2009;25 (10):671.
65. Batdelger D, Dandii D, Jirathitikal V, Bourinbaiar AS. Open-label trial of therapeutic immunization with oral V-5 Immunitor (V5) vaccine in patients with chronic hepatitis C. *Vaccine.* 2008;26(22):2733–7.
66. Ghany MG, Nelson DR, Strader DB, Thomas DL, Seeff LB. An update on treatment of genotype 1 chronic hepatitis C virus infection: 2011 practice guideline by the American Association for the Study of Liver Diseases. *Hepatology.* 2011;54(4):1433–44.
67. Villela-Nogueira CA, Perez RM, de Segadas Soares JA, Coelho HSM. Gamma-glutamyl transferase (GGT) as an independent predictive factor of sustained virologic response in patients with hepatitis C treated with interferon-alpha and ribavirin. *Journal of clinical gastroenterology.* 2005;39(8):728–30.
68. Rauch A, Gaudieri S, Thio C, Bochud P-Y. Host genetic determinants of spontaneous hepatitis C clearance. *Pharmacogenomics.* 2009;10(11):1819–37.
69. Napoli N, Giannelli G, Parisi CV, Antonaci A, Maddalena G, Antonaci S. Predictive value of early virological response to treatment with different interferon-based regimens plus ribavirin in patients with chronic hepatitis C. *The new microbiologica.* 2005;28(1):13–21.
70. Reau N, Hamzeh F, Lentz E, Zhou X, Jensen D. Characterization of nonrapid virologic response patients infected with HCV genotype 1 who may relapse after standard therapy with peginterferon plus ribavirin. *Journal of Viral Hepatitis.* 2012;19(2):94–102.
71. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçales FL Jr, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *New England Journal of Medicine.* 2002;347(13):975–82.
72. Rodriguez-Torres M, Sulkowski M, Chung R, Hamzeh F, Jensen D. Factors associated with rapid and early virologic response to peginterferon alfa-2a/ribavirin treatment in HCV genotype 1 patients representative of the general chronic hepatitis C population. *Journal of Viral Hepatitis.* 2010;17(2):139–47.
73. Wedemeyer H, Mazur W, Nevens F, Horsmans Y, Adler M, Blum H, et al. 61 FACTORS INFLUENCING PROGRESSION OF LIVER FIBROSIS IN PATIENTS WITH CHRONIC HEPATITIS C: RESULTS OF THE 3-YEAR T2S-918-HCV STUDY WITH HCVE1 THERAPEUTIC VACCINATION. *Journal of hepatology.* 2008;48:S27–8.
74. Wedemeyer H, Janczewska E, Wlodzimierz M, Stanciu C, Habersetzer F, Carreno V, et al. 1403 SIGNIFICANT IMPROVEMENT OF COMPLETE EVR IN HCVAC PHASE II CLINICAL TRIAL WHEN ADDING TG4040 THERAPEUTIC VACCINE TO PEGIF-Nα2A AND RIBAVIRIN. *Journal of hepatology.* 2012;56:S552.
75. Poordad F, Reddy KR, Martin P. Rapid virologic response: a new milestone in the management of chronic hepatitis C. *Clinical infectious diseases.* 2008;46 (1):78–84.
76. Jensen DM, Morgan TR, Marcellin P, Pockros PJ, Reddy KR, Hadziyannis SJ, et al. Early identification of HCV genotype 1 patients responding to 24 weeks peginterferon α-2a (40 kd)/ribavirin therapy. *Hepatology.* 2006;43(5):954–60.
77. Zeuzem S, Buti M, Ferenci P, Sperl J, Horsmans Y, Cianciara J, et al. Efficacy of 24 weeks treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C infected with genotype 1 and low pretreatment viremia. *Journal of hepatology.* 2006;44(1):97–103.
78. Hudacko R, Theise N. Liver biopsies in chronic viral hepatitis: beyond grading and staging. *Archives of pathology & laboratory medicine.* 2011;135(10): 1320–8.
79. Reiss G, Keeffe E. Role of liver biopsy in the management of chronic liver disease: selective rather than routine. *Reviews in gastroenterological disorders.* 2004;5(4):195–205.
80. Pockros P, Jacobson I, Boyer TD, Schiff ER, Everson GT, Lee WM, et al. GI-5005 therapeutic vaccine plus pegIFN/ribavirin improves sustained virologic response versus pegIFN/ribavirin in prior non-responders with genotype 1 chronic HCV infection. *Hepatology.* 2010;52:107A.