Viusid, a Nutritional Supplement, Increases Survival and Reduces Disease Progression in HCV-Related Decompensated Cirrhosis. A Randomized and Controlled Trial.

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Author names:

Eduardo Vilar Gomez

Email: vilar@infomed.sld.cu

Director of Researches. National Institute of Gastroenterology, Havana, Cuba.

Yoan Sanchez Rodriguez

Email: yoan.sanchez@infomed.sld.cu

Head of the Department of Hepatology. National Institute of Gastroenterology, Havana, Cuba.

Ana Torres Gonzalez

Email: anylut@infomed.sld.cu

Department of Hepatology. National Institute of Gastroenterology, Havana, Cuba.

Luis Calzadilla Bertot

Email: lcbertot@infomed.sld.cu

Department of Hepatology. National Institute of Gastroenterology, Havana, Cuba.

Enrique Arus Soler

Email: anayda@infomed.sld.cu

General Director. National Institute of Gastroenterology, Havana, Cuba.

Yadina Martinez Perez

Email: yadinamartinez@infomed.sld.cu

Department of Gastroenterology. National Institute of Gastroenterology, Havana, Cuba.

Ali Yasells Garcia

Email: yasellsg@gmail.com

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Department of Gastroenterology. National Institute of Gastroenterology

Havana, Cuba

Maria del Rosario Abreu Vazquez

Email: charo@infomed.sld.cu

Department of Biostatistics. National Institute of Gastroenterology, Havana, Cuba

Corresponding author:

EDUARDO VILAR GOMEZ.

National Institute of Gastroenterology, Havana, Cuba.

Mailing address: 25th Avenue, 503, Vedado, Havana, Cuba

E-mail address: vilar@infomed.sld.cu

Phone number: 53 7 8325067

Fax number: 53 7 8333253

Abbreviations: HCV, hepatitis C virus; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HR, hazard ratio; CI, confidence interval; OS, overall survival; HCC, hepatocellular carcinoma; MELD, model end-stage liver disease; CHC, chronic hepatitis C; OLT, orthotopic liver transplantation; SOC, standard of care; PEG-IFN, peginterferon; RBV, ribavirin; SVR, sustained virological response; IL, interleukin; TNF, tumor necrosis factor; IFN-γ, interferon-gamma; HIV, human immunodeficiency virus; PCR, polymerase chain reaction; SD, standard deviation; ITT, intention-to-treat analysis; SBP, spontaneous bacterial peritonitis; MDA, malondialdehyde; HALT-C, the hepatitis C antiviral long-term treatment against cirrhosis; CO-PILOT, colchicine versus pegintron long-term therapy; EPIC, evaluation of pegintron in control of hepatitis C cirrhosis.

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Abstract

Objectives: Viusid is a nutritional supplement with recognized antioxidant and immunomodulatory properties which could have beneficial effects on cirrhosis-related clinical outcomes such as survival, disease progression and development of hepatocellular carcinoma. Our study evaluated the efficacy and safety of viusid in patients with HCV-related decompensated cirrhosis. **Design:** A randomized double-blind and placebo-controlled study was conducted in a tertiary care academic center (National Institute of Gastroenterology, Havana, Cuba). We randomly assigned 100 patients with HCV-related decompensated cirrhosis to receive viusid (3 oral sachets daily, n=50) or placebo (n=50) during 96 weeks. Primary and secondary end points were comparisons of overall survival (OS), time to disease progression and time to hepatocellular carcinoma (HCC) diagnosis between groups in the intention-to-treat population. **Results:** Viusid led to a significant improvement in overall survival (90%) versus placebo (74%) (hazard ratio [HR] 0.27, 95% CI: 0.08-0.92; *P*=0.036). A similar improvement in disease progression was seen in viusid-treated patients (28%), compared to placebo-treated patients (48%) (HR 0.47, 95% CI: 0.22-0.89; *P*=0.044). The cumulative incidence of HCC was significantly reduced in patients treated with viusid (2%) as compared to placebo (12%) (HR 0.15, 95% CI: 0.019-0.90; *P*=0.046). Viusid was well tolerated. **Conclusions:** Our results indicate that treatment with viusid leads to a notable improvement in overall clinical outcomes such as survival, disease progression and development of HCC in patients with HCV-related decompensated cirrhosis. The trial had been registered at ClinicalTrials.gov (NCT00502086).

Key words: chronic hepatitis C, cirrhosis of the liver, survival.

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Article summary

- HCV-related decompensated cirrhotic patients have a poor therapeutic response and reduced tolerance to the current standard of care (SOC) therapy.
- Therapeutic goals in these patients should be directed towards reducing liver-related morbidity and mortality, and the need for liver transplantation.
- Viusid is a nutritional supplement with recognized antioxidant and immunomodulatory properties that could modulate the histological pattern of CHC, especially inflammation and fibrosis in an attempt to halt disease progression and consequently improve liver function and liver-related morbidity and mortality, and prevent development of HCC.

Key messages

- The administration of viusid to HCV-related decompensated cirrhotic patients induced a significant improvement of overall survival, a significant reduction in the disease progression and development of hepatocellular carcinoma.
- The benefit of Viusid was also seen in the secondary end point of worsening of the prognostic scores such as MELD and CP scores.
- Viusid was well tolerated and only minor transient adverse events such as nausea and diarrhea were reported.

Strengths and limitations of this study

- The main strength of this study was to demonstrate that Viusid improves overall clinical outcomes (survival, HCC, and disease progression) in cirrhotic patients who have failed to achieve SVR with SOC, and these benefits appear to be not associated to viral suppression rates.
- The study was designed with a small sample size.

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- Further multicentre and large-scale studies are needed to corroborate the impact of Viusid on the clinical outcomes in patients with HCV-related decompensated cirrhosis.

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Specific author contributions:

The authors were collectively responsible for the study design, data collection, statistical analysis, and interpretation of data; the writing of the manuscript; and the decision to submit the manuscript for publication.

Eduardo Vilar Gomez was involved in the study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis; and obtained funding. He has approved the final draft submitted.

Yoan Sanchez Rodriguez was involved in the study concept and design; acquisition of data; analysis and interpretation of data; critical revision of the manuscript for important intellectual content; and drafting of the manuscript. He has approved the final draft submitted.

Ana Torres Gonzalez was involved in the study concept and design; acquisition of data; analysis and interpretation of data; and drafting of the manuscript. She has approved the final draft submitted.

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Luis Calzadilla Bertot was involved in the study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; and critical revision of the manuscript for important intellectual content. He has approved the final draft submitted.

Enrique Arus Soler was involved in the study concept and design; critical revision of the manuscript for important intellectual content; and drafting of the manuscript. He has approved the final draft submitted.

Yadina Martinez Perez was involved in the study concept and design; acquisition of data; analysis and interpretation of data; and drafting of the manuscript. She has approved the final draft submitted.

Ali Yasells Garcia was involved in the study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; and critical revision of the manuscript for important intellectual content. He has approved the final draft submitted.

Maria del Rosario Abreu Vazquez was involved in the study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; and statistical analysis. She has approved the final draft submitted.

Introduction

Chronic hepatitis C virus (HCV) infection is a leading cause of end-stage liver disease and hepatocellular carcinoma (HCC) worldwide [1], and the most common indication for orthotopic liver transplantation (OLT) in the western world [2]. Once HCV cirrhosis has developed, the risk of clinical decompensation is about 5% per year [3-5], and the risk of mortality is considerably high with a survival rate of 50% at 5 years [6-7]. Cumulative data of patients with compensated cirrhosis indicate that the 5-year risk of decompensation is

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estimated to be 15-28%, and the annual risk of developing HCC is 1.4-6.7% [3-4 8]. Liver-related mortality increases considerably as soon as decompensation is established, and then liver transplantation is the only successful therapeutic option. However, the limited number of liver donors as well as age-related impairment of cardiovascular, renal, and pulmonary functions makes this option unavailable to the greater part of patients. Unfortunately, once the liver is grafted, disease recurrence is universal. The recurrence of the infection leads to cirrhosis in approximately 25% of the transplant recipients within 5 – 10 years after transplantation. The cumulative probability of decompensation 1 year after cirrhosis is in the order of 30%, and 1-year survival is 46% [9].

HCV-related cirrhotic patients have a poor therapeutic response and reduced tolerance to the current standard of care (SOC) therapy [10-15] . Peginterferon (PEG-IFN) plus ribavirin (RBV) is the recommended treatment strategy for patients with compensated cirrhosis [16]. However, the efficacy of antiviral therapy is in this group significantly lower than in noncirrhotic patients, achieving the poorest rates of sustained virological response (SVR, 5-25%) in patients with genotype 1-4[17]. Current evidence indicates that antiviral treatment with PEG-IFN alone or in combination with RBV reduces the rate of clinical decompensation, improves liver-related survival, and decreases the development of HCC, but only in those patients who achieved SVR [18-20]. However, this benefit should be balanced with severe side effects that led to therapy discontinuation and derangement of liver function in a high proportion of patients with Child-Pugh class B-C cirrhosis.

Thus, an effective treatment is needed immediately in cirrhotic patients who have failed to achieve SVR or with advanced disease to avoid further deterioration and death. Therefore, therapeutic goals in these patients should be directed towards reducing liver-related morbidity and mortality, and the need for liver transplantation.

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Several studies have demonstrated an important association between increased levels of products related to oxidative stress and advanced stages of the disease [21-22]. Likewise, cytokine dysregulation is thought to play a crucial role in the persistence of viral infection and as a key mediator in inflammatory and fibrogenic processes in patients with HCV infection [23].

Therefore, the administration of compounds with antioxidant and immunomodulatory properties could be a plausible strategy to halt the natural course of the disease, particularly in cirrhotic patients with non-response to SOC or advanced disease.

Viusid (Catalyis laboratory, Madrid, Spain) is a nutritional supplement that contains different molecules (ascorbic acid, zinc, and glycyrrhizic acid) with recognized antioxidant and immunomodulatory properties (Table 1) [24-26].

Encouraging effects of Viusid on liver histology have been reported in patients with chronic hepatitis C and nonalcoholic fatty liver disease [27-28].

Recent data suggest that Viusid improves oxidative stress through reduction of lipid peroxidation products and has an immunomodulatory effect on cytokine secretion via increased production of IFN-γ and IL-10, decreased production of IL-1α, and stabilized TNF-α secretion in patients with HCV who have failed previous antiviral treatment [29].

All of these effects could modulate the histological pattern of CHC, especially inflammation and fibrosis in an attempt to halt disease progression and consequently improve liver function and liver-related morbidity and mortality, and prevent development of HCC.

Thus, a randomized double-blind and placebo-controlled study was conducted to evaluate whether Viusid may have a beneficial effect on survival, time to disease progression and time to diagnosis of HCC in HCV-related cirrhotic patients with decompensated disease.

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Materials and Methods

Participants

We recruited 100 patients with HCV liver-related cirrhosis at a tertiary care academic center (National Institute of Gastroenterology, Havana, Cuba) between May 2005 and June 2007 and who fulfilled the following inclusion criteria: male and female patients of 18 to 70 years of age, clinical or histological diagnosis of cirrhosis, naïve patients or non-responders to previous treatment with PEG-IFN plus RBV with decompensated cirrhosis, defined as a Child-Pugh score ≥ 7 or clinical evidence or history of ascites, encephalopathy, upper gastrointestinal bleeding, and/or impaired hepatic synthetic function, who had contraindicated the antiviral treatment, absence of active alcoholism (alcohol abstinence was monitored at each clinic visit in the course of patient interview), and ability to provide informed consent. Patients were excluded if they had presence of other causes of liver disease, uncontrollable clinical or biochemical complications related to severe liver failure (hepatic encephalopathy, hepatorenal syndrome, gastrointestinal bleeding, serum total bilirubin greater than 85 mmol/L (5 mg/dL), international normalized ratio greater than 2.5), serum creatinine greater than 180 mmol/L (2 mg/dL), positive screening for viral hepatitis A and B and HIV, pregnancy or lactation, concomitant disease with reduced life expectancy, severe psychiatric conditions, drug dependence, and evidence of liver cancer at entry into the study on the basis of ultrasonography and α-fetoprotein levels higher than 200 ng/L.

Ethics

The study was conducted in compliance with the Declaration of Helsinki and approved by the ethics committee and the institutional review board of the National Institute of Gastroenterology. All patients provided written informed consent for participation.

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Interventions

After initial evaluation, all patients who met the eligibility criteria were consecutively enrolled in the study. They were randomly assigned to receive: Viusid (3 oral sachets daily, n=50) or placebo (3 oral sachets daily, n=50) for 96 weeks.

Randomization was conducted by blocks of 4 (block randomization). It was performed by a health worker experienced in randomization techniques who was not involved in the evaluation or treatment of the participants. The physicians, study coordinators, and patients did not have access to the randomization scheme.

The researchers, study coordinators, and patients were blinded as to the treatment administered. When the patients were allocated, they brought their entry code to the pharmacy which was provided with the randomized list. The code was revealed to the researchers at the end of the study protocol. Catalysis, Spain provided the Viusid and placebo sachets. There was no difference in appearance, smell, and flavor between Viusid and placebo.

Treatment started 4 weeks after the clinical evidence of decompensation had been treated and controlled with appropriate therapy.

Clinical and laboratory assessment

All patients were closely monitored for clinical, biochemical, and hematological assessment at baseline, weekly for the first eight weeks, and every eight weeks thereafter until the end of the study.

Clinical assessment included physical examination along with compliance to the study medication (verified through sachet count). Biochemical and hematological evaluations included complete blood count, liver tests, glucose, coagulation, and renal function tests.

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We defined overweight as BMI 25 to 30 kg/m^2 and defined obesity as BMI > 30 kg/m^2.

Patient's data with diagnosis of diabetes mellitus at baseline, elevated fasting glucose levels (> 6.1 mmol/L), a positive glucose tolerance test and used antidiabetic medication were recorded.

Liver ultrasonography and serum α-fetoprotein determinations were carried out at baseline and every 24 weeks during the study to screen for hepatocellular carcinoma.

An upper digestive endoscopy was performed before admission.

The HCV-RNA level was quantified by PCR assay (Amplicor Monitor HCV v.2.0; Roche Molecular System; lower limit of detection, 600 IU/ml). HCV genotyping was performed by reverse hybridization (Inno-LiPA HCV; Innogenetics, Ghent, Belgium).

Definition of Outcomes

The primary outcome of the study was overall survival (OS) which was measured from the date of randomization until the date of death (related to liver disease). Patients with liver-unrelated death or lost to follow-up were censored at the time of death or discontinuation, and patients undergoing liver transplantation were censored at the transplant date.

Secondary outcomes included the time to disease progression, time to diagnosis of HCC, time to worsening of the prognostic scoring systems Child-Pugh and MELD, time to a new occurrence or relapse for each one of the main clinical complications secondary to portal hypertension, and safety.

The time to disease progression was reflected as the time between random assignment and disease progression, defined as the incidence of liver-related death, the development of hepatocellular carcinoma, or the first occurrence or relapse (only for those patients with a previous history of clinical decompensation) of at least one of the following clinical

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conditions: ascites, hepatic encephalopathy, spontaneous bacterial peritonitis, hepatorenal syndrome, or upper gastrointestinal bleeding secondary to portal hypertension.

The time to diagnosis of hepatocellular carcinoma was calculated from the date of randomization to the date of occurrence of HCC. Diagnosis of HCC was implemented using currently accepted diagnostic criteria for HCC [30-32].

The time to worsening of the prognostic scores was defined as the time from randomization to worsening of the Child-Pugh score in at least 2 points and the MELD score in at least 4 points on the basis of independent clinical evaluation on two consecutive study visits. The Child-Pugh and MELD scores are measures of the severity of liver disease, with higher numbers indicating greater decompensation.

The time to a new occurrence or relapse for each one of the main clinical complications secondary to portal hypertension was defined from the date of randomization to the date of a new occurrence or relapse (only for those patients with a previous history of clinical complications) of the following clinical conditions: ascites, hepatic encephalopathy, upper gastrointestinal bleeding, hepatorenal syndrome, and spontaneous bacterial peritonitis.

The evidence for each end point was verified and confirmed by two blinded independent hepatologists.

Safety was assessed by dynamic reports of adverse events (AEs), clinical laboratory test (hematological and biochemical analysis), physical examination, and measurement of vital sign. The presence of sepsis and hospitalization were included in the safety reports. Episodes of sepsis were recorded, and they were diagnosed and treated according to recommended guidelines. Sepsis was graded as severe if requiring hospitalization or treatment discontinuation.

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Statistical methods

The baseline characteristics were summarized in percentage for categorical variables and as means ±SD for continuous variables. The chi-square test was applied to categorical variables. The two-sample *t*-test was used to compare means, and the Mann-Whitney *U*-test if they were not normally distributed. Outcome measurements included all patients who were randomized and received at least one dose of study medication (intention-to-treat analysis). The safety analysis included all treated patients who had at least one safety evaluation after baseline.

Both primary and secondary outcomes were analyzed by the Kaplan-Meier method and differences were compared using Cox proportional hazard models adjusted by sex and age, baseline CP and MELD scores, previous history of clinical decompensation, and current use of diuretics and propranolol.

We defined overall survival time at 96 weeks as a primary end point to compute sample size. The study was designed to have a statistical power of 80% to detect an absolute difference of 25% in the survival rates at 96 weeks (95% in the group with Viusid versus 70% in the control group). Considering a type I error of 0.05 and a type II error of 0.20, 43 patients per arm were needed to reach statistical significance. After considering patient loss as a result of dropout, we set the target number of patients at 50 per arm, or 100 in total.

All confidence intervals, significance tests, and resulting *P* values were two-sided, with an alpha level of 0.05.

Statistical analyses were performed using STATA software, release 11.

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The study was designed by Catalysis Laboratory in conjunction with the principal investigator. The data were collected and analyzed by the investigators. All authors had access to the data.

Results

Patients

Between May 2005 and June 2007, 124 patients were screened. 100 of these patients met the eligibility criteria and were randomly assigned to the Viusid (n=50) and the placebo arms (n=50). These patients were all included in the intention-to-treat analysis. 24 patients were excluded from the study during the screening period because they did not meet the inclusion criteria, met one or more of the exclusion criteria, or withdrew their consent. The flow of the participants through the trial is presented in Figure 1. None of the patients received co-interventions during the trial that could have affected the outcomes. One death secondary to myocardial infarction occurred in each group of treatment during the study. Four of the 7 patients with HCC were not discontinued and completed the study because diagnosis was made only at the end of the treatment.

Demographic and baseline disease characteristics of the ITT population were generally well balanced between treatment arms (Table 2). The patients' mean age was 57.5 years and 60% were women. All patients had genotype 1 infection. The mean Child-Pugh and MELD scores at baseline were 6.32 and 12.94, respectively.

All patients with a previous history of hepatic decompensation were controlled and treated with appropriate therapy before trial admission.

At study entry, none of the patients had evidence of hepatocellular carcinoma, ascites, hepatic encephalopathy, renal failure, upper gastrointestinal bleeding, or spontaneous bacterial peritonitis.

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Efficacy (primary end point)

Overall survival at 96 weeks was significantly higher in the patients assigned to Viusid (90% with a 95% CI, 75 to 95) as compared to the patients assigned to placebo (74% with a 95% CI, 56 to 83) (HR = 0.27; 95% CI, 0.08 to 0.92; *P*=0.036; Figure 2A).

Efficacy (secondary end points)

The Kaplan-Meier estimates of the proportion of patients with disease progression at 96 weeks (Figure 2B) were 28% (95% CI, 19 to 45) in the experimental group and 48% (95% CI, 38 to 66) in the control group. The hazard ratio for the Viusid arm was 0.47 (95% CI, 0.22 to 0.89; *P*=0.044).

The cumulative incidence of hepatocellular carcinoma at 96 weeks was 2% (95% CI, 0.3 to 15) in the Viusid-treated patients and 12% (95% CI, 6 to 33) in the placebo group, with a hazard ratio for the Viusid group of 0.15 (95% CI, 0.019 to 0.90; *P*=0.046) (Figure 2C). All patients with HCC were diagnosed during the second year after randomization. 2 of the 7 patients with HCC were eligible for liver transplantation and 3 had transarterial chemoembolization.

An increase in the Child-Pugh score (Figure 3A) occurred in 7 patients (14%; 95% CI, 8 to 32) allocated to the Viusid group as compared to 19 patients (38%; 95% CI, 30 to 59) allocated to the placebo group. The hazard ratio for the Viusid arm was 0.34 (95% CI, 0.14 to 0.81; *P*=0.015). Likewise, a significant worsening in the MELD score (Figure 3B) was observed in 15 individuals (30%; 95% CI, 21 to 49) assigned to placebo as compared to 6 individuals (12%; 95% CI, 6 to 26) assigned to Viusid, with a hazard ratio for the Viusid group of 0.39 (95% CI, 0.15 to 0.92, *P*=0.042).

The cumulative incidence of ascites at 96 weeks was significantly higher in the patients assigned to placebo (32%; 95% CI, 14 to 39) than in the patients assigned to Viusid (14%,

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95% CI, 7 to 28). The hazard ratio for the Viusid arm was 0.32 (95% CI, 0.11 to 0.90; *P*=0.031), but the differences were not statistically significant for hepatic encephalopathy, upper gastrointestinal bleeding, and spontaneous bacterial peritonitis. Type 2 hepatorenal syndrome was reported in one patient of each group of treatment. The primary and secondary outcome measures are summarized in Table 3.

Safety

Cramps (33%), asthenia (32%), sepsis (27%), predominantly bacterial infections, and muscle pain (24%) were the most frequent adverse events. The main causes of sepsis were urinary infection (11%), SBP (6%), pneumonia (5%), and lymphangitis (3%). None of the patients had infections related to leukopenia or neutropenia.

A lesser proportion of patients treated with Viusid than treated with placebo had fatigue (Viusid, 10%; placebo, 26%; *P*=0.04), cramps (Viusid, 22%; placebo, 44%; *P*=0.02), and sepsis (Viusid, 14%; placebo, 40%; *P*<0.01), respectively.

A high percentage of patients (24%) were hospitalized during the study secondary to episodes of hepatic decompensation or severe sepsis; however, there was no difference between the treatment groups. A summary of adverse events is given in Table 4. There were no significant laboratory abnormalities in the two study groups.

Neither was there any incidence of Viusid discontinuation or dose modification secondary to adverse events.

Discussion

HCV-related cirrhotic patients represent an important population with increased morbidity and mortality rates. Unfortunately, current antiviral therapy, especially for patients with decompensated disease, is generally limited by side effects and early discontinuation is common. Therefore, liver transplantation is the most appropriate therapeutic option for

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these patients. Recent studies have demonstrated encouraging SVR rates and, consequently, clinical outcome improvements (overall survival, HCC, and hepatic decompensation) in decompensated cirrhotic patients, but this was only achieved in a minority of patients (SVR, 5-25%) infected with genotype 1-4 [17]. Therefore, there is a critical need to explore new therapeutic options for patients with HCV-related end-stage liver disease who are never listed for liver transplant and could receive a beneficial impact on their clinical outcomes.

The study was designed to evaluate the efficacy and safety of Viusid in a particular population of elderly cirrhotic patients who had a previous history or current evidence of clinical hepatic decompensation and genotype 1 infection, and therefore the poorest chance of achieving SVR and elevated probabilities of adverse clinical outcome in their next years of follow-up.

In the current study, we demonstrated that administration of viusid to HCV-related decompensated cirrhotic patients induced a significant improvement of overall survival, as compared to placebo. Similarly, a significant reduction in the disease progression, defined as the presence liver-related death, the development of hepatocellular carcinoma or a first occurrence or relapse of at least one of the main portal hypertension-related clinical complications, was observed in patients treated with viusid in comparison to those patients treated with placebo. Interestingly, the cumulative incidence of HCC was notably reduced in those patients assigned to viusid arm, compared to placebo arm.

In the present study, we found increased rates of mortality, disease progression and cumulative incidence of HCC in the placebo group than previously reported rates in a large, prospective and multicenter trial [33]. The most likely explanation for the disparity between these rates appears to be related to the difference in the study design. Our study

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was designed to include a large proportion of patients who had a previous history or current evidence of clinical hepatic decompensation (poor hepatic reserve), subjects who were excluded from the HALT-C Trial [33]. A recent controlled study has validated the efficacy and safety of IFN-based therapy for HCV-related decompensated cirrhotic patients [14]. One of the main advantages of the study was to include a group of untreated patients (controls) with decompensated events who were enrolled to define survival and progression disease during 30 months of follow-up. The results obtained in this study show that this group of patients have a poor chance to survive (68%) and increased rates of hepatic decompensation (88%) and HCC (10%). These results suggest that natural history of HCV-related cirrhotic patients is more accelerated in patients with previous history or current evidence of clinical hepatic decompensation.

Data from the HALT-C study show an increased annual risk of HCC in patients with a low platelet count and the presence of esophageal varices. It could be another reasonable theory to explain the increased risk of HCC in our study. In the current study, an elevated percentage of patients (~50%) had evidence of esophageal varices and/or thrombocytopenia (<100 x 10^3/μL) [34].

Finally, an increased prevalence of diabetes was reported in our study (42% in placebo group and 34% in viusid group), which has been associated with development of HCC and accelerated disease progression [35].

In the current study, the rate of new occurrence or relapse of overall clinical outcome secondary to portal hypertension was statistically reduced in the patients assigned to Viusid in comparison to those allocated to placebo. The cumulative incidence of ascites was the only remarkable clinical condition reduced in the patients treated with Viusid as compared to placebo. In contrast, no differences were observed between the treatment

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groups for hepatic encephalopathy, spontaneous bacterial peritonitis, hepatorenal syndrome, and upper gastrointestinal bleeding.

The benefit of Viusid was also seen in the secondary end point of worsening of the prognostic scores. A significant increase in the Child-Pugh and MELD scores was observed in the placebo-treated patients compared to the experimental group.

During the Viusid therapy, the risk of bacterial infections decreased independently from neutropenia, which could suggest an improvement in the qualitative neutrophil function, but this effect should be additionally studied.

Viusid was well tolerated and only minor transient adverse events such as nausea and diarrhea were reported.

The mechanisms responsible for the beneficial effects of Viusid on the clinical outcomes such as survival, development of HCC, and disease progression have not yet been fully studied. However, there are several reasons to understand why its administration might improve overall clinical end points.

A recent trial has suggested that Viusid therapy combined with standard of care (SOC) in patients with chronic hepatitis C may reduce inflammation and fibrosis, irrespective of virological response [28]. Another recently published study has reported a dual role to explain possible mechanisms of action of Viusid on liver histology [29]. The authors found that MDA and 4-hydroxyalkenal levels were significantly reduced in patients treated with Viusid, indicating an important effect on lipid peroxidation products. Furthermore, Viusid provided immunomodulatory effects on cytokine secretion via increased production of anti-inflammatory cytokines (IL-10) and decreased or stabilized production of pro-inflammatory cytokines (IL-1α and TNF-α). Current studies are focusing on the biological effects of Viusid on hepatic stellate cell apoptosis as a critical step to clarify the potential mechanism

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of Viusid in liver fibrogenesis. On the other hand, it would be important to evaluate whether the Viusid effects on the clinical outcomes are directly related to the significant reduction of portal pressure in cirrhotic patients. Further studies should be addressed to answer this concern.

Recently, the HALT-C study was designed to determine whether low-dose peginterferon alpha 2a maintenance therapy over 3.5 years could reduce hepatic decompensation, HCC, and mortality in patients with advanced fibrosis or cirrhosis who failed to achieve SVR with SOC [33]. Unfortunately, no overall reduction in any of these clinical end points was achieved. Like the HALT-C study, 2 other studies (COPILOT and EPIC) failed to demonstrate overall benefit on clinical outcomes in HCV-related cirrhotic patients [36-37]. A recent analysis of the HALT-C trial has demonstrated that benefits on clinical outcomes could only be reached in patients with profound viral suppression obtained with full-dose peginterferon and ribavirin [19].

The main strength of this study was to demonstrate that Viusid improves overall clinical outcomes (survival, HCC, and disease progression) in cirrhotic patients who have failed to achieve SVR with SOC, and these benefits appear to be not associated to viral suppression rates [28].

Our study was designed with a small sample size. Therefore, further multicentre and large-scale studies are needed to corroborate the impact of Viusid on the clinical outcomes in patients with HCV-related decompensated cirrhosis.

In conclusion, the study supports the use of Viusid in patients with HCV-related decompensated cirrhosis who have failed to achieve SVR, with full-dose peginterferon and ribavirin in an attempt to prevent disease progression and improve overall survival.

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However, additional studies are required to confirm the long-term effect of Viusid in these patients.

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Table 1. Ingredients of Viusid

Malic acid	0.666 g	Ascorbic acid	0.020 g
Glycyrrhizic acid	0.033 g	Folic acid	66 mcg
Glucosamine	0.666 g	Cyanocobalamine	0.3 mcg
Arginine	0.666 g	Zinc sulfate	0.005 g
Glycine	0.333 g	Pyridoxal	0.6 mg
Calcium pantothenate	0.002 g		

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Table 2. Baseline characteristics.

Variable	Viusid (n=50)	Placebo (n=50)	P value*
Age (y)	58.5±8.9	56.6±8.4	0.29
Sex, n (%)			
Male	22 (44%)	18 (36%)	0.41
Female	28 (56%)	32 (64%)	
BMI (kg/m^2)	25.4±4.6	26.7±4.5	0.16
BMI > 25 (kg/m^2), n (%)	28 (56%)	31 (62%)	0.54
HCV RNA >600,000 IU/ml	42 (84%)	38 (76%)	0.45
Genotype 1, n (%)	50 (100%)	50 (100%)	1.0
Clinical scores			
Child-Pugh Class A	32 (64%)	29 (58%)	
Child-Pugh Class B	15 (30%)	15 (30%)	0.56
Child-Pugh Class C	3 (6%)	6 (12%)	
MELD	12.5±3.7	13.3±4.7	0.46
History of diabetes or fasting glucose ≥ 7 (mmol/L), n (%)	17 (34%)	21 (42%)	0.41
Previous history of clinical decompensation, n (%)†			
Ascites	22 (44%)	14 (32%)	0.10
Upper gastrointestinal bleeding	9 (18%)	5 (10%)	0.25
Spontaneous bacterial peritonitis	2 (4%)	2 (4%)	1.00
Hepatic encephalopathy	4 (8%)	3 (6%)	0.69
Evidence of esophageal varices	23 (46%)	18 (36%)	0.31

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Current propranolol use, n (%)	13 (26%)	10 (20%)	0.65
Average doses	70±17.7	80±37.7	0.37
Current spironolactone use, n (%)	21 (42%)	12 (24%)	0.09
Average doses	84.5±39.1	111±40	0.14
Current furosemide use, n (%)	4 (8%)	5 (10%)	1.00
Average doses	40±10	64±22	0.19
ALT (U/L)	92.2±76.6	82.7±49.5	0.86
AST (U/L)	105±80.2	94.1±56.6	0.72
Fasting plasma glucose (mmol/L)	4.9±1.2	5.1±1.3	0.70
Alkaline phosphatase (mmol/L)	290.4±108	281±78.8	0.96
Creatinine (mmol/L)	1±0.3	1±0.3	0.88
Hemoglobin (g/L)	125.8±13.8	129.5±17.6	0.32
Cholesterol (mmol/L)	3.85±0.9	3.85±1	0.50
Total bilirubin (mmol/L)	24.3±17.6	23.9±17.7	0.98
Albumin (g/L)	38.9±4.3	38.9±4.3	0.52
Partial thromboplastin time (s)	38.4±9.7	39.3±12.3	0.73
Prothrombin time (s)	4.7±2.5	5.5±3.7	0.38
INR	1.49±0.3	1.58±0.4	0.38
White blood cells (x 10^3/μL)	6.1±1.9	5.9±1.7	0.70
Platelets (x 10^3/μL)	133.7±57.9	130.6±65.7	0.48
Platelets < 100 x 10^3/μL	20 (40%)	24 (48%)	0.42
α-fetoprotein (ng/ml)	11±16.9	10±12.7	0.25

* P values are for the comparison between Viusid and placebo.

† Previous history of clinical decompensation within one year before enrollment.

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Plus-minus values are means ± standard deviations.

For all laboratory measures and for continuous demographics: P value Mann-Whitney *U*-test.

Proportions: percentage, P value chi-square.

MELD, Model for End-Stage Liver Disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

The Child-Pugh and MELD scores are measures of the severity of liver disease.

Prothrombin time (s): value is expressed in seconds upper the control.

Partial thromboplastin time (s): value in seconds.

To convert mmol/L of bilirubin to mg/dL, multiply by 0.0585.

To convert mmol/L of creatinine to mg/dL, multiply by 0.01131.

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Table 3. Summary of outcome measures.

Variable	Viusid (N=50)	Placebo (N=50)	Hazard Ratio* (95% CI)	P value
	No. of patients (%)			
Primary outcomes – no. (%)				
Survival	45 (90%)	37 (74%)	0.27 (0.08-0.92)	0.036
Secondary outcomes – no. (%)				
Time to disease progression	14 (28%)	24 (48%)	0.47 (0.22-0.89)	0.044
Time to diagnosis of HCC†	1 (2%)	6 (12%)	0.15 (0.019-0.90)	0.046
Worsening of CP score in at least 2 points	7 (14%)	19 (38%)	0.34 (0.14-0.81)	0.015
Worsening of MELD score in at least 4 points	6 (12%)	15 (30%)	0.39 (0.15-0.92)	0.042
Ascites	7 (14%)	16 (32%)	0.32 (0.11-0.90)	0.031
Hepatic encephalopathy	1 (2%)	5 (10%)	0.20 (0.10-1.7)	0.10
Spontaneous bacterial peritonitis	1 (2%)	5 (10%)	0.20 (0.13-1.7)	0.09

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Upper gastrointestinal bleeding	8 (16%)	10 (20%)	0.78 (0.31-1.99)	0.67

*Hazard ratios were computed using Cox proportional hazard model adjusted for sex and age, baseline CP and MELD scores, previous history of clinical decompensation, and current use of diuretics and propranolol. CI denotes confidence interval for HR.

†All cases of HCC were diagnosed during the second year of treatment.

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Table 4. Incidence of adverse events*.

Variable	Viusid (n=50) No (%)	Placebo (n=50) No (%)	P value†
Asthenia	12 (24%)	20 (40%)	0.08
Fatigue or malaise	5 (10%)	13 (26%)	0.04
Muscle pain	8 (16%)	16 (32%)	0.06
Anorexia	5 (10%)	9 (18%)	0.24
Cramps	11 (22%)	22 (44%)	0.02
Discomfort on the RUC‡	7 (14%)	13 (26%)	0.13
Gingival bleeding	5 (10%)	10 (20%)	0.16
Epistaxis	5 (10%)	10 (20%)	0.16
Nausea	5 (10%)	1 (2%)	0.12
Diarrhea	5 (5%)	1 (2%)	0.12
Sepsis	7 (14%)	20 (40%)	<0.01
Hospitalization	9 (18%)	15 (30%)	0.24

* The adverse events listed are those recorded in at least 5% of the patients in either study group.

† P values were calculated on the basis of the two-sided chi-square.

‡ RUC: right upper quadrant.

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FIGURE LEGENDS:

Fig. 1. Flow of patients through the study.

*Four patients with HCC were not discontinued because diagnosis was made at the end of the treatment.

Fig. 2. Kaplan-Meier curves for overall survival (Panel A), time to disease progression (Panel B)*, and time to diagnosis of hepatocellular carcinoma (Panel C), according to intention-to-treat analysis.

*Time to disease progression was defined as the incidence of liver-related death, the development of hepatocellular carcinoma, or the first occurrence or relapse (only for those patients with a previous history of hepatic decompensation) of at least one of the following clinical conditions: ascites, hepatic encephalopathy, spontaneous bacterial peritonitis, hepatorenal syndrome, or upper gastrointestinal bleeding secondary to portal hypertension. Parentheses show number of events.

Fig. 3. Kaplan-Meier estimates of the time to worsening of the Child-Pugh (Panel A) and MELD (Panel B) scores during the treatment.

Parentheses show number of events.

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CONSORT Statement 2001 - Checklist

Items to include when reporting a randomized trial

PAPER SECTION And topic	Item	Descriptor	Reported on Page #
TITLE & ABSTRACT	1	How participants were allocated to interventions (*e.g.*, "random allocation", "randomized", or "randomly assigned").	1,3
INTRODUCTION Background	2	Scientific background and explanation of rationale.	6-8
METHODS Participants	3	Eligibility criteria for participants and the settings and locations where the data were collected.	9
Interventions	4	Precise details of the interventions intended for each group and how and when they were actually administered.	10
Objectives	5	Specific objectives and hypotheses.	8
Outcomes	6	Clearly defined primary and secondary outcome measures and, when applicable, any methods used to enhance the quality of measurements (*e.g.*, multiple observations, training of assessors).	11-12
Sample size	7	How sample size was determined and, when applicable, explanation of any interim analyses and stopping rules.	13
Randomization -- Sequence generation	8	Method used to generate the random allocation sequence, including details of any restrictions (*e.g.*, blocking, stratification)	10
Randomization -- Allocation concealment	9	Method used to implement the random allocation sequence (*e.g.*, numbered containers or central telephone), clarifying whether the sequence was concealed until interventions were assigned.	10
Randomization -- Implementation	10	Who generated the allocation sequence, who enrolled participants, and who assigned participants to their groups.	10
Blinding (masking)	11	Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to group assignment. If done, how the success of blinding was evaluated.	10
Statistical methods	12	Statistical methods used to compare groups for primary outcome(s); Methods for additional analyses, such as subgroup analyses and adjusted analyses.	13
RESULTS Participant flow	13	Flow of participants through each stage (a diagram is strongly recommended). Specifically, for each group report the numbers of participants randomly assigned, receiving intended treatment, completing the study protocol, and analyzed for the primary outcome. Describe protocol deviations from study as planned, together with reasons.	14 Figure 1
Recruitment	14	Dates defining the periods of recruitment and follow-up.	14
Baseline data	15	Baseline demographic and clinical characteristics of each group.	14
Numbers analyzed	16	Number of participants (denominator) in each group included in each analysis and whether the analysis was by "intention-to-treat". State the results in absolute numbers when feasible (*e.g.*, 10/20, not 50%).	14
Outcomes and estimation	17	For each primary and secondary outcome, a summary of results for each group, and the estimated effect size and its precision (*e.g.*, 95% confidence interval).	15-16
Ancillary analyses	18	Address multiplicity by reporting any other analyses performed, including subgroup analyses and adjusted analyses, indicating those pre-specified and those exploratory.	15-16
Adverse events	19	All important adverse events or side effects in each intervention group.	16
DISCUSSION Interpretation	20	Interpretation of the results, taking into account study hypotheses, sources of potential bias or imprecision and the dangers associated with multiplicity of analyses and outcomes.	16-21
Generalizability	21	Generalizability (external validity) of the trial findings.	16-21
Overall evidence	22	General interpretation of the results in the context of current evidence.	16-21

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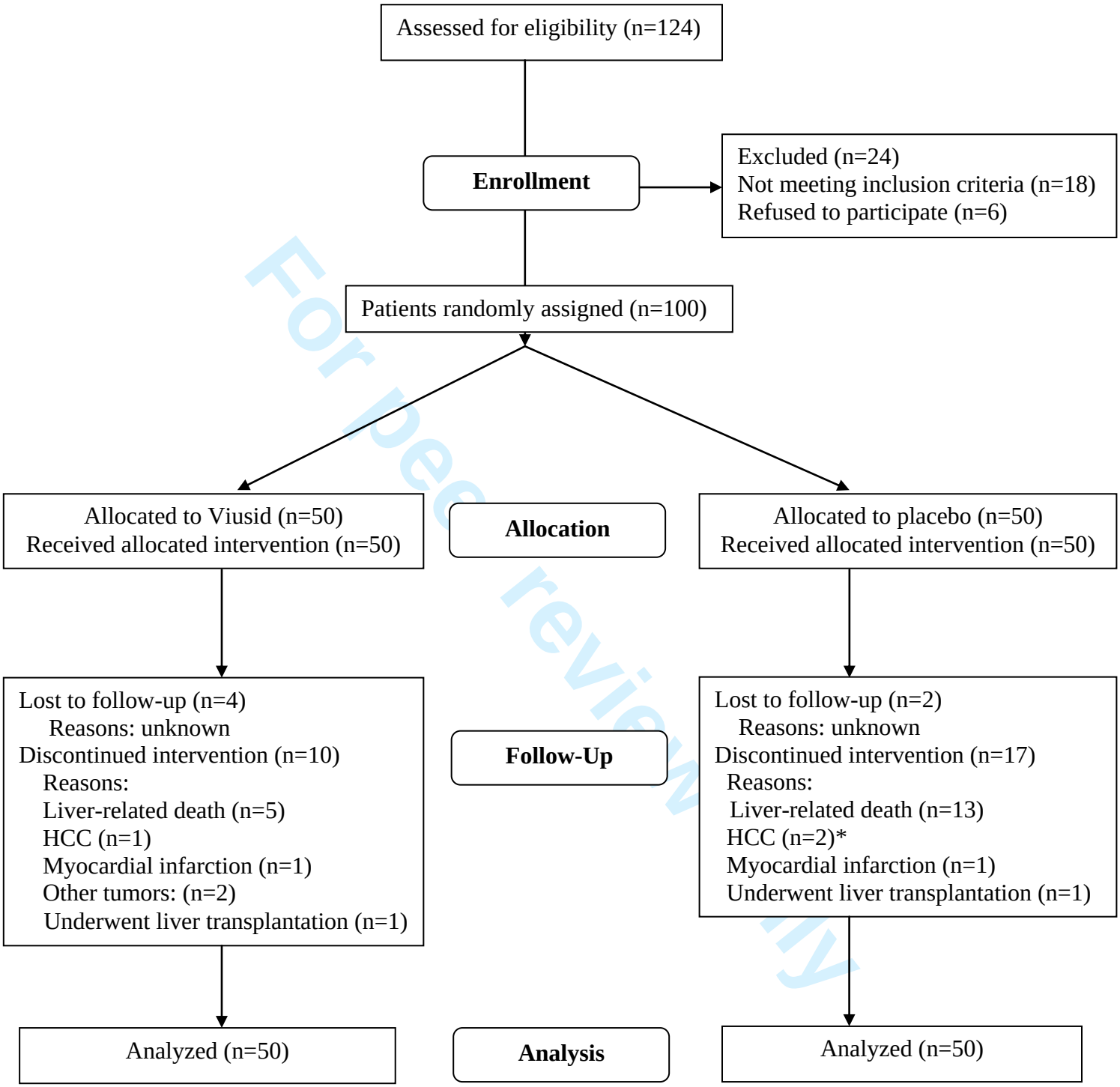

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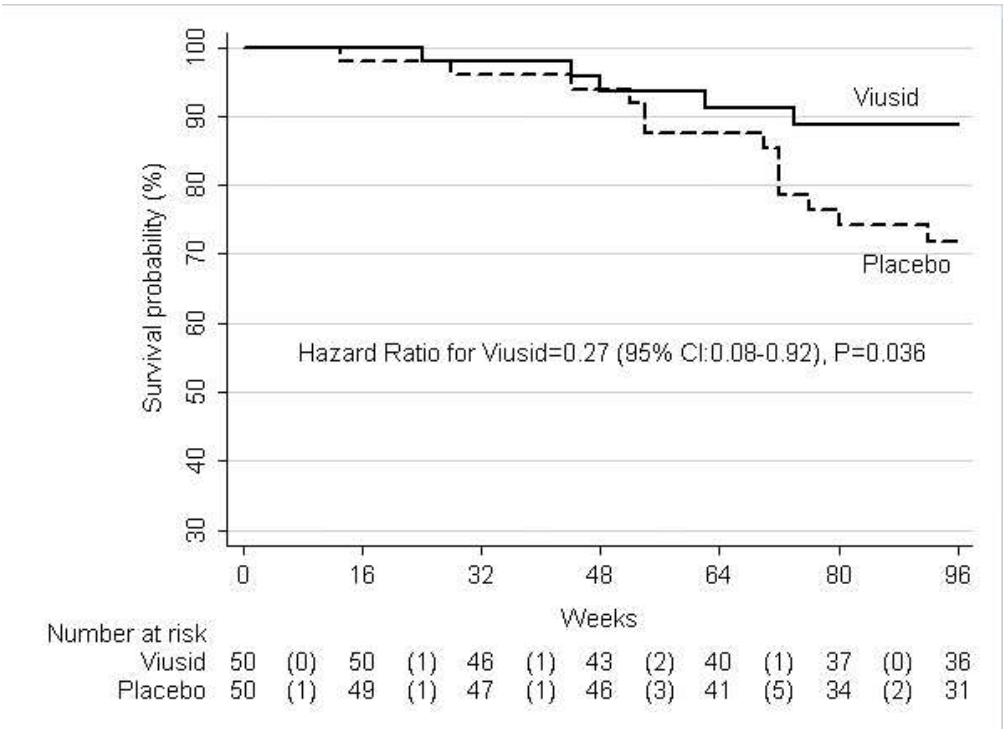

Figure 2A. Overall survival
50x36mm (300 x 300 DPI)

View only

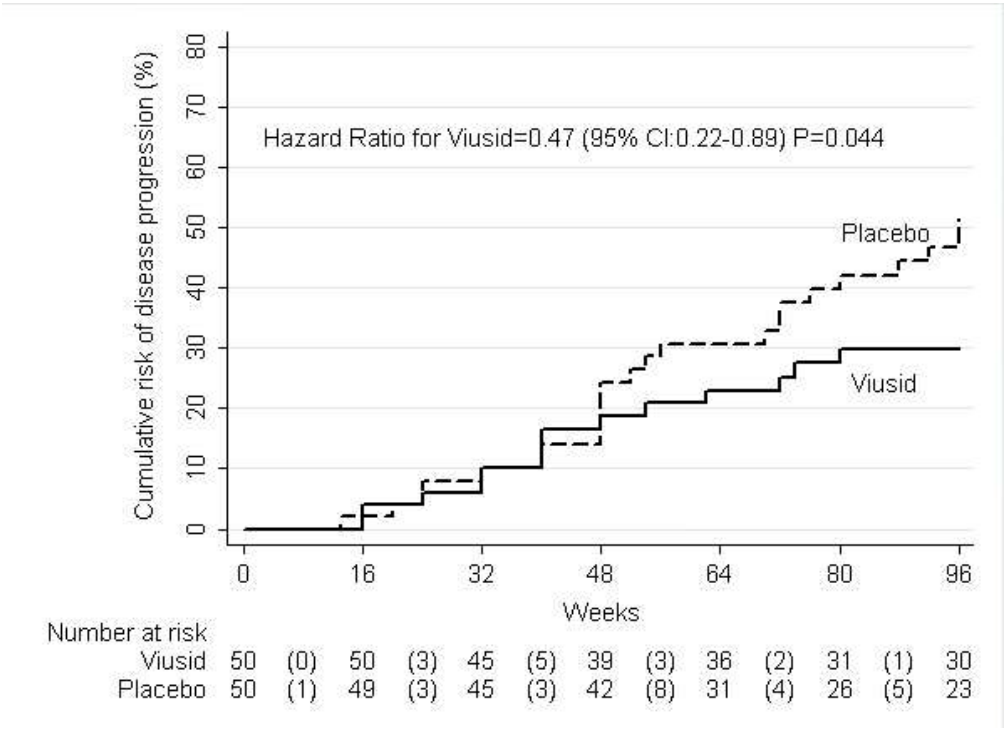

Figure 2B. Time to disease progression
50x36mm (300 x 300 DPI)

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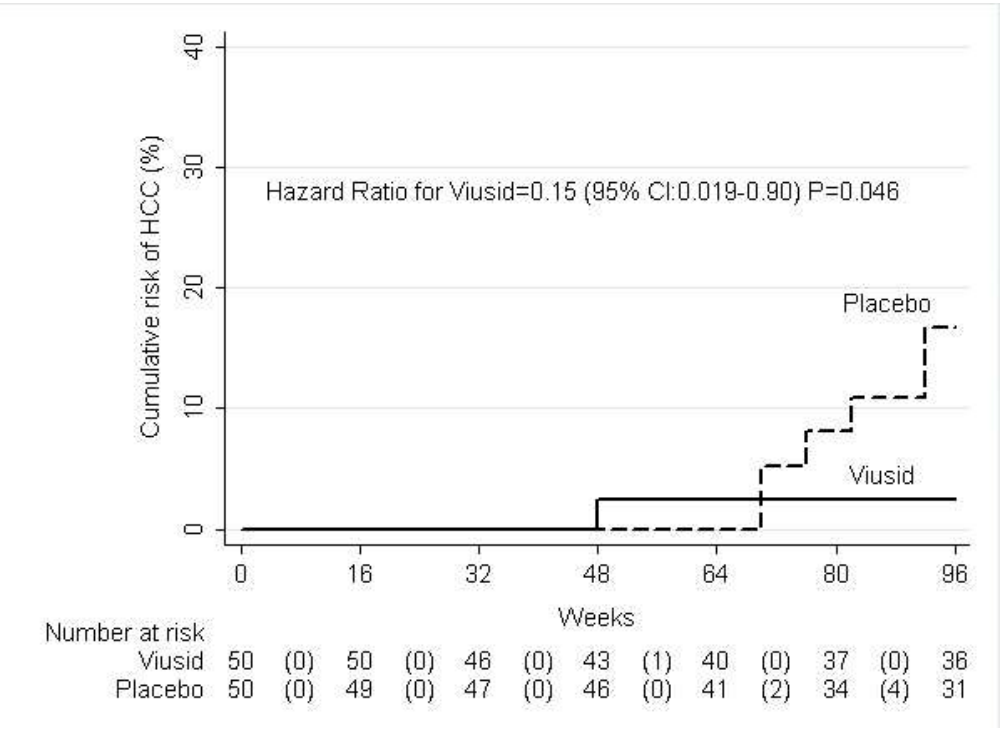

Figure 2C. Time to HCC diagnosis
50x36mm (300 x 300 DPI)

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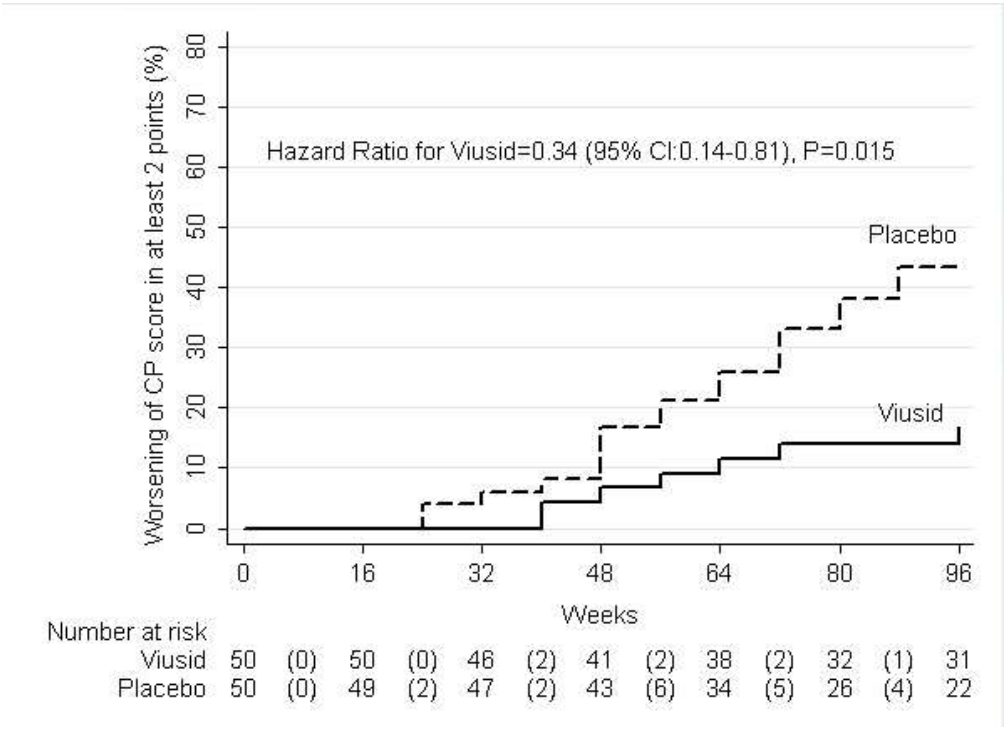

Figure 3A. Time to worsening CP score
50x36mm (300 x 300 DPI)

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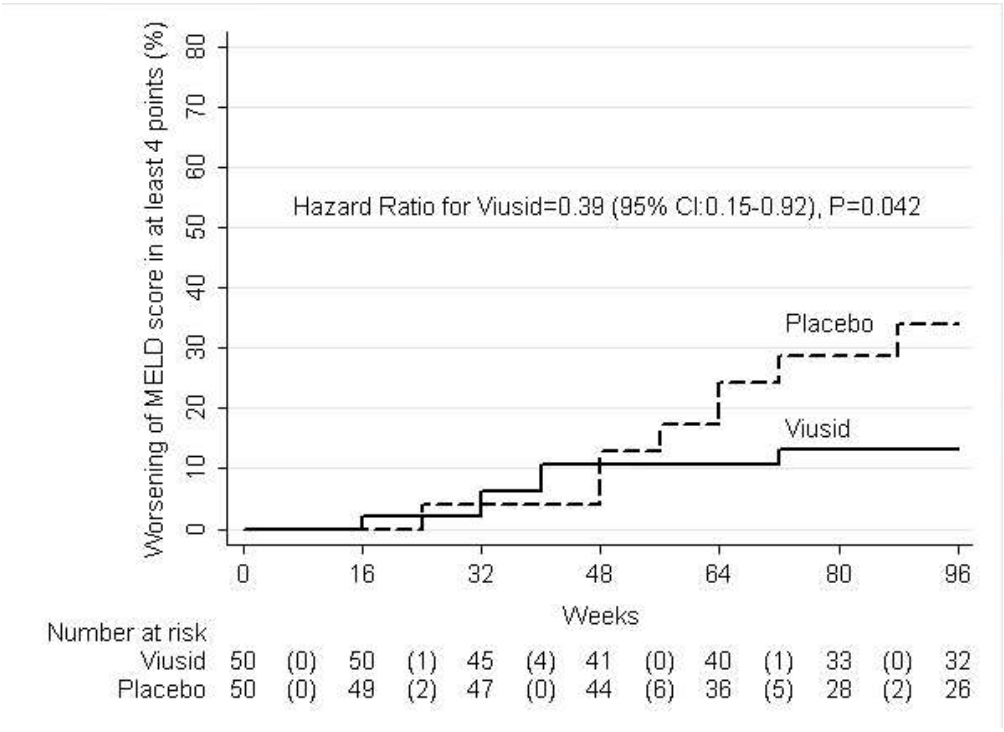

Figure 3B. Time to worsening MELD score
50x36mm (300 x 300 DPI)

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Viusid, a Nutritional Supplement, Increases Survival and Reduces Disease Progression in HCV-Related Decompensated Cirrhosis. A Randomized and Controlled Trial.

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Manuscripts

Viusid, a Nutritional Supplement, Increases Survival and Reduces Disease Progression in HCV-Related Decompensated Cirrhosis. A Randomized and Controlled Trial.

Author names:

Eduardo Vilar Gomez

Email: vilar@infomed.sld.cu

Director of Researches. National Institute of Gastroenterology, Havana, Cuba.

Yoan Sanchez Rodriguez

Email: yoan.sanchez@infomed.sld.cu

Head of the Department of Hepatology. National Institute of Gastroenterology, Havana, Cuba.

Ana Torres Gonzalez

Email: anylut@infomed.sld.cu

Department of Hepatology. National Institute of Gastroenterology, Havana, Cuba.

Luis Calzadilla Bertot

Email: lcbertot@infomed.sld.cu

Department of Hepatology. National Institute of Gastroenterology, Havana, Cuba.

Enrique Arus Soler

Email: anayda@infomed.sld.cu

General Director. National Institute of Gastroenterology, Havana, Cuba.

Yadina Martinez Perez

Email: yadinamartinez@infomed.sld.cu

Department of Gastroenterology. National Institute of Gastroenterology, Havana, Cuba.

Ali Yasells Garcia

Email: yasellsg@gmail.com

For peer review only

Department of Gastroenterology. National Institute of Gastroenterology

Havana, Cuba

Maria del Rosario Abreu Vazquez

Email: charo@infomed.sld.cu

Department of Biostatistics. National Institute of Gastroenterology, Havana, Cuba

Corresponding author:

EDUARDO VILAR GOMEZ.

National Institute of Gastroenterology, Havana, Cuba.

Mailing address: 25th Avenue, 503, Vedado, Havana, Cuba

E-mail address: vilar@infomed.sld.cu

Phone number: 53 7 8325067

Fax number: 53 7 8333253

Abbreviations: HCV, hepatitis C virus; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HR, hazard ratio; CI, confidence interval; OS, overall survival; HCC, hepatocellular carcinoma; MELD, model end-stage liver disease; CHC, chronic hepatitis C; OLT, orthotopic liver transplantation; SOC, standard of care; PEG-IFN, peginterferon; RBV, ribavirin; SVR, sustained virological response; IL, interleukin; TNF, tumor necrosis factor; IFN-γ, interferon-gamma; HIV, human immunodeficiency virus; PCR, polymerase chain reaction; SD, standard deviation; ITT, intention-to-treat analysis; SBP, spontaneous bacterial peritonitis; MDA, malondialdehyde; HALT-C, the hepatitis C antiviral long-term treatment against cirrhosis; CO-PILOT, colchicine versus pegintron long-term therapy; EPIC, evaluation of pegintron in control of hepatitis C cirrhosis; CP, Child-Pugh.

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Abstract

Objectives: Viusid is a nutritional supplement with recognized antioxidant and immunomodulatory properties which could have beneficial effects on cirrhosis-related clinical outcomes such as survival, disease progression and development of hepatocellular carcinoma. Our study evaluated the efficacy and safety of viusid in patients with HCV-related decompensated cirrhosis. **Design:** A randomized double-blind and placebo-controlled study was conducted in a tertiary care academic center (National Institute of Gastroenterology, Havana, Cuba). We randomly assigned 100 patients with HCV-related decompensated cirrhosis to receive viusid (3 oral sachets daily, n=50) or placebo (n=50) during 96 weeks. Primary and secondary end points were comparisons of overall survival (OS), time to disease progression and time to hepatocellular carcinoma (HCC) diagnosis between groups in the intention-to-treat population. **Results:** Viusid led to a significant improvement in overall survival (90%) versus placebo (74%) (hazard ratio [HR] 0.27, 95% CI: 0.08-0.92; *P*=0.036). A similar improvement in disease progression was seen in viusid-treated patients (28%), compared to placebo-treated patients (48%) (HR 0.47, 95% CI: 0.22-0.89; *P*=0.044). However, the beneficial effects of viusid were wholly observed among patients with Child-Pugh classes B or C, but not among patients with Child-Pugh classes A. The cumulative incidence of HCC was significantly reduced in patients treated with viusid (2%) as compared to placebo (12%) (HR 0.15, 95% CI: 0.019-0.90; *P*=0.046). Viusid was well tolerated. **Conclusions:** Our results indicate that treatment with viusid leads to a notable improvement in overall clinical outcomes such as survival, disease progression and development of HCC in patients with HCV-related decompensated cirrhosis. The trial had been registered at ClinicalTrials.gov (NCT00502086).

Key words: chronic hepatitis C, cirrhosis of the liver, survival.

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Article summary

- HCV-related decompensated cirrhotic patients have a poor therapeutic response and reduced tolerance to the current standard of care (SOC) therapy.
- Therapeutic goals in these patients should be directed towards reducing liver-related morbidity and mortality, and the need for liver transplantation.
- Viusid is a nutritional supplement with recognized antioxidant and immunomodulatory properties that could modulate the histological pattern of CHC, especially inflammation and fibrosis in an attempt to halt disease progression and consequently improve liver function and liver-related morbidity and mortality, and prevent development of HCC.

Key messages

- The administration of viusid to HCV-related decompensated cirrhotic patients induced a significant improvement of overall survival, a significant reduction in the disease progression and development of hepatocellular carcinoma.
- The benefit of viusid was also seen in the secondary end point of worsening of the prognostic scores such as MELD and CP scores.
- The viusid effects on survival and disease progression were selective for patients with advanced stage of liver disease (Child-Pugh B or C).
- Viusid was well tolerated and only minor transient adverse events such as nausea and diarrhea were reported.

Strengths and limitations of this study

- The main strength of this study was to demonstrate that viusid improves overall clinical outcomes (survival, HCC, and disease progression) in cirrhotic patients who have failed to achieve SVR with SOC, and these benefits appear to be more prominent in patients with poorer liver function (Child-Pugh B or C).

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- The study was designed with a small sample size.
- Further multicentre and large-scale studies are needed to corroborate the impact of viusid on the clinical outcomes in patients with HCV-related decompensated cirrhosis.

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Competing interest statement: Nothing to declare

Specific author contributions:

The authors were collectively responsible for the study design, data collection, statistical analysis, and interpretation of data; the writing of the manuscript; and the decision to submit the manuscript for publication.

Eduardo Vilar Gomez was involved in the study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis; and obtained funding. He has approved the final draft submitted.

Yoan Sanchez Rodriguez was involved in the study concept and design; acquisition of data; analysis and interpretation of data; critical revision of the manuscript for important intellectual content; and drafting of the manuscript. He has approved the final draft submitted.

Ana Torres Gonzalez was involved in the study concept and design; acquisition of data; analysis and interpretation of data; and drafting of the manuscript. She has approved the final draft submitted.

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Luis Calzadilla Bertot was involved in the study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; and critical revision of the manuscript for important intellectual content. He has approved the final draft submitted.

Enrique Arus Soler was involved in the study concept and design; critical revision of the manuscript for important intellectual content; and drafting of the manuscript. He has approved the final draft submitted.

Yadina Martinez Perez was involved in the study concept and design; acquisition of data; analysis and interpretation of data; and drafting of the manuscript. She has approved the final draft submitted.

Ali Yasells Garcia was involved in the study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; and critical revision of the manuscript for important intellectual content. He has approved the final draft submitted.

Maria del Rosario Abreu Vazquez was involved in the study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; and statistical analysis. She has approved the final draft submitted.

Introduction

Chronic hepatitis C virus (HCV) infection is a leading cause of end-stage liver disease and hepatocellular carcinoma (HCC) worldwide [1], and the most common indication for orthotopic liver transplantation (OLT) in the western world [2]. Once HCV cirrhosis has developed, the risk of clinical decompensation is about 5% per year [3-5], and the risk of mortality is considerably high with a survival rate of 50% at 5 years [6-7]. Cumulative data of patients with compensated cirrhosis indicate that the 5-year risk of decompensation is

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estimated to be 15-28%, and the annual risk of developing HCC is 1.4-6.7% [3-4 8]. Liver-related mortality increases considerably as soon as decompensation is established, and then liver transplantation is the only successful therapeutic option. Unfortunately, once the liver is grafted, disease recurrence is universal. The recurrence of the infection leads to cirrhosis in approximately 25% of the transplant recipients within 5 – 10 years after transplantation. The cumulative probability of decompensation 1 year after cirrhosis is in the order of 30%, and 1-year survival is 46% [9].

HCV-related cirrhotic patients have a poor therapeutic response and reduced tolerance to the current standard of care (SOC) therapy [10-15] . Peginterferon (PEG-IFN) plus ribavirin (RBV) is the recommended treatment strategy for patients with compensated cirrhosis [16]. However, the efficacy of antiviral therapy is in this group significantly lower than in noncirrhotic patients, achieving the poorest rates of sustained virological response (SVR, 5-25%) in patients with genotype 1-4[17]. Current evidence indicates that antiviral treatment with PEG-IFN alone or in combination with RBV reduces the rate of clinical decompensation, improves liver-related survival, and decreases the development of HCC, but only in those patients who achieved SVR [18-20]. However, this benefit should be balanced with severe side effects that led to therapy discontinuation and derangement of liver function in a high proportion of patients with Child-Pugh (CP) class B-C cirrhosis. Thus, an effective treatment is needed immediately in cirrhotic patients who have failed to achieve SVR or with advanced disease to avoid further deterioration and death.

Several studies have demonstrated an important association between increased levels of products related to oxidative stress and advanced stages of the disease [21-22]. Likewise, cytokine dysregulation is thought to play a crucial role in the persistence of viral infection and as a key mediator in inflammatory and fibrogenic processes in patients with HCV

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infection [23]. Therefore, the administration of compounds with antioxidant and immunomodulatory properties could be a plausible strategy to halt the natural course of the disease, particularly in cirrhotic patients with advanced disease.

Viusid (Catalyis laboratory, Madrid, Spain) is a nutritional supplement that contains different molecules (ascorbic acid, zinc, and glycyrrhizic acid) with recognized antioxidant and immunomodulatory properties (Table 1) [24-26].

Encouraging effects of viusid on liver histology have been reported in patients with chronic hepatitis C and nonalcoholic fatty liver disease [27-28].

Recent data suggest that viusid improves oxidative stress through reduction of lipid peroxidation products and has an immunomodulatory effect on cytokine secretion via increased production of IFN-γ and IL-10, decreased production of IL-1α, and stabilized TNF-α secretion in patients with HCV who have failed previous antiviral treatment [29].

All of these effects could modulate the histological pattern of CHC, especially inflammation and fibrosis in an attempt to halt disease progression and consequently improve liver function and liver-related morbidity and mortality, and prevent development of HCC.

Thus, a randomized double-blind and placebo-controlled study was conducted to evaluate whether viusid may have a beneficial effect on survival, time to disease progression and time to diagnosis of HCC in HCV-related cirrhotic patients with decompensated disease.

Materials and Methods

Participants

We recruited 100 patients with HCV liver-related cirrhosis at a tertiary care academic center (National Institute of Gastroenterology, Havana, Cuba) between May 2005 and June 2007 and who fulfilled the following inclusion criteria: male and female patients of 18 to 70 years of age, clinical or histological diagnosis of cirrhosis, naïve patients or non-

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responders to previous treatment with PEG-IFN plus RBV with decompensated cirrhosis, defined as a Child-Pugh score ≥ 7 or clinical evidence or history of ascites, encephalopathy, upper gastrointestinal bleeding, and/or impaired hepatic synthetic function, who had contraindicated the antiviral treatment, absence of active alcoholism (alcohol abstinence was monitored at each clinic visit in the course of patient interview), and ability to provide informed consent. Patients were excluded if they had presence of other causes of liver disease, uncontrollable clinical or biochemical complications related to severe liver failure (hepatic encephalopathy, hepatorenal syndrome, gastrointestinal bleeding, serum total bilirubin greater than 85 mmol/L (5 mg/dL), international normalized ratio greater than 2.5), serum creatinine greater than 180 mmol/L (2 mg/dL), positive screening for viral hepatitis A and B and HIV, pregnancy or lactation, concomitant disease with reduced life expectancy, severe psychiatric conditions, drug dependence, and evidence of liver cancer at entry into the study on the basis of ultrasonography and α-fetoprotein levels higher than 200 ng/L.

Ethics

The study was conducted in compliance with the Declaration of Helsinki and approved by the ethics committee and the institutional review board of the National Institute of Gastroenterology. All patients provided written informed consent for participation.

Interventions

After initial evaluation, all patients who met the eligibility criteria were consecutively enrolled in the study. They were randomly assigned to receive: viusid (3 oral sachets daily, n=50) or placebo (3 oral sachets daily, n=50) for 96 weeks.

Randomization was conducted by blocks of 4 (block randomization). It was performed by a health worker experienced in randomization techniques who was not involved in the

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evaluation or treatment of the participants. The physicians, study coordinators, and patients did not have access to the randomization scheme.

The researchers, study coordinators, and patients were blinded as to the treatment administered. When the patients were allocated, they brought their entry code to the pharmacy which was provided with the randomized list. The code was revealed to the researchers at the end of the study protocol. Catalysis, Spain provided the viusid and placebo sachets. There was no difference in appearance, smell, and flavor between viusid and placebo.

Treatment started 4 weeks after the clinical evidence of decompensation had been treated and controlled with appropriate therapy.

Clinical and laboratory assessment

All patients were closely monitored for clinical, biochemical, and hematological assessment at baseline, weekly for the first eight weeks, and every eight weeks thereafter until the end of the study.

Clinical assessment included physical examination along with compliance to the study medication (verified through sachet count). Biochemical and hematological evaluations included complete blood count, liver tests, glucose, coagulation, and renal function tests. We defined overweight as BMI 25 to 30 kg/m^2 and defined obesity as BMI > 30 kg/m^2. Patient's data with diagnosis of diabetes mellitus at baseline, elevated fasting glucose levels (> 6.1 mmol/L), a positive glucose tolerance test and used antidiabetic medication were recorded.

Liver ultrasonography and serum α-fetoprotein determinations were carried out at baseline and every 24 weeks during the study to screen for hepatocellular carcinoma.

An upper digestive endoscopy was performed before admission.

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The HCV-RNA level was quantified by PCR assay (Amplicor Monitor HCV v.2.0; Roche Molecular System; lower limit of detection, 600 IU/ml). HCV genotyping was performed by reverse hybridization (Inno-LiPA HCV; Innogenetics, Ghent, Belgium).

Definition of Outcomes

The primary outcome of the study was overall survival (OS) which was measured from the date of randomization until the date of death (related to liver disease). Patients with liver-unrelated death or lost to follow-up were censored at the time of death or discontinuation, and patients undergoing liver transplantation were censored at the transplant date.

Secondary outcomes included the time to disease progression, time to diagnosis of HCC, time to worsening of the prognostic scoring systems Child-Pugh and MELD, time to a new occurrence or relapse for each one of the main clinical complications secondary to portal hypertension, and safety.

The time to disease progression was reflected as the time between random assignment and disease progression, defined as the incidence of liver-related death, the development of hepatocellular carcinoma, or the first occurrence or relapse (only for those patients with a previous history of clinical decompensation) of at least one of the following clinical conditions: ascites, hepatic encephalopathy, spontaneous bacterial peritonitis, hepatorenal syndrome, or upper gastrointestinal bleeding secondary to portal hypertension.

The time to diagnosis of hepatocellular carcinoma was calculated from the date of randomization to the date of occurrence of HCC. Diagnosis of HCC was implemented using currently accepted diagnostic criteria for HCC [30-32].

The time to worsening of the prognostic scores was defined as the time from randomization to worsening of the Child-Pugh score in at least 2 points and the MELD score in at least 4 points on the basis of independent clinical evaluation on two

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consecutive study visits. The Child-Pugh and MELD scores are measures of the severity of liver disease, with higher numbers indicating greater decompensation.

The time to a new occurrence or relapse for each one of the main clinical complications secondary to portal hypertension was defined from the date of randomization to the date of a new occurrence or relapse (only for those patients with a previous history of clinical complications) of the following clinical conditions: ascites, hepatic encephalopathy, upper gastrointestinal bleeding, hepatorenal syndrome, and spontaneous bacterial peritonitis. The evidence for each end point was verified and confirmed by two blinded independent hepatologists.

Safety was assessed by dynamic reports of adverse events (AEs), clinical laboratory test (hematological and biochemical analysis), physical examination, and measurement of vital sign. The presence of sepsis and hospitalization were included in the safety reports. Episodes of sepsis were recorded, and they were diagnosed and treated according to recommended guidelines. Sepsis was graded as severe if requiring hospitalization or treatment discontinuation.

Statistical methods

The baseline characteristics were summarized in percentage for categorical variables and as means ±SD for continuous variables. The chi-square test was applied to categorical variables. The two-sample *t*-test was used to compare means, and the Mann-Whitney *U*-test if they were not normally distributed. Outcome measurements included all patients who were randomized and received at least one dose of study medication (intention-to-treat analysis). The safety analysis included all treated patients who had at least one safety evaluation after baseline.

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Both primary and secondary outcomes were analyzed by the Kaplan-Meier method and differences were compared using Cox proportional hazard models adjusted by sex and age, baseline CP and MELD scores, previous history of clinical decompensation, and current use of diuretics and propranolol.

We defined overall survival time at 96 weeks as a primary end point to compute sample size. The study was designed to have a statistical power of 80% to detect an absolute difference of 25% in the survival rates at 96 weeks (95% in the group with viusid versus 70% in the control group). Considering a type I error of 0.05 and a type II error of 0.20, 43 patients per arm were needed to reach statistical significance. After considering patient loss as a result of dropout, we set the target number of patients at 50 per arm, or 100 in total.

All confidence intervals, significance tests, and resulting *P* values were two-sided, with an alpha level of 0.05.

Statistical analyses were performed using STATA software, release 11.

The study was designed by Catalysis Laboratory in conjunction with the principal investigator. The data were collected and analyzed by the investigators. All authors had access to the data.

Results

Patients

Between May 2005 and June 2007, 124 patients were screened. 100 of these patients met the eligibility criteria and were randomly assigned to the viusid (n=50) and the placebo arms (n=50). These patients were all included in the intention-to-treat analysis. 24 patients were excluded from the study during the screening period because they did not meet the inclusion criteria, met one or more of the exclusion criteria, or withdrew their consent. The

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flow of the participants through the trial is presented in Figure 1. None of the patients received co-interventions during the trial that could have affected the outcomes. One death secondary to myocardial infarction occurred in each group of treatment during the study. Four of the 7 patients with HCC were not discontinued and completed the study because diagnosis was made only at the end of the treatment.

Demographic and baseline disease characteristics of the ITT population were generally well balanced between treatment arms (Table 2). The patients' mean age was 57.5 years and 60% were women. All patients had genotype 1 infection. The mean CP and MELD scores at baseline were 6.32 and 12.94, respectively.

All patients with a previous history of hepatic decompensation were controlled and treated with appropriate therapy before trial admission.

At study entry, none of the patients had evidence of hepatocellular carcinoma, ascites, hepatic encephalopathy, renal failure, upper gastrointestinal bleeding, or spontaneous bacterial peritonitis.

Efficacy (primary end point)

Overall survival at 96 weeks was significantly higher in the patients assigned to viusid (90% with a 95% CI, 75 to 95) as compared to the patients assigned to placebo (74% with a 95% CI, 56 to 83) (HR = 0.27; 95% CI, 0.08 to 0.92; *P*=0.036; Table 3). However, the beneficial effects of viusid on survival appear to be selective for patients with poor hepatic reserve (Child-Pugh B or C) (Figure 2A). Survival in patients with CP classes B or C was significantly higher in the viusid group that in the placebo group (80% vs. 48%; HR in the viusid group, 0.36; 95% CI, 0.10 to 0.91; *P*=0.041).

Efficacy (secondary end points)

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The Kaplan-Meier estimates of the proportion of patients with disease progression at 96 weeks (Table 3) were 28% (95% CI, 19 to 45) in the experimental group and 48% (95% CI, 38 to 66) in the control group. The hazard ratio for the viusid arm was 0.47 (95% CI, 0.22 to 0.89; *P*=0.044). Nevertheless, this effect was seen among patients classified as Child-Pugh B or C, but not among patients with CP classes A (Figure 2B). Among patients with CP scores B or C, the disease progression rates were lower in patients treated with viusid (47%) and were progressively higher in patients assigned to placebo arm (80%) (HR in the viusid arm, 0.51; 95% CI, 0.23 to 0.96; *P*=0.047; Figure 2B).

The cumulative incidence of hepatocellular carcinoma at 96 weeks was 2% (95% CI, 0.3 to 15) in the viusid-treated patients and 12% (95% CI, 6 to 33) in the placebo group, with a hazard ratio for the viusid group of 0.15 (95% CI, 0.019 to 0.90; *P*=0.046) (Table 3). All patients with HCC were diagnosed during the second year after randomization. 2 of the 7 patients with HCC were eligible for liver transplantation and 3 had transarterial chemoembolization.

An increase in the CP score (Figure 3A) occurred in 7 patients (14%; 95% CI, 8 to 32) allocated to the viusid group as compared to 19 patients (38%; 95% CI, 30 to 59) allocated to the placebo group. The hazard ratio for the viusid arm was 0.34 (95% CI, 0.14 to 0.81; *P*=0.015). Likewise, a significant worsening in the MELD score (Figure 3B) was observed in 15 individuals (30%; 95% CI, 21 to 49) assigned to placebo as compared to 6 individuals (12%; 95% CI, 6 to 26) assigned to viusid, with a hazard ratio for the Viusid group of 0.39 (95% CI, 0.15 to 0.92, *P*=0.042).

The cumulative incidence of ascites at 96 weeks was significantly higher in the patients assigned to placebo (32%; 95% CI, 14 to 39) than in the patients assigned to viusid (14%, 95% CI, 7 to 28). The hazard ratio for the viusid arm was 0.32 (95% CI, 0.11 to 0.90;

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P=0.031), but the differences were not statistically significant for hepatic encephalopathy, upper gastrointestinal bleeding, and spontaneous bacterial peritonitis. Type 2 hepatorenal syndrome was reported in one patient of each group of treatment. The primary and secondary outcome measures are summarized in Table 3.

Safety

Cramps (33%), asthenia (32%), sepsis (27%), predominantly bacterial infections, and muscle pain (24%) were the most frequent adverse events. The main causes of sepsis were urinary infection (11%), SBP (6%), pneumonia (5%), and lymphangitis (3%). None of the patients had infections related to leukopenia or neutropenia.

A lesser proportion of patients treated with viusid than treated with placebo had fatigue (viusid, 10%; placebo, 26%; *P*=0.04), cramps (viusid, 22%; placebo, 44%; *P*=0.02), and sepsis (viusid, 14%; placebo, 40%; *P*<0.01), respectively.

A high percentage of patients (24%) were hospitalized during the study secondary to episodes of hepatic decompensation or severe sepsis; however, there was no difference between the treatment groups. A summary of adverse events is given in Table 4. There were no significant laboratory abnormalities in the two study groups.

Neither was there any incidence of viusid discontinuation or dose modification secondary to adverse events.

Discussion

HCV-related cirrhotic patients represent an important population with increased morbidity and mortality rates. Unfortunately, current antiviral therapy, especially for patients with decompensated disease, is generally limited by side effects and early discontinuation is common. Therefore, liver transplantation is the most appropriate therapeutic option for these patients. Recent studies have demonstrated encouraging SVR rates and,

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consequently, clinical outcome improvements (overall survival, HCC, and hepatic decompensation) in decompensated cirrhotic patients, but this was only achieved in a minority of patients (SVR, 5-25%) infected with genotype 1-4 [17]. Therefore, there is a critical need to explore new therapeutic options for patients with HCV-related end-stage liver disease who are never listed for liver transplant and could receive a beneficial impact on their clinical outcomes.

The study was designed to evaluate the efficacy and safety of viusid in a particular population of elderly cirrhotic patients who had a previous history or current evidence of clinical hepatic decompensation and genotype 1 infection, and therefore the poorest chance of achieving SVR and elevated probabilities of adverse clinical outcome in their next years of follow-up.

In the current study, we demonstrated that administration of viusid to HCV-related decompensated cirrhotic patients induced a significant improvement of overall survival, as compared to placebo. Similarly, a significant reduction in the disease progression, defined as the presence liver-related death, the development of hepatocellular carcinoma or a first occurrence or relapse of at least one of the main portal hypertension-related clinical complications, was observed in patients treated with viusid in comparison to those patients treated with placebo. However, the effect of viusid on survival and disease progression was irrelevant for patients with CP classes A, in contrast to those patients with CP classes B or C. Interestingly; the cumulative incidence of HCC was notably reduced in those patients assigned to viusid arm, compared to placebo arm. However, a stratified analysis according to Child-Pugh classes was not performed, due to a small proportion of patients with presence of HCC which could generate a bias in the interpretation of the results.

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In the present study, we found increased rates of mortality, disease progression and cumulative incidence of HCC in the placebo group than previously reported rates in a large, prospective and multicenter trial [33]. The most likely explanation for the disparity between these rates appears to be related to the difference in the study design. Our study was designed to include a large proportion of patients who had a previous history or current evidence of clinical hepatic decompensation (poor hepatic reserve), subjects who were excluded from the HALT-C Trial [33]. A recent controlled study has validated the efficacy and safety of IFN-based therapy for HCV-related decompensated cirrhotic patients [14]. One of the main advantages of the study was to include a group of untreated patients (controls) with decompensated events who were enrolled to define survival and progression disease during 30 months of follow-up. The results obtained in this study show that this group of patients have a poor chance to survive (68%) and increased rates of hepatic decompensation (88%) and HCC (10%). These results suggest that natural history of HCV-related cirrhotic patients is more accelerated in patients with previous history or current evidence of clinical hepatic decompensation.

Data from the HALT-C study show an increased annual risk of HCC in patients with a low platelet count and the presence of esophageal varices. It could be another reasonable theory to explain the increased risk of HCC in our study. In the current study, an elevated percentage of patients (~50%) had evidence of esophageal varices and/or thrombocytopenia (<100 x 10^3/μL) [34].

Finally, an increased prevalence of diabetes was reported in our study (42% in placebo group and 34% in viusid group), which has been associated with development of HCC and accelerated disease progression [35].

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In the current study, the rate of new occurrence or relapse of overall clinical outcome secondary to portal hypertension was statistically reduced in the patients assigned to viusid in comparison to those allocated to placebo. The cumulative incidence of ascites was the only remarkable clinical condition reduced in the patients treated with viusid as compared to placebo. In contrast, no differences were observed between the treatment groups for hepatic encephalopathy, spontaneous bacterial peritonitis, hepatorenal syndrome, and upper gastrointestinal bleeding.

The benefit of viusid was also seen in the secondary end point of worsening of the prognostic scores. A significant increase in the CP and MELD scores was observed in the placebo-treated patients compared to the experimental group.

During the viusid therapy, the risk of bacterial infections decreased independently from neutropenia, which could suggest an improvement in the qualitative neutrophil function, but this effect should be additionally studied.

Viusid was well tolerated and only minor transient adverse events such as nausea and diarrhea were reported.

The mechanisms responsible for the beneficial effects of viusid on the clinical outcomes such as survival, development of HCC, and disease progression have not yet been fully studied. However, there are several reasons to understand why its administration might improve overall clinical end points.

A recent trial has suggested that viusid therapy combined with standard of care (SOC) in patients with chronic hepatitis C may reduce inflammation and fibrosis, irrespective of virological response [28]. Another recently published study has reported a dual role to explain possible mechanisms of action of viusid on liver histology [29]. The authors found that MDA and 4-hydroxyalkenal levels were significantly reduced in patients treated with

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viusid, indicating an important effect on lipid peroxidation products. Furthermore, viusid provided immunomodulatory effects on cytokine secretion via increased production of anti-inflammatory cytokines (IL-10) and decreased or stabilized production of pro-inflammatory cytokines (IL-1α and TNF-α). Current studies are focusing on the biological effects of viusid on hepatic stellate cell apoptosis as a critical step to clarify the potential mechanism of viusid in liver fibrogenesis. On the other hand, it would be important to evaluate whether the viusid effects on the clinical outcomes are directly related to the significant reduction of portal pressure in cirrhotic patients. Further studies should be addressed to answer this concern.

Recently, the HALT-C study was designed to determine whether low-dose peginterferon alpha 2a maintenance therapy over 3.5 years could reduce hepatic decompensation, HCC, and mortality in patients with advanced fibrosis or cirrhosis who failed to achieve SVR with SOC [33]. Unfortunately, no overall reduction in any of these clinical end points was achieved. Like the HALT-C study, 2 other studies (COPILOT and EPIC) failed to demonstrate overall benefit on clinical outcomes in HCV-related cirrhotic patients [36-37]. A recent analysis of the HALT-C trial has demonstrated that benefits on clinical outcomes could only be reached in patients with profound viral suppression obtained with full-dose peginterferon and ribavirin [19].

The main strength of this study was to demonstrate that viusid improves overall clinical outcomes (survival, HCC, and disease progression) in cirrhotic patients who have failed to achieve SVR with SOC, and these benefits appear to be not associated to viral suppression rates [28].

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Our study was designed with a small sample size. Therefore, further multicentre and large-scale studies are needed to corroborate the impact of viusid on the clinical outcomes in patients with HCV-related decompensated cirrhosis.

In conclusion, the study supports the use of viusid in patients with HCV-related decompensated cirrhosis who have failed to achieve SVR, with full-dose peginterferon and ribavirin in an attempt to prevent disease progression and improve overall survival. However, additional studies are required to confirm the long-term effect of viusid in patients with poorer liver function (Child-Pugh B or C).

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Table 1. Ingredients of viusid

Malic acid	0.666 g	Ascorbic acid	0.020 g
Glycyrrhizic acid	0.033 g	Folic acid	66 mcg
Glucosamine	0.666 g	Cyanocobalamine	0.3 mcg
Arginine	0.666 g	Zinc sulfate	0.005 g
Glycine	0.333 g	Pyridoxal	0.6 mg
Calcium pantothenate	0.002 g		

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Table 2. Baseline characteristics.

Variable	Viusid (n=50)	Placebo (n=50)	P value*
Age (y)	58.5±8.9	56.6±8.4	0.29
Sex, n (%)			
Male	22 (44%)	18 (36%)	0.41
Female	28 (56%)	32 (64%)	
BMI (kg/m^2)	25.4±4.6	26.7±4.5	0.16
BMI > 25 (kg/m^2), n (%)	28 (56%)	31 (62%)	0.54
HCV RNA >600,000 IU/ml	42 (84%)	38 (76%)	0.45
Genotype 1, n (%)	50 (100%)	50 (100%)	1.0
Clinical scores			
Child-Pugh Class A	32 (64%)	29 (58%)	
Child-Pugh Class B	15 (30%)	15 (30%)	0.56
Child-Pugh Class C	3 (6%)	6 (12%)	
MELD	12.5±3.7	13.3±4.7	0.46
History of diabetes or fasting glucose ≥ 7 (mmol/L), n (%)	17 (34%)	21 (42%)	0.41
Previous history of clinical decompensation, n (%)†			
Ascites	22 (44%)	14 (32%)	0.10
Upper gastrointestinal bleeding	9 (18%)	5 (10%)	0.25
Spontaneous bacterial peritonitis	2 (4%)	2 (4%)	1.00
Hepatic encephalopathy	4 (8%)	3 (6%)	0.69
Evidence of esophageal varices	23 (46%)	18 (36%)	0.31

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Current propranolol use, n (%)	13 (26%)	10 (20%)	0.65
Average doses	70±17.7	80±37.7	0.37
Current spironolactone use, n (%)	21 (42%)	12 (24%)	0.09
Average doses	84.5±39.1	111±40	0.14
Current furosemide use, n (%)	4 (8%)	5 (10%)	1.00
Average doses	40±10	64±22	0.19
ALT (U/L)	92.2±76.6	82.7±49.5	0.86
AST (U/L)	105±80.2	94.1±56.6	0.72
Fasting plasma glucose (mmol/L)	4.9±1.2	5.1±1.3	0.70
Alkaline phosphatase (mmol/L)	290.4±108	281±78.8	0.96
Creatinine (mmol/L)	1±0.3	1±0.3	0.88
Hemoglobin (g/L)	125.8±13.8	129.5±17.6	0.32
Cholesterol (mmol/L)	3.85±0.9	3.85±1	0.50
Total bilirubin (mmol/L)	24.3±17.6	23.9±17.7	0.98
Albumin (g/L)	38.9±4.3	38.9±4.3	0.52
Partial thromboplastin time (s)	38.4±9.7	39.3±12.3	0.73
Prothrombin time (s)	4.7±2.5	5.5±3.7	0.38
INR	1.49±0.3	1.58±0.4	0.38
White blood cells (x 10^3/μL)	6.1±1.9	5.9±1.7	0.70
Platelets (x 10^3/μL)	133.7±57.9	130.6±65.7	0.48
Platelets < 100 x 10^3/μL	20 (40%)	24 (48%)	0.42
α-fetoprotein (ng/ml)	11±16.9	10±12.7	0.25

* P values are for the comparison between viusid and placebo.

† Previous history of clinical decompensation within one year before enrollment.

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Plus-minus values are means ± standard deviations.

For all laboratory measures and for continuous demographics: P value Mann-Whitney *U*-test.

Proportions: percentage, P value chi-square.

MELD, Model for End-Stage Liver Disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

The Child-Pugh and MELD scores are measures of the severity of liver disease.

Prothrombin time (s): value is expressed in seconds upper the control.

Partial thromboplastin time (s): value in seconds.

To convert mmol/L of bilirubin to mg/dL, multiply by 0.0585.

To convert mmol/L of creatinine to mg/dL, multiply by 0.01131.

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Table 3. Summary of outcome measures.

Variable	Viusid (N=50)	Placebo (N=50)	Hazard Ratio* (95% CI)	P value
	No. of patients (%)			
Primary outcomes – no. (%)				
Overall survival	45 (90%)	37 (74%)	0.27 (0.08-0.92)	0.036
Secondary outcomes – no. (%)				
Time to disease progression	14 (28%)	24 (48%)	0.47 (0.22-0.89)	0.044
Time to diagnosis of HCC†	1 (2%)	6 (12%)	0.15 (0.019-0.90)	0.046
Worsening of CP score in at least 2 points	7 (14%)	19 (38%)	0.34 (0.14-0.81)	0.015
Worsening of MELD score in at least 4 points	6 (12%)	15 (30%)	0.39 (0.15-0.92)	0.042
Ascites	7 (14%)	16 (32%)	0.32 (0.11-0.90)	0.031
Hepatic encephalopathy	1 (2%)	5 (10%)	0.20 (0.10-1.7)	0.10
Spontaneous bacterial peritonitis	1 (2%)	5 (10%)	0.20 (0.13-1.7)	0.09

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Upper gastrointestinal bleeding	8 (16%)	10 (20%)	0.78 (0.31-1.99)	0.67

*Hazard ratios were computed using Cox proportional hazard model adjusted for sex and age, baseline CP and MELD scores, previous history of clinical decompensation, and current use of diuretics and propranolol. CI denotes confidence interval for HR.

†All cases of HCC were diagnosed during the second year of treatment.

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Table 4. Incidence of adverse events*.

Variable	Viusid (n=50) No (%)	Placebo (n=50) No (%)	P value†
Asthenia	12 (24%)	20 (40%)	0.08
Fatigue or malaise	5 (10%)	13 (26%)	0.04
Muscle pain	8 (16%)	16 (32%)	0.06
Anorexia	5 (10%)	9 (18%)	0.24
Cramps	11 (22%)	22 (44%)	0.02
Discomfort on the RUC‡	7 (14%)	13 (26%)	0.13
Gingival bleeding	5 (10%)	10 (20%)	0.16
Epistaxis	5 (10%)	10 (20%)	0.16
Nausea	5 (10%)	1 (2%)	0.12
Diarrhea	5 (5%)	1 (2%)	0.12
Sepsis	7 (14%)	20 (40%)	<0.01
Hospitalization	9 (18%)	15 (30%)	0.24

* The adverse events listed are those recorded in at least 5% of the patients in either study group.

† P values were calculated on the basis of the two-sided chi-square.

‡ RUC: right upper quadrant.

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FIGURE LEGENDS:

Fig. 1. Flow of patients through the study.

*Four patients with HCC were not discontinued because diagnosis was made at the end of the treatment.

Fig. 2. Kaplan-Meier curves for survival (Panel A) and time to disease progression (Panel B)* according to Child-Pugh classes (A versus B or C).

*Time to disease progression was defined as the incidence of liver-related death, the development of hepatocellular carcinoma, or the first occurrence or relapse (only for those patients with a previous history of hepatic decompensation) of at least one of the following clinical conditions: ascites, hepatic encephalopathy, spontaneous bacterial peritonitis, hepatorenal syndrome, or upper gastrointestinal bleeding secondary to portal hypertension. The Child-Pugh score is a measure of the severity of liver disease.

Parentheses show number of events.

CP, Child-Pugh class.

Fig. 3. Kaplan-Meier estimates of the time to worsening of the Child-Pugh (Panel A) and MELD (Panel B) scores during the treatment.

Parentheses show number of events.

The MELD and Child-Pugh score are measures of the severity of liver disease.

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CONSORT Statement 2001 - Checklist

Items to include when reporting a randomized trial

PAPER SECTION And topic	Item	Descriptor	Reported on Page #
TITLE & ABSTRACT	1	How participants were allocated to interventions (*e.g.*, "random allocation", "randomized", or "randomly assigned").	1,3
INTRODUCTION Background	2	Scientific background and explanation of rationale.	6-8
METHODS Participants	3	Eligibility criteria for participants and the settings and locations where the data were collected.	9
Interventions	4	Precise details of the interventions intended for each group and how and when they were actually administered.	10
Objectives	5	Specific objectives and hypotheses.	8
Outcomes	6	Clearly defined primary and secondary outcome measures and, when applicable, any methods used to enhance the quality of measurements (*e.g.*, multiple observations, training of assessors).	11-12
Sample size	7	How sample size was determined and, when applicable, explanation of any interim analyses and stopping rules.	13
Randomization -- Sequence generation	8	Method used to generate the random allocation sequence, including details of any restrictions (*e.g.*, blocking, stratification)	10
Randomization -- Allocation concealment	9	Method used to implement the random allocation sequence (*e.g.*, numbered containers or central telephone), clarifying whether the sequence was concealed until interventions were assigned.	10
Randomization -- Implementation	10	Who generated the allocation sequence, who enrolled participants, and who assigned participants to their groups.	10
Blinding (masking)	11	Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to group assignment. If done, how the success of blinding was evaluated.	10
Statistical methods	12	Statistical methods used to compare groups for primary outcome(s); Methods for additional analyses, such as subgroup analyses and adjusted analyses.	13
RESULTS Participant flow	13	Flow of participants through each stage (a diagram is strongly recommended). Specifically, for each group report the numbers of participants randomly assigned, receiving intended treatment, completing the study protocol, and analyzed for the primary outcome. Describe protocol deviations from study as planned, together with reasons.	14 Figure 1
Recruitment	14	Dates defining the periods of recruitment and follow-up.	14
Baseline data	15	Baseline demographic and clinical characteristics of each group.	14
Numbers analyzed	16	Number of participants (denominator) in each group included in each analysis and whether the analysis was by "intention-to-treat". State the results in absolute numbers when feasible (*e.g.*, 10/20, not 50%).	14
Outcomes and estimation	17	For each primary and secondary outcome, a summary of results for each group, and the estimated effect size and its precision (*e.g.*, 95% confidence interval).	15-16
Ancillary analyses	18	Address multiplicity by reporting any other analyses performed, including subgroup analyses and adjusted analyses, indicating those pre-specified and those exploratory.	15-16
Adverse events	19	All important adverse events or side effects in each intervention group.	16
DISCUSSION Interpretation	20	Interpretation of the results, taking into account study hypotheses, sources of potential bias or imprecision and the dangers associated with multiplicity of analyses and outcomes.	16-21
Generalizability	21	Generalizability (external validity) of the trial findings.	16-21
Overall evidence	22	General interpretation of the results in the context of current evidence.	16-21

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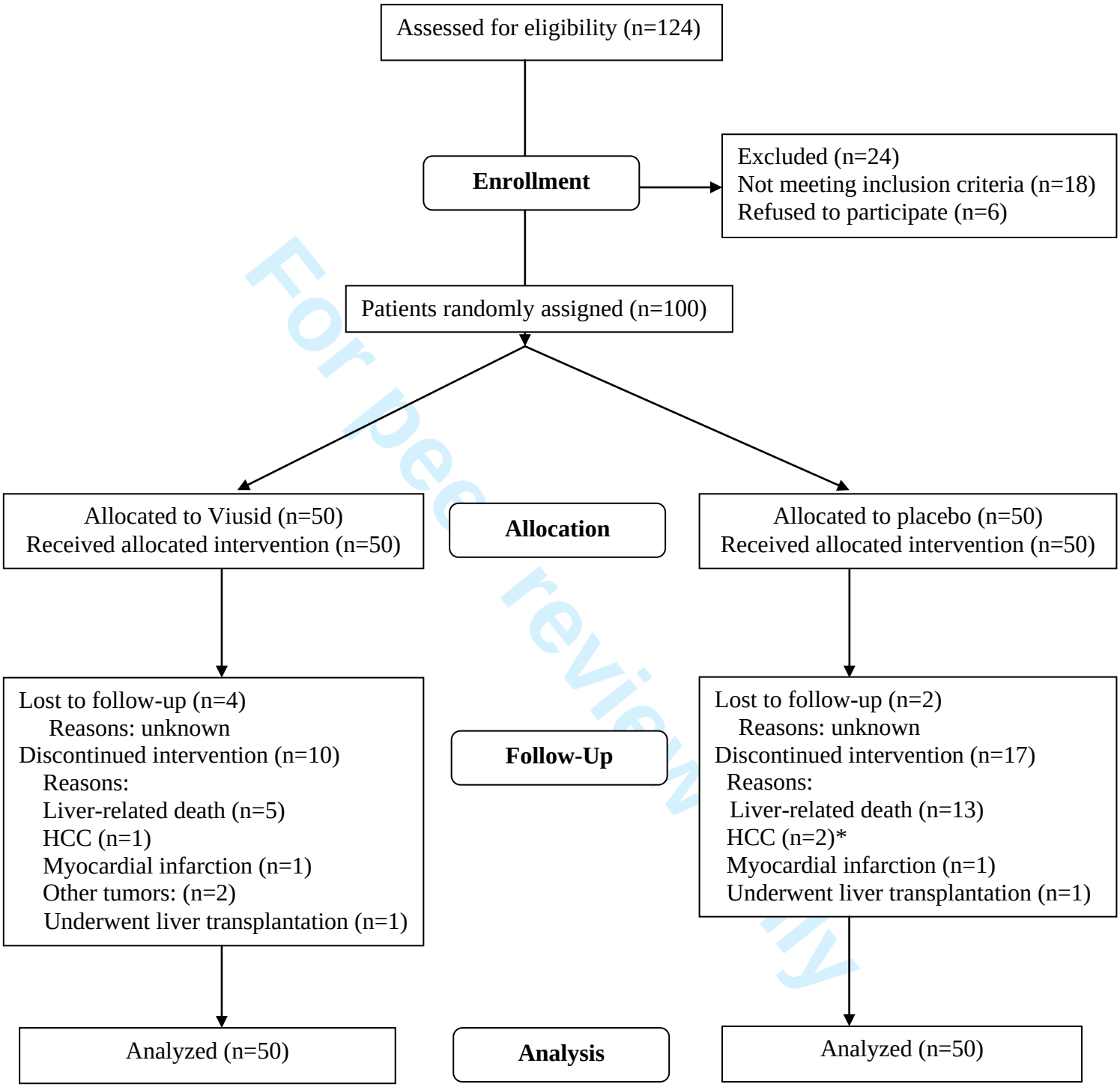

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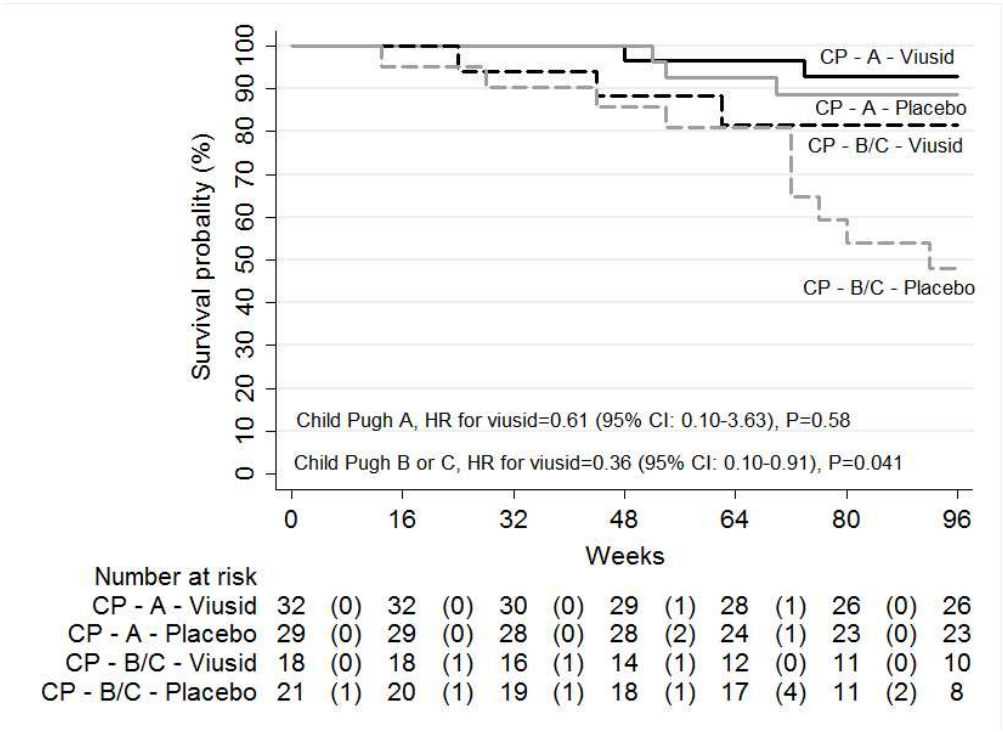

Survival according to CP classes
290x211mm (72 x 72 DPI)

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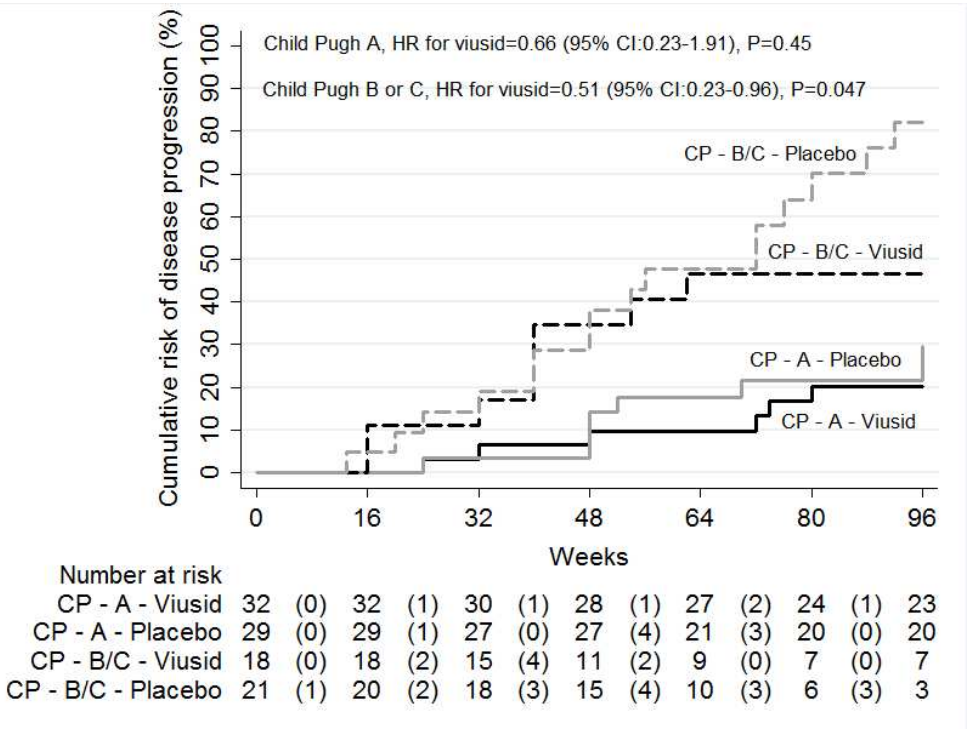

Time to disease progression according to CP classes
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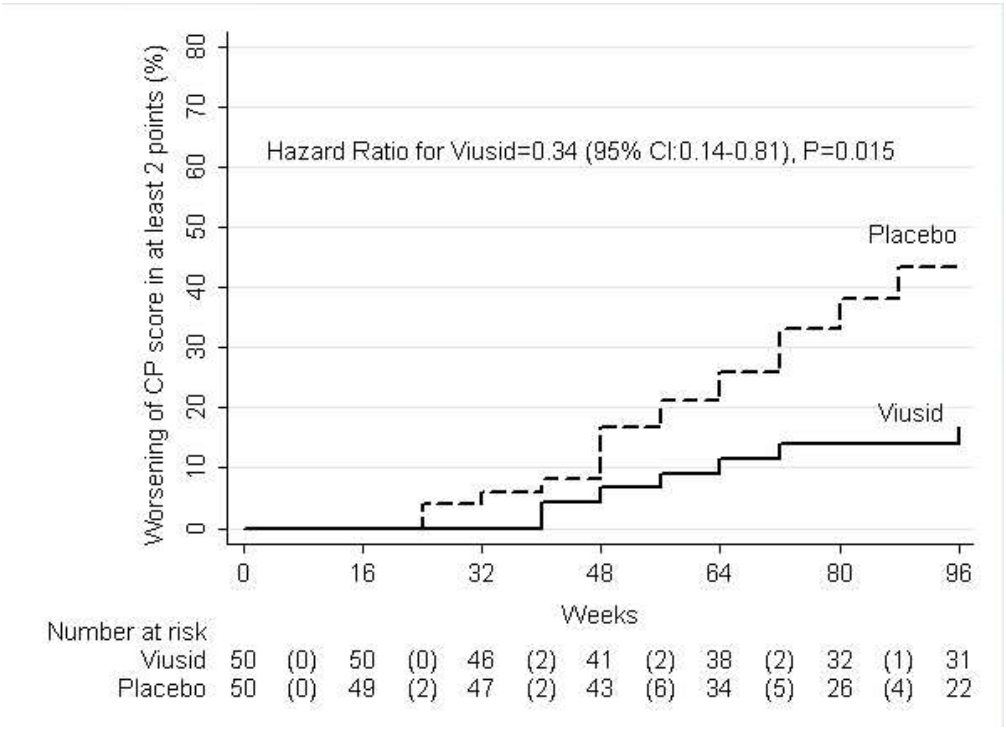

Figure 3A. Time to worsening CP score
50x36mm (300 x 300 DPI)

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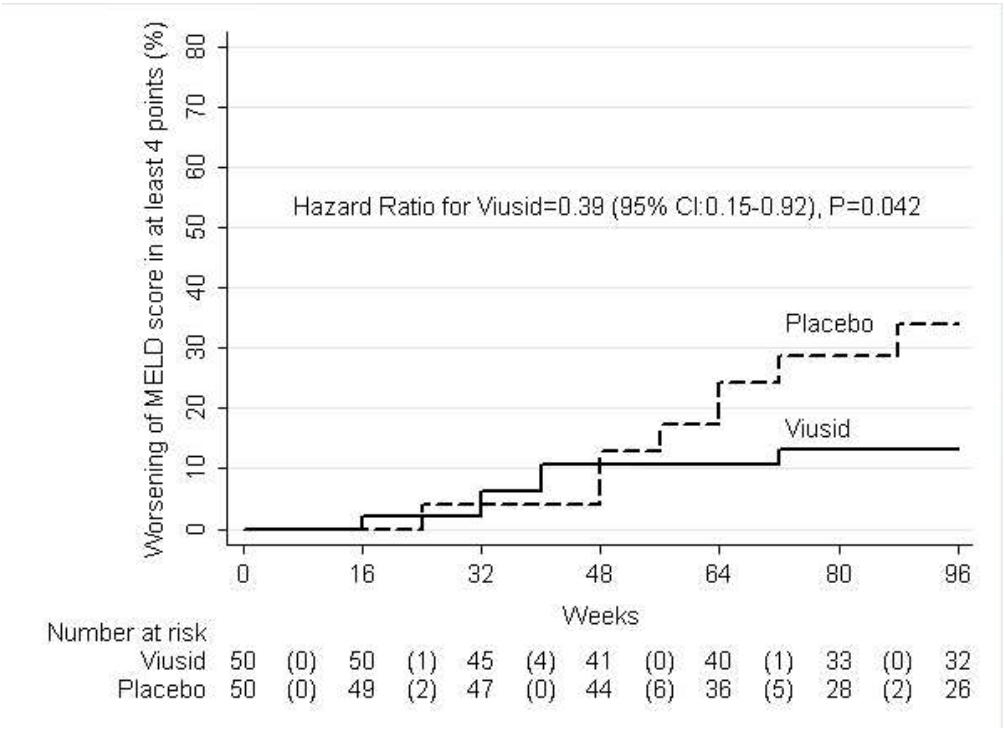

Figure 3B. Time to worsening MELD score
50x36mm (300 x 300 DPI)

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Viusid, a Nutritional Supplement, Increases Survival and Reduces Disease Progression in HCV-Related Decompensated Cirrhosis. A Randomized and Controlled Trial.

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Manuscripts

Viusid, a Nutritional Supplement, Increases Survival and Reduces Disease Progression in HCV-Related Decompensated Cirrhosis. A Randomized and Controlled Trial.

Author names:

Eduardo Vilar Gomez

Email: vilar@infomed.sld.cu

Director of Researches. National Institute of Gastroenterology, Havana, Cuba.

Yoan Sanchez Rodriguez

Email: yoan.sanchez@infomed.sld.cu

Head of the Department of Hepatology. National Institute of Gastroenterology, Havana, Cuba.

Ana Torres Gonzalez

Email: anylut@infomed.sld.cu

Department of Hepatology. National Institute of Gastroenterology, Havana, Cuba.

Luis Calzadilla Bertot

Email: lcbertot@infomed.sld.cu

Department of Hepatology. National Institute of Gastroenterology, Havana, Cuba.

Enrique Arus Soler

Email: anayda@infomed.sld.cu

General Director. National Institute of Gastroenterology, Havana, Cuba.

Yadina Martinez Perez

Email: yadinamartinez@infomed.sld.cu

Department of Gastroenterology. National Institute of Gastroenterology, Havana, Cuba.

Ali Yasells Garcia

Email: yasellsg@gmail.com

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Department of Gastroenterology. National Institute of Gastroenterology

Havana, Cuba

Maria del Rosario Abreu Vazquez

Email: charo@infomed.sld.cu

Department of Biostatistics. National Institute of Gastroenterology, Havana, Cuba

Corresponding author:

EDUARDO VILAR GOMEZ.

National Institute of Gastroenterology, Havana, Cuba.

Mailing address: 25th Avenue, 503, Vedado, Havana, Cuba

E-mail address: vilar@infomed.sld.cu

Phone number: 53 7 8325067

Fax number: 53 7 8333253

Abbreviations: HCV, hepatitis C virus; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HR, hazard ratio; CI, confidence interval; OS, overall survival; HCC, hepatocellular carcinoma; MELD, model end-stage liver disease; CHC, chronic hepatitis C; OLT, orthotopic liver transplantation; SOC, standard of care; PEG-IFN, peginterferon; RBV, ribavirin; SVR, sustained virological response; IL, interleukin; TNF, tumor necrosis factor; IFN-γ, interferon-gamma; HIV, human immunodeficiency virus; PCR, polymerase chain reaction; SD, standard deviation; ITT, intention-to-treat analysis; SBP, spontaneous bacterial peritonitis; MDA, malondialdehyde; HALT-C, the hepatitis C antiviral long-term treatment against cirrhosis; CO-PILOT, colchicine versus pegintron long-term therapy; EPIC, evaluation of pegintron in control of hepatitis C cirrhosis; CP, Child-Pugh.

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Abstract

Objectives: Viusid is a nutritional supplement with recognized antioxidant and immunomodulatory properties which could have beneficial effects on cirrhosis-related clinical outcomes such as survival, disease progression and development of hepatocellular carcinoma. Our study evaluated the efficacy and safety of viusid in patients with HCV-related decompensated cirrhosis. **Design:** A randomized double-blind and placebo-controlled study was conducted in a tertiary care academic center (National Institute of Gastroenterology, Havana, Cuba). We randomly assigned 100 patients with HCV-related decompensated cirrhosis to receive viusid (3 oral sachets daily, n=50) or placebo (n=50) during 96 weeks. The primary outcome of the study was overall survival (OS) at 96 weeks and the secondary outcomes included time to disease progression, time to hepatocellular carcinoma (HCC) diagnosis, time to worsening of the prognostic scoring systems Child-Pugh and MELD and time to a new occurrence or relapse for each one of the main clinical complications secondary to portal hypertension at 96 weeks. **Results:** Viusid led to a significant improvement in overall survival (90%) versus placebo (74%) (hazard ratio [HR] 0.27, 95% CI: 0.08-0.92; *P*=0.036). A similar improvement in disease progression was seen in viusid-treated patients (28%), compared to placebo-treated patients (48%) (HR 0.47, 95% CI: 0.22-0.89; *P*=0.044). However, the beneficial effects of viusid were wholly observed among patients with Child-Pugh classes B or C, but not among patients with Child-Pugh classes A. The cumulative incidence of HCC was significantly reduced in patients treated with viusid (2%) as compared to placebo (12%) (HR 0.15, 95% CI: 0.019-0.90; *P*=0.046). Viusid was well tolerated. **Conclusions:** Our results indicate that treatment with viusid leads to a notable improvement in overall clinical outcomes such as survival, disease progression and

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development of HCC in patients with HCV-related decompensated cirrhosis. The trial had been registered at ClinicalTrials.gov (NCT00502086).

Key words: chronic hepatitis C, cirrhosis of the liver, survival.

Article summary

- HCV-related decompensated cirrhotic patients have a poor therapeutic response and reduced tolerance to the current standard of care (SOC) therapy.
- Therapeutic goals in these patients should be directed towards reducing liver-related morbidity and mortality, and the need for liver transplantation.
- Viusid is a nutritional supplement with recognized antioxidant and immunomodulatory properties that could modulate the histological pattern of CHC, especially inflammation and fibrosis in an attempt to halt disease progression and consequently improve liver function and liver-related morbidity and mortality, and prevent development of HCC.

Key messages

- The administration of viusid to HCV-related decompensated cirrhotic patients induced a significant improvement of overall survival, a significant reduction in the disease progression and development of hepatocellular carcinoma.
- The benefit of viusid was also seen in the secondary end point of worsening of the prognostic scores such as MELD and CP scores.
- The viusid effects on survival and disease progression were selective for patients with advanced stage of liver disease (Child-Pugh B or C).
- Viusid was well tolerated and only minor transient adverse events such as nausea and diarrhea were reported.

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Strengths and limitations of this study

- The main strength of this study was to demonstrate that viusid improves overall clinical outcomes (survival, HCC, and disease progression) in cirrhotic patients who have failed to achieve SVR with SOC, and these benefits appear to be more prominent in patients with poorer liver function (Child-Pugh B or C).
- The study was designed with a small sample size.
- Further multicentre and large-scale studies are needed to corroborate the impact of viusid on the clinical outcomes in patients with HCV-related decompensated cirrhosis.

Funding statement: This work was supported in part by a grant from Catalysis Laboratories, Spain. They provided the viusid and placebo sachets. The supporting sources had no role in the study design, collection of data, interpretation of data, writing of the manuscript, or submission for publication.

All studies related to viusid have been investigator-initiated researches, and Catalysis had no direct involvement in the design of the study, data collection, or preparation of the manuscript. The National Institute of Gastroenterology had no financial interest with the nutritional supplement viusid.

Competing interest statement: Nothing to declare

Specific author contributions:

The authors were collectively responsible for the study design, data collection, statistical analysis, and interpretation of data; the writing of the manuscript; and the decision to submit the manuscript for publication.

Eduardo Vilar Gomez was involved in the study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the

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manuscript for important intellectual content; statistical analysis; and obtained funding. He has approved the final draft submitted.

Yoan Sanchez Rodriguez was involved in the study concept and design; acquisition of data; analysis and interpretation of data; critical revision of the manuscript for important intellectual content; and drafting of the manuscript. He has approved the final draft submitted.

Ana Torres Gonzalez was involved in the study concept and design; acquisition of data; analysis and interpretation of data; and drafting of the manuscript. She has approved the final draft submitted.

Luis Calzadilla Bertot was involved in the study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; and critical revision of the manuscript for important intellectual content. He has approved the final draft submitted.

Enrique Arus Soler was involved in the study concept and design; critical revision of the manuscript for important intellectual content; and drafting of the manuscript. He has approved the final draft submitted.

Yadina Martinez Perez was involved in the study concept and design; acquisition of data; analysis and interpretation of data; and drafting of the manuscript. She has approved the final draft submitted.

Ali Yasells Garcia was involved in the study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; and critical revision of the manuscript for important intellectual content. He has approved the final draft submitted.

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Maria del Rosario Abreu Vazquez was involved in the study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; and statistical analysis. She has approved the final draft submitted.

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Introduction

Chronic hepatitis C virus (HCV) infection is a leading cause of end-stage liver disease and hepatocellular carcinoma (HCC) worldwide [1], and the most common indication for orthotopic liver transplantation (OLT) in the western world [2]. Once HCV cirrhosis has developed, the risk of clinical decompensation is about 5% per year [3-5], and the risk of mortality is considerably high with a survival rate of 50% at 5 years [6-7]. Cumulative data of patients with compensated cirrhosis indicate that the 5-year risk of decompensation is estimated to be 15-28%, and the annual risk of developing HCC is 1.4-6.7% [3-4 8]. Liver-related mortality increases considerably as soon as decompensation is established, and then liver transplantation is the only successful therapeutic option. Unfortunately, once the liver is grafted, disease recurrence is universal. The recurrence of the infection leads to cirrhosis in approximately 25% of the transplant recipients within 5 – 10 years after transplantation. The cumulative probability of decompensation 1 year after cirrhosis is in the order of 30%, and 1-year survival is 46% [9].

HCV-related cirrhotic patients have a poor therapeutic response and reduced tolerance to the current standard of care (SOC) therapy [10-15] . Peginterferon (PEG-IFN) plus ribavirin (RBV) is the recommended treatment strategy for patients with compensated cirrhosis [16]. However, the efficacy of antiviral therapy is in this group significantly lower than in noncirrhotic patients, achieving the poorest rates of sustained virological response (SVR, 5-25%) in patients with genotype 1-4[17]. Current evidence indicates that antiviral treatment with PEG-IFN alone or in combination with RBV reduces the rate of clinical decompensation, improves liver-related survival, and decreases the development of HCC, but only in those patients who achieved SVR [18-20]. However, this benefit should be

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balanced with severe side effects that led to therapy discontinuation and derangement of liver function in a high proportion of patients with Child-Pugh (CP) class B-C cirrhosis. Thus, an effective treatment is needed immediately in cirrhotic patients who have failed to achieve SVR or with advanced disease to avoid further deterioration and death.

Several studies have demonstrated an important association between increased levels of products related to oxidative stress and advanced stages of the disease [21-22]. Likewise, cytokine dysregulation is thought to play a crucial role in the persistence of viral infection and as a key mediator in inflammatory and fibrogenic processes in patients with HCV infection [23]. Therefore, the administration of compounds with antioxidant and immunomodulatory properties could be a plausible strategy to halt the natural course of the disease, particularly in cirrhotic patients with advanced disease.

Viusid (Catalyis laboratory, Madrid, Spain) is a nutritional supplement that contains different molecules (ascorbic acid, zinc, and glycyrrhizic acid) with recognized antioxidant and immunomodulatory properties (Table 1) [24-26]. Glycyrrhizin (0.033 g), the most important active ingredient of the supplement, is known to have various immune-modulating, antiviral and biological response-modifier activities. It has different anti-inflammatory properties (increased production of IL-10 [is a potent anti-inflammatory cytokine which inhibits the syntheses of many pro-inflammatory proteins]), anti-apoptotic effect, hepatocyte proliferation, and stabilization of hepatic cellular membranes [27-30].

Encouraging effects of viusid on liver histology have been reported in patients with nonalcoholic fatty liver disease and chronic hepatitis C [31-32]. The authors reported that the addition of viusid to the conventional interferon/ribavirin therapy was associated with significant histologic and biochemical improvements, especially in patients without sustained virological response. In another study the same authors showed that the

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administration of viusid combined to a lifestyle modification based on a hypocaloric diet and exercise during 6 months was associated with marked histological improvements on steatosis, lobular inflammation, ballooning and NAFLD activity score in patients with nonalcoholic fatty liver disease. No significant clinical and laboratory adverse events have been reported with the use of viusid in previous trials.

Recent data suggest that viusid improves oxidative stress through reduction of lipid peroxidation products and has an immunomodulatory effect on cytokine secretion via increased production of IFN-γ and IL-10, decreased production of IL-1α, and stabilized TNF-α secretion in patients with HCV who have failed previous antiviral treatment [33].

All of these effects could modulate the histological pattern of CHC, especially inflammation and fibrosis in an attempt to halt disease progression and consequently improve liver function and liver-related morbidity and mortality, and prevent development of HCC.

Thus, a randomized double-blind and placebo-controlled study was conducted to evaluate whether viusid may have a beneficial effect on survival, time to disease progression and time to diagnosis of HCC in HCV-related cirrhotic patients with decompensated disease.

Materials and Methods

Participants

We recruited 100 patients with HCV liver-related cirrhosis at a tertiary care academic center (National Institute of Gastroenterology, Havana, Cuba) between May 2005 and June 2007 and who fulfilled the following inclusion criteria: male and female patients of 18 to 70 years of age, clinical or histological diagnosis of cirrhosis, naïve patients or non-responders to previous treatment with PEG-IFN plus RBV with decompensated cirrhosis, defined as a Child-Pugh score ≥ 7 or clinical evidence or history of ascites, encephalopathy, upper gastrointestinal bleeding, and/or impaired hepatic synthetic

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function, who had contraindicated the antiviral treatment, absence of active alcoholism (alcohol abstinence was monitored at each clinic visit in the course of patient interview), and ability to provide informed consent. Patients were excluded if they had presence of other causes of liver disease, uncontrollable clinical or biochemical complications related to severe liver failure (hepatic encephalopathy, hepatorenal syndrome, gastrointestinal bleeding, serum total bilirubin greater than 85 mmol/L (5 mg/dL), international normalized ratio greater than 2.5), serum creatinine greater than 180 mmol/L (2 mg/dL), positive screening for viral hepatitis A and B and HIV, pregnancy or lactation, concomitant disease with reduced life expectancy, severe psychiatric conditions, drug dependence, and evidence of liver cancer at entry into the study on the basis of ultrasonography and α-fetoprotein levels higher than 200 ng/L.

Ethics

The study was conducted in compliance with the Declaration of Helsinki and approved by the ethics committee and the institutional review board of the National Institute of Gastroenterology. All patients provided written informed consent for participation.

Interventions

After initial evaluation, all patients who met the eligibility criteria were consecutively enrolled in the study. They were randomly assigned to receive: viusid (3 oral sachets daily, n=50) or placebo (3 oral sachets daily, n=50) for 96 weeks.

Randomization was conducted by blocks of 4 (block randomization). It was performed by a health worker experienced in randomization techniques who was not involved in the evaluation or treatment of the participants. The physicians, study coordinators, and patients did not have access to the randomization scheme.

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The researchers, study coordinators, and patients were blinded as to the treatment administered. When the patients were allocated, they brought their entry code to the pharmacy which was provided with the randomized list. The code was revealed to the researchers at the end of the study protocol. Catalysis, Spain provided the viusid and placebo sachets. There was no difference in appearance, smell, and flavor between viusid and placebo.

Treatment started 4 weeks after the clinical evidence of decompensation had been treated and controlled with appropriate therapy.

Clinical and laboratory assessment

All patients were closely monitored for clinical, biochemical, and hematological assessment at baseline, weekly for the first eight weeks, and every eight weeks thereafter until the end of the study.

Clinical assessment included physical examination along with compliance to the study medication (verified through sachet count). Biochemical and hematological evaluations included complete blood count, liver tests, glucose, coagulation, and renal function tests. We defined overweight as BMI 25 to 30 kg/m^2 and defined obesity as BMI > 30 kg/m^2. Patient's data with diagnosis of diabetes mellitus at baseline, elevated fasting glucose levels (> 6.1 mmol/L), a positive glucose tolerance test and used antidiabetic medication were recorded.

Liver ultrasonography and serum α-fetoprotein determinations were carried out at baseline and every 24 weeks during the study to screen for hepatocellular carcinoma.

An upper digestive endoscopy was performed before admission.

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The HCV-RNA level was quantified by PCR assay (Amplicor Monitor HCV v.2.0; Roche Molecular System; lower limit of detection, 600 IU/ml). HCV genotyping was performed by reverse hybridization (Inno-LiPA HCV; Innogenetics, Ghent, Belgium).

Definition of Outcomes

The terminology to define outcomes in this study was slightly modified as compared with the original protocol because it is more precise and less subjective to assess time dependent clinical complications in cirrhotic patients. Additionally, primary and secondary outcomes are in accordance to standardized terminology used in the majority of the trials evaluating the impact of treatments on patients with HCV-related cirrhosis.

The primary outcome of the study was overall survival (OS) which was measured from the date of randomization until the date of death (related to liver disease). Patients with liver-unrelated death or lost to follow-up were censored at the time of death or discontinuation, and patients undergoing liver transplantation were censored at the transplant date.

Secondary outcomes included the time to disease progression, time to diagnosis of HCC, time to worsening of the prognostic scoring systems Child-Pugh and MELD, time to a new occurrence or relapse for each one of the main clinical complications secondary to portal hypertension, and safety.

The time to disease progression was reflected as the time between random assignment and disease progression, defined as the incidence of liver-related death, the development of hepatocellular carcinoma, or the first occurrence or relapse (only for those patients with a previous history of clinical decompensation) of at least one of the following clinical conditions: ascites, hepatic encephalopathy, spontaneous bacterial peritonitis, hepatorenal syndrome, or upper gastrointestinal bleeding secondary to portal hypertension.

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The time to diagnosis of hepatocellular carcinoma was calculated from the date of randomization to the date of occurrence of HCC. Diagnosis of HCC was implemented using currently accepted diagnostic criteria for HCC [34-36].

The time to worsening of the prognostic scores was defined as the time from randomization to worsening of the Child-Pugh score in at least 2 points and the MELD score in at least 4 points on the basis of independent clinical evaluation on two consecutive study visits. The Child-Pugh and MELD scores are measures of the severity of liver disease, with higher numbers indicating greater decompensation.

The time to a new occurrence or relapse for each one of the main clinical complications secondary to portal hypertension was defined from the date of randomization to the date of a new occurrence or relapse (only for those patients with a previous history of clinical complications) of the following clinical conditions: ascites, hepatic encephalopathy, upper gastrointestinal bleeding, hepatorenal syndrome, and spontaneous bacterial peritonitis. The evidence for each end point was verified and confirmed by two blinded independent hepatologists.

Safety was assessed by dynamic reports of adverse events (AEs), clinical laboratory test (hematological and biochemical analysis), physical examination, and measurement of vital sign. The presence of sepsis and hospitalization were included in the safety reports. Episodes of sepsis were recorded, and they were diagnosed and treated according to recommended guidelines. Sepsis was graded as severe if requiring hospitalization or treatment discontinuation.

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Statistical methods

The baseline characteristics were summarized in percentage for categorical variables and as means ±SD for continuous variables. The chi-square test was applied to categorical variables. The two-sample *t*-test was used to compare means, and the Mann-Whitney *U*-test if they were not normally distributed. Outcome measurements included all patients who were randomized and received at least one dose of study medication (intention-to-treat analysis). The safety analysis included all treated patients who had at least one safety evaluation after baseline.

Both primary and secondary outcomes were analyzed by the Kaplan-Meier method and differences were compared using Cox proportional hazard models adjusted by sex and age, baseline CP and MELD scores, previous history of clinical decompensation, and current use of diuretics and propranolol.

We defined overall survival time at 96 weeks as a primary end point to compute sample size. The study was designed to have a statistical power of 80% to detect an absolute difference of 25% in the survival rates at 96 weeks (95% in the experimental group versus 70% in the control group). Considering a type I error of 0.05 and a type II error of 0.20, 43 patients per arm were needed to reach statistical significance. After considering patient loss as a result of dropout, we set the target number of patients at 50 per arm, or 100 in total.

All confidence intervals, significance tests, and resulting *P* values were two-sided, with an alpha level of 0.05.

Statistical analyses were performed using STATA software, release 11.

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The study was designed by Catalysis Laboratory in conjunction with the principal investigator. The data were collected and analyzed by the investigators. All authors had access to the data.

Results

Patients

Between May 2005 and June 2007, 124 patients were screened. 100 of these patients met the eligibility criteria and were randomly assigned to the viusid (n=50) and the placebo arms (n=50). These patients were all included in the intention-to-treat analysis. 24 patients were excluded from the study during the screening period because they did not meet the inclusion criteria, met one or more of the exclusion criteria, or withdrew their consent. The flow of the participants through the trial is presented in Figure 1. None of the patients received co-interventions during the trial that could have affected the outcomes. One death secondary to myocardial infarction occurred in each group of treatment during the study. Four of the 7 patients with HCC were not discontinued and completed the study because diagnosis was made only at the end of the treatment.

Demographic and baseline disease characteristics of the ITT population were generally well balanced between treatment arms (Table 2). The patients' mean age was 57.5 years and 60% were women. All patients had genotype 1 infection. The mean CP and MELD scores at baseline were 6.32 and 12.94, respectively.

All patients with a previous history of hepatic decompensation were controlled and treated with appropriate therapy before trial admission.

At study entry, none of the patients had evidence of hepatocellular carcinoma, ascites, hepatic encephalopathy, renal failure, upper gastrointestinal bleeding, or spontaneous bacterial peritonitis.

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Efficacy (primary end point)

Overall survival at 96 weeks was significantly higher in the patients assigned to the nutritional supplement (90% with a 95% CI, 75 to 95) as compared to the patients assigned to placebo (74% with a 95% CI, 56 to 83) (HR = 0.27; 95% CI, 0.08 to 0.92; *P*=0.036; Table 3). However, the beneficial effects of viusid on survival appear to be selective for patients with poor hepatic reserve (Child-Pugh B or C) (Figure 2A). Survival in patients with CP classes B or C was significantly higher in the experimental group that in the placebo group (80% vs. 48%; HR in the viusid group, 0.36; 95% CI, 0.10 to 0.91; *P*=0.041).

Efficacy (secondary end points)

The Kaplan-Meier estimates of the proportion of patients with disease progression at 96 weeks (Table 3) were 28% (95% CI, 19 to 45) in the experimental group and 48% (95% CI, 38 to 66) in the control group. The hazard ratio for the viusid arm was 0.47 (95% CI, 0.22 to 0.89; *P*=0.044). Nevertheless, this effect was seen among patients classified as Child-Pugh B or C, but not among patients with CP classes A (Figure 2B). Among patients with CP scores B or C, the disease progression rates were lower in patients treated with the experimental intervention (47%) and were progressively higher in patients assigned to placebo arm (80%) (HR in the viusid arm, 0.51; 95% CI, 0.23 to 0.96; *P*=0.047; Figure 2B). The cumulative incidence of hepatocellular carcinoma at 96 weeks was 2% (95% CI, 0.3 to 15) in the active product-treated patients and 12% (95% CI, 6 to 33) in the placebo group, with a hazard ratio for the group assigned to active product of 0.15 (95% CI, 0.019 to 0.90; *P*=0.046) (Table 3). All patients with HCC were diagnosed during the second year after randomization. 2 of the 7 patients with HCC were eligible for liver transplantation and 3 had transarterial chemoembolization.

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An increase in the CP score (Figure 3A) occurred in 7 patients (14%; 95% CI, 8 to 32) allocated to the nutritional supplement group as compared to 19 patients (38%; 95% CI, 30 to 59) allocated to the placebo group. The hazard ratio for the viusid arm was 0.34 (95% CI, 0.14 to 0.81; *P*=0.015). Likewise, a significant worsening in the MELD score (Figure 3B) was observed in 15 individuals (30%; 95% CI, 21 to 49) assigned to placebo as compared to 6 individuals (12%; 95% CI, 6 to 26) assigned to nutritional supplement, with a hazard ratio for the viusid group of 0.39 (95% CI, 0.15 to 0.92, *P*=0.042).

The cumulative incidence of ascites at 96 weeks was significantly higher in the patients assigned to placebo (32%; 95% CI, 14 to 39) than in the patients assigned to viusid (14%, 95% CI, 7 to 28). The hazard ratio for the viusid arm was 0.32 (95% CI, 0.11 to 0.90; *P*=0.031), but the differences were not statistically significant for hepatic encephalopathy, upper gastrointestinal bleeding, and spontaneous bacterial peritonitis. Type 2 hepatorenal syndrome was reported in one patient of each group of treatment. The primary and secondary outcome measures are summarized in Table 3.

Safety

Cramps (33%), asthenia (32%), sepsis (27%), predominantly bacterial infections, and muscle pain (24%) were the most frequent adverse events. The main causes of sepsis were urinary infection (11%), SBP (6%), pneumonia (5%), and lymphangitis (3%). None of the patients had infections related to leukopenia or neutropenia.

A lesser proportion of patients treated with the nutritional product than treated with placebo had fatigue (experimental group, 10%; placebo, 26%; *P*=0.04), cramps (experimental group, 22%; placebo, 44%; *P*=0.02), and sepsis (experimental group, 14%; placebo, 40%; *P*<0.01), respectively.

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A high percentage of patients (24%) were hospitalized during the study secondary to episodes of hepatic decompensation or severe sepsis; however, there was no difference between the treatment groups. A summary of adverse events is given in Table 4. There were no significant laboratory abnormalities in the two study groups.

Neither was there any incidence of viusid discontinuation or dose modification secondary to adverse events.

Discussion

HCV-related cirrhotic patients represent an important population with increased morbidity and mortality rates. Unfortunately, current antiviral therapy, especially for patients with decompensated disease, is generally limited by side effects and early discontinuation is common. Therefore, liver transplantation is the most appropriate therapeutic option for these patients. Recent studies have demonstrated encouraging SVR rates and, consequently, clinical outcome improvements (overall survival, HCC, and hepatic decompensation) in decompensated cirrhotic patients, but this was only achieved in a minority of patients (SVR, 5-25%) infected with genotype 1-4 [17]. Therefore, there is a critical need to explore new therapeutic options for patients with HCV-related end-stage liver disease who are never listed for liver transplant and could receive a beneficial impact on their clinical outcomes.

The study was designed to evaluate the efficacy and safety of viusid in a particular population of elderly cirrhotic patients who had a previous history or current evidence of clinical hepatic decompensation and genotype 1 infection, and therefore the poorest chance of achieving SVR and elevated probabilities of adverse clinical outcome in their next years of follow-up.

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In the current study, we demonstrated that administration of a nutritional supplement to HCV-related decompensated cirrhotic patients induced a significant improvement of overall survival, as compared to placebo. Similarly, a significant reduction in the disease progression, defined as the presence liver-related death, the development of hepatocellular carcinoma or a first occurrence or relapse of at least one of the main portal hypertension-related clinical complications, was observed in patients treated with viusid in comparison to those patients treated with placebo. However, the effect of viusid on survival and disease progression was irrelevant for patients with CP classes A, in contrast to those patients with CP classes B or C. Interestingly; the cumulative incidence of HCC was notably reduced in those patients assigned to experimental arm, compared to placebo arm. However, a stratified analysis according to Child-Pugh classes was not performed, due to a small proportion of patients with presence of HCC which could generate a bias in the interpretation of the results.

In the present study, we found increased rates of mortality, disease progression and cumulative incidence of HCC in the placebo group than previously reported rates in a large, prospective and multicenter trial [37]. The most likely explanation for the disparity between these rates appears to be related to the difference in the study design. Our study was designed to include a large proportion of patients who had a previous history or current evidence of clinical hepatic decompensation (poor hepatic reserve), subjects who were excluded from the HALT-C Trial [37]. A recent controlled study has validated the efficacy and safety of IFN-based therapy for HCV-related decompensated cirrhotic patients [14]. One of the main advantages of the study was to include a group of untreated patients (controls) with decompensated events who were enrolled to define survival and progression disease during 30 months of follow-up. The results obtained in this study show

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that this group of patients have a poor chance to survive (68%) and increased rates of hepatic decompensation (88%) and HCC (10%). These results suggest that natural history of HCV-related cirrhotic patients is more accelerated in patients with previous history or current evidence of clinical hepatic decompensation.

Data from the HALT-C study show an increased annual risk of HCC in patients with a low platelet count and the presence of esophageal varices. It could be another reasonable theory to explain the increased risk of HCC in our study. In the current study, an elevated percentage of patients (~50%) had evidence of esophageal varices and/or thrombocytopenia (<100 x 10^3/μL) [38].

Finally, an increased prevalence of diabetes was reported in our study (42% in placebo group and 34% in viusid group), which has been associated with development of HCC and accelerated disease progression [39].

In the current study, the rate of new occurrence or relapse of overall clinical outcome secondary to portal hypertension was statistically reduced in the patients assigned to viusid in comparison to those allocated to placebo. The cumulative incidence of ascites was the only remarkable clinical condition reduced in the patients treated with viusid as compared to placebo. In contrast, no differences were observed between the treatment groups for hepatic encephalopathy, spontaneous bacterial peritonitis, hepatorenal syndrome, and upper gastrointestinal bleeding.

The benefit of viusid was also seen in the secondary end point of worsening of the prognostic scores. A significant increase in the CP and MELD scores was observed in the placebo-treated patients compared to the experimental group.

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During the viusid therapy, the risk of bacterial infections decreased independently from neutropenia, which could suggest an improvement in the qualitative neutrophil function, but this effect should be additionally studied.

Viusid was well tolerated and only minor transient adverse events such as nausea and diarrhea were reported.

The mechanisms responsible for the beneficial effects of viusid on the clinical outcomes such as survival, development of HCC, and disease progression have not yet been fully studied. However, there are several reasons to understand why its administration might improve overall clinical end points.

A recent trial has suggested that viusid therapy combined with standard of care (SOC) in patients with chronic hepatitis C may reduce inflammation and fibrosis, irrespective of virological response [32]. Another recently published study has reported a dual role to explain possible mechanisms of action of viusid on liver histology [33]. The authors found that MDA and 4-hydroxyalkenal levels were significantly reduced in patients treated with viusid, indicating an important effect on lipid peroxidation products. Furthermore, viusid provided immunomodulatory effects on cytokine secretion via increased production of anti-inflammatory cytokines (IL-10) and decreased or stabilized production of pro-inflammatory cytokines (IL-1α and TNF-α). Current studies are focusing on the biological effects of viusid on hepatic stellate cell apoptosis as a critical step to clarify the potential mechanism of viusid in liver fibrogenesis. On the other hand, it would be important to evaluate whether the viusid effects on the clinical outcomes are directly related to the significant reduction of portal pressure in cirrhotic patients. Further studies should be addressed to answer this concern.

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Recently, the HALT-C study was designed to determine whether low-dose peginterferon alpha 2a maintenance therapy over 3.5 years could reduce hepatic decompensation, HCC, and mortality in patients with advanced fibrosis or cirrhosis who failed to achieve SVR with SOC [37]. Unfortunately, no overall reduction in any of these clinical end points was achieved. Like the HALT-C study, 2 other studies (COPILOT and EPIC) failed to demonstrate overall benefit on clinical outcomes in HCV-related cirrhotic patients [40-41]. A recent analysis of the HALT-C trial has demonstrated that benefits on clinical outcomes could only be reached in patients with profound viral suppression obtained with full-dose peginterferon and ribavirin [19].

The main strength of this study was to demonstrate that viusid improves overall clinical outcomes (survival, HCC, and disease progression) in cirrhotic patients who have failed to achieve SVR with SOC, and these benefits appear to be not associated to viral suppression rates [32].

Our study was designed with a small sample size. Therefore, further multicentre and large-scale studies are needed to corroborate the impact of viusid on the clinical outcomes in patients with HCV-related decompensated cirrhosis.

In conclusion, the study supports the use of viusid in patients with HCV-related decompensated cirrhosis who have failed to achieve SVR, with full-dose peginterferon and ribavirin in an attempt to prevent disease progression and improve overall survival. However, additional studies are required to confirm the long-term effect of viusid in patients with poorer liver function (Child-Pugh B or C).

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Table 1. Ingredients of viusid

Malic acid	0.666 g	Ascorbic acid	0.020 g
Glycyrrhizic acid	0.033 g	Folic acid	66 mcg
Glucosamine	0.666 g	Cyanocobalamine	0.3 mcg
Arginine	0.666 g	Zinc sulfate	0.005 g
Glycine	0.333 g	Pyridoxal	0.6 mg
Calcium pantothenate	0.002 g		

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Table 2. Baseline characteristics.

Variable	Viusid (n=50)	Placebo (n=50)	P value*
Age (y)	58.5±8.9	56.6±8.4	0.29
Sex, n (%)			
Male	22 (44%)	18 (36%)	0.41
Female	28 (56%)	32 (64%)	
BMI (kg/m^2)	25.4±4.6	26.7±4.5	0.16
BMI > 25 (kg/m^2), n (%)	28 (56%)	31 (62%)	0.54
HCV RNA >600,000 IU/ml	42 (84%)	38 (76%)	0.45
Genotype 1, n (%)	50 (100%)	50 (100%)	1.0
Clinical scores			
Child-Pugh Class A	32 (64%)	29 (58%)	
Child-Pugh Class B	15 (30%)	15 (30%)	0.56
Child-Pugh Class C	3 (6%)	6 (12%)	
MELD	12.5±3.7	13.3±4.7	0.46
History of diabetes or fasting glucose ≥ 7 (mmol/L), n (%)	17 (34%)	21 (42%)	0.41
Previous history of clinical decompensation, n (%)†			
Ascites	22 (44%)	14 (32%)	0.10
Upper gastrointestinal bleeding	9 (18%)	5 (10%)	0.25
Spontaneous bacterial peritonitis	2 (4%)	2 (4%)	1.00
Hepatic encephalopathy	4 (8%)	3 (6%)	0.69
Evidence of esophageal varices	23 (46%)	18 (36%)	0.31

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Current propranolol use, n (%)	13 (26%)	10 (20%)	0.65
Average doses	70±17.7	80±37.7	0.37
Current spironolactone use, n (%)	21 (42%)	12 (24%)	0.09
Average doses	84.5±39.1	111±40	0.14
Current furosemide use, n (%)	4 (8%)	5 (10%)	1.00
Average doses	40±10	64±22	0.19
ALT (U/L)	92.2±76.6	82.7±49.5	0.86
AST (U/L)	105±80.2	94.1±56.6	0.72
Fasting plasma glucose (mmol/L)	4.9±1.2	5.1±1.3	0.70
Alkaline phosphatase (mmol/L)	290.4±108	281±78.8	0.96
Creatinine (mmol/L)	1±0.3	1±0.3	0.88
Hemoglobin (g/L)	125.8±13.8	129.5±17.6	0.32
Cholesterol (mmol/L)	3.85±0.9	3.85±1	0.50
Total bilirubin (mmol/L)	24.3±17.6	23.9±17.7	0.98
Albumin (g/L)	38.9±4.3	38.9±4.3	0.52
Partial thromboplastin time (s)	38.4±9.7	39.3±12.3	0.73
Prothrombin time (s)	4.7±2.5	5.5±3.7	0.38
INR	1.49±0.3	1.58±0.4	0.38
White blood cells (x 10^3/μL)	6.1±1.9	5.9±1.7	0.70
Platelets (x 10^3/μL)	133.7±57.9	130.6±65.7	0.48
Platelets < 100 x 10^3/μL	20 (40%)	24 (48%)	0.42
α-fetoprotein (ng/ml)	11±16.9	10±12.7	0.25

* P values are for the comparison between viusid and placebo.

† Previous history of clinical decompensation within one year before enrollment.

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Plus-minus values are means ± standard deviations.

For all laboratory measures and for continuous demographics: P value Mann-Whitney *U*-test.

Proportions: percentage, P value chi-square.

MELD, Model for End-Stage Liver Disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

The Child-Pugh and MELD scores are measures of the severity of liver disease.

Prothrombin time (s): value is expressed in seconds upper the control.

Partial thromboplastin time (s): value in seconds.

To convert mmol/L of bilirubin to mg/dL, multiply by 0.0585.

To convert mmol/L of creatinine to mg/dL, multiply by 0.01131.

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Table 3. Summary of outcome measures.

Variable	Viusid (N=50)	Placebo (N=50)	Hazard Ratio* (95% CI)	P value
	No. of patients (%)			
Primary outcomes – no. (%)				
Overall survival	45 (90%)	37 (74%)	0.27 (0.08-0.92)	0.036
Secondary outcomes – no. (%)				
Time to disease progression	14 (28%)	24 (48%)	0.47 (0.22-0.89)	0.044
Time to diagnosis of HCC†	1 (2%)	6 (12%)	0.15 (0.019-0.90)	0.046
Worsening of CP score in at least 2 points	7 (14%)	19 (38%)	0.34 (0.14-0.81)	0.015
Worsening of MELD score in at least 4 points	6 (12%)	15 (30%)	0.39 (0.15-0.92)	0.042
Ascites	7 (14%)	16 (32%)	0.32 (0.11-0.90)	0.031
Hepatic encephalopathy	1 (2%)	5 (10%)	0.20 (0.10-1.7)	0.10
Spontaneous bacterial peritonitis	1 (2%)	5 (10%)	0.20 (0.13-1.7)	0.09

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Upper gastrointestinal bleeding	8 (16%)	10 (20%)	0.78 (0.31-1.99)	0.67

*Hazard ratios were computed using Cox proportional hazard model adjusted for sex and age, baseline CP and MELD scores, previous history of clinical decompensation, and current use of diuretics and propranolol. CI denotes confidence interval for HR.

†All cases of HCC were diagnosed during the second year of treatment.

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Table 4. Incidence of adverse events*.

Variable	Viusid (n=50) No (%)	Placebo (n=50) No (%)	P value†
Asthenia	12 (24%)	20 (40%)	0.08
Fatigue or malaise	5 (10%)	13 (26%)	0.04
Muscle pain	8 (16%)	16 (32%)	0.06
Anorexia	5 (10%)	9 (18%)	0.24
Cramps	11 (22%)	22 (44%)	0.02
Discomfort on the RUC‡	7 (14%)	13 (26%)	0.13
Gingival bleeding	5 (10%)	10 (20%)	0.16
Epistaxis	5 (10%)	10 (20%)	0.16
Nausea	5 (10%)	1 (2%)	0.12
Diarrhea	5 (5%)	1 (2%)	0.12
Sepsis	7 (14%)	20 (40%)	<0.01
Hospitalization	9 (18%)	15 (30%)	0.24

* The adverse events listed are those recorded in at least 5% of the patients in either study group.

† P values were calculated on the basis of the two-sided chi-square.

‡ RUC: right upper quadrant.

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FIGURE LEGENDS:

Fig. 1. Flow of patients through the study.

*Four patients with HCC were not discontinued because diagnosis was made at the end of the treatment.

Fig. 2. Kaplan-Meier curves for survival (Panel A) and time to disease progression (Panel B)* according to Child-Pugh classes (A versus B or C).

*Time to disease progression was defined as the incidence of liver-related death, the development of hepatocellular carcinoma, or the first occurrence or relapse (only for those patients with a previous history of hepatic decompensation) of at least one of the following clinical conditions: ascites, hepatic encephalopathy, spontaneous bacterial peritonitis, hepatorenal syndrome, or upper gastrointestinal bleeding secondary to portal hypertension. The Child-Pugh score is a measure of the severity of liver disease.

Parentheses show number of events.

CP, Child-Pugh class.

Fig. 3. Kaplan-Meier estimates of the time to worsening of the Child-Pugh (Panel A) and MELD (Panel B) scores during the treatment.

Parentheses show number of events.

The MELD and Child-Pugh score are measures of the severity of liver disease.

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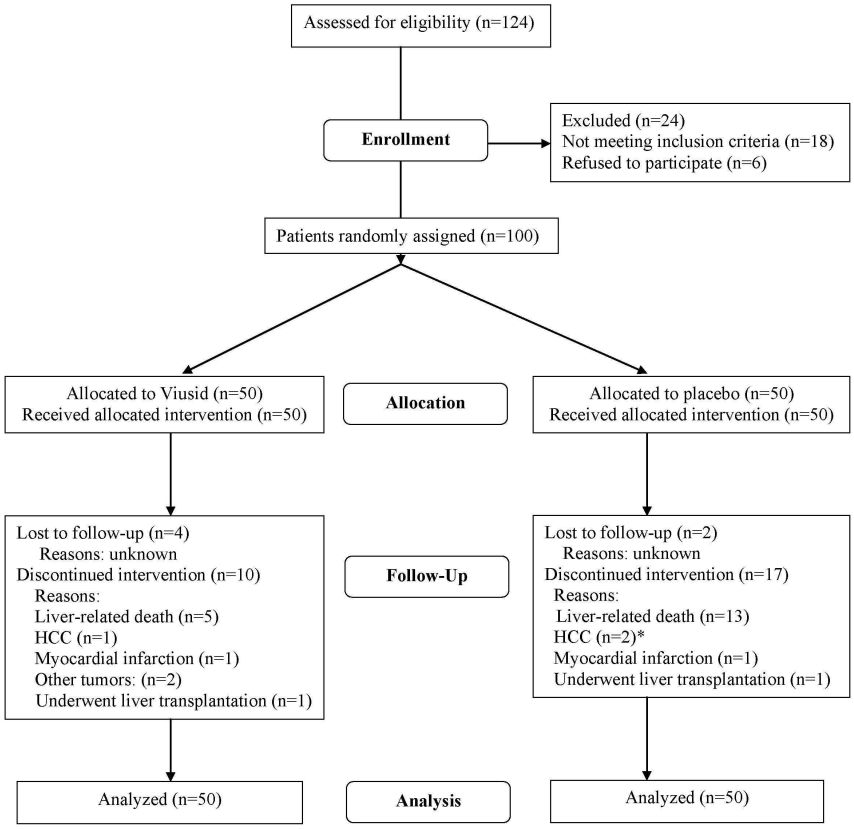

Figure 1. Patient flow
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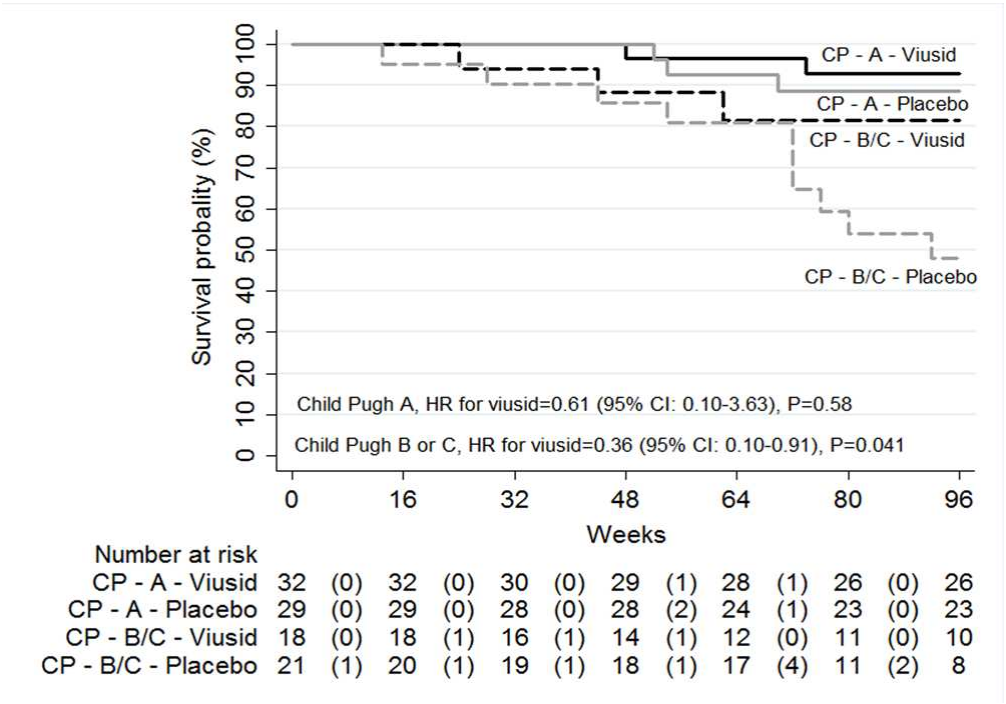

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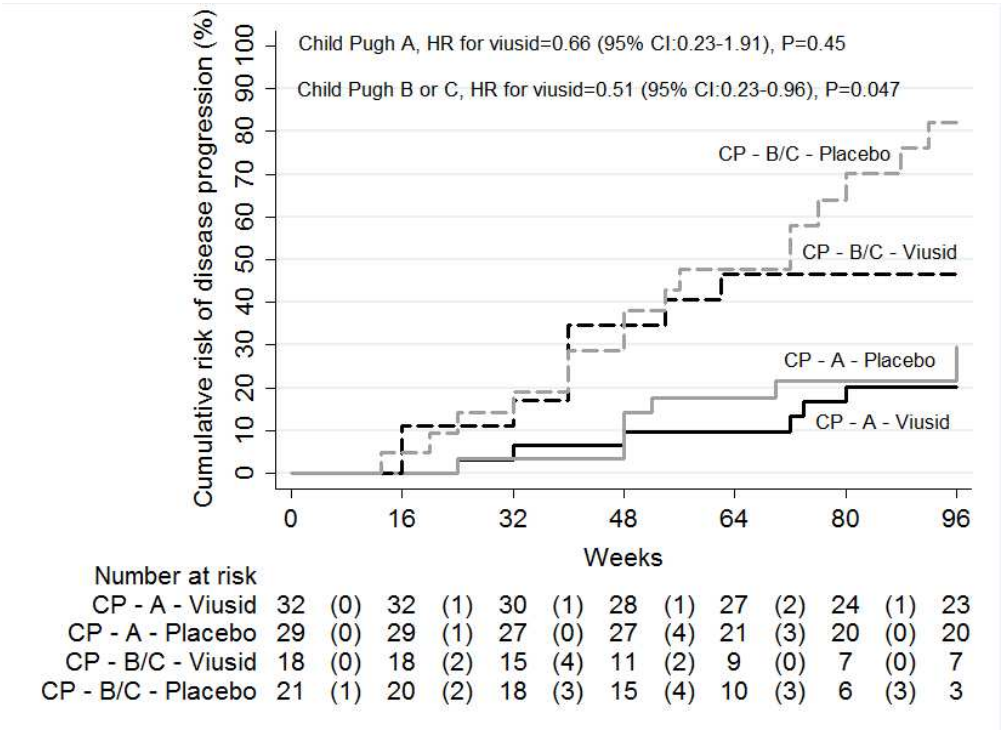

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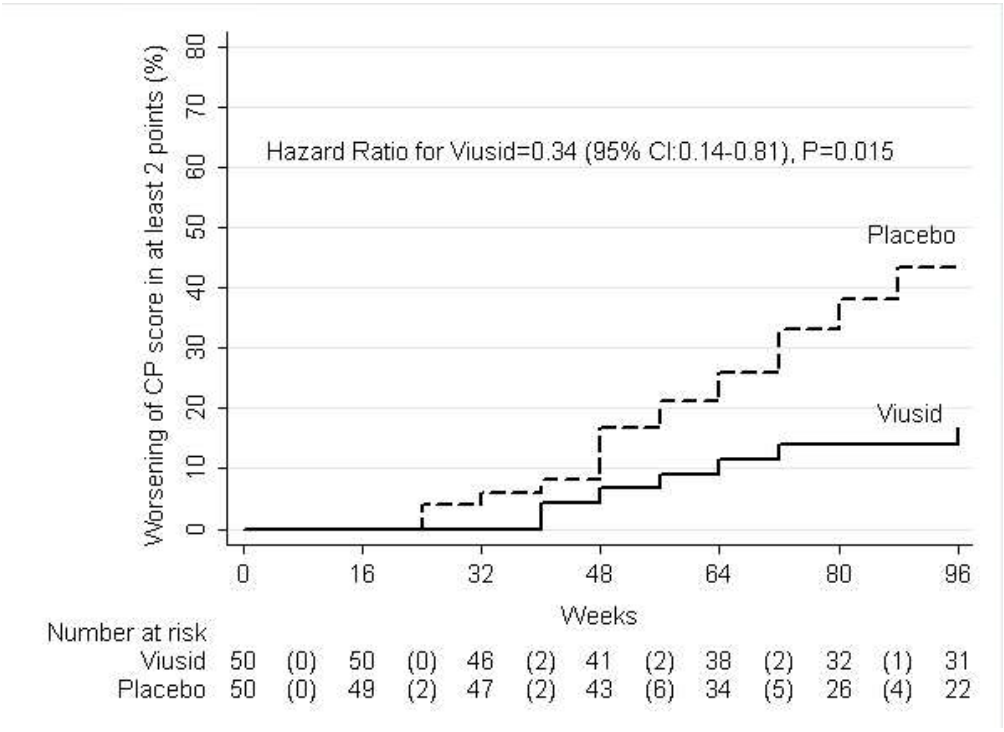

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CONSORT Statement 2001 - Checklist

Items to include when reporting a randomized trial

PAPER SECTION And topic	Item	Descriptor	Reported on Page #
TITLE & ABSTRACT	1	How participants were allocated to interventions (*e.g.*, "random allocation", "randomized", or "randomly assigned").	1,3
INTRODUCTION Background	2	Scientific background and explanation of rationale.	6-8
METHODS Participants	3	Eligibility criteria for participants and the settings and locations where the data were collected.	9
Interventions	4	Precise details of the interventions intended for each group and how and when they were actually administered.	10
Objectives	5	Specific objectives and hypotheses.	8
Outcomes	6	Clearly defined primary and secondary outcome measures and, when applicable, any methods used to enhance the quality of measurements (*e.g.*, multiple observations, training of assessors).	11-12
Sample size	7	How sample size was determined and, when applicable, explanation of any interim analyses and stopping rules.	13
Randomization -- Sequence generation	8	Method used to generate the random allocation sequence, including details of any restrictions (*e.g.*, blocking, stratification)	10
Randomization -- Allocation concealment	9	Method used to implement the random allocation sequence (*e.g.*, numbered containers or central telephone), clarifying whether the sequence was concealed until interventions were assigned.	10
Randomization -- Implementation	10	Who generated the allocation sequence, who enrolled participants, and who assigned participants to their groups.	10
Blinding (masking)	11	Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to group assignment. If done, how the success of blinding was evaluated.	10
Statistical methods	12	Statistical methods used to compare groups for primary outcome(s); Methods for additional analyses, such as subgroup analyses and adjusted analyses.	13
RESULTS Participant flow	13	Flow of participants through each stage (a diagram is strongly recommended). Specifically, for each group report the numbers of participants randomly assigned, receiving intended treatment, completing the study protocol, and analyzed for the primary outcome. Describe protocol deviations from study as planned, together with reasons.	14 Figure 1
Recruitment	14	Dates defining the periods of recruitment and follow-up.	14
Baseline data	15	Baseline demographic and clinical characteristics of each group.	14
Numbers analyzed	16	Number of participants (denominator) in each group included in each analysis and whether the analysis was by "intention-to-treat". State the results in absolute numbers when feasible (*e.g.*, 10/20, not 50%).	14
Outcomes and estimation	17	For each primary and secondary outcome, a summary of results for each group, and the estimated effect size and its precision (*e.g.*, 95% confidence interval).	15-16
Ancillary analyses	18	Address multiplicity by reporting any other analyses performed, including subgroup analyses and adjusted analyses, indicating those pre-specified and those exploratory.	15-16
Adverse events	19	All important adverse events or side effects in each intervention group.	16
DISCUSSION Interpretation	20	Interpretation of the results, taking into account study hypotheses, sources of potential bias or imprecision and the dangers associated with multiplicity of analyses and outcomes.	16-21
Generalizability	21	Generalizability (external validity) of the trial findings.	16-21
Overall evidence	22	General interpretation of the results in the context of current evidence.	16-21

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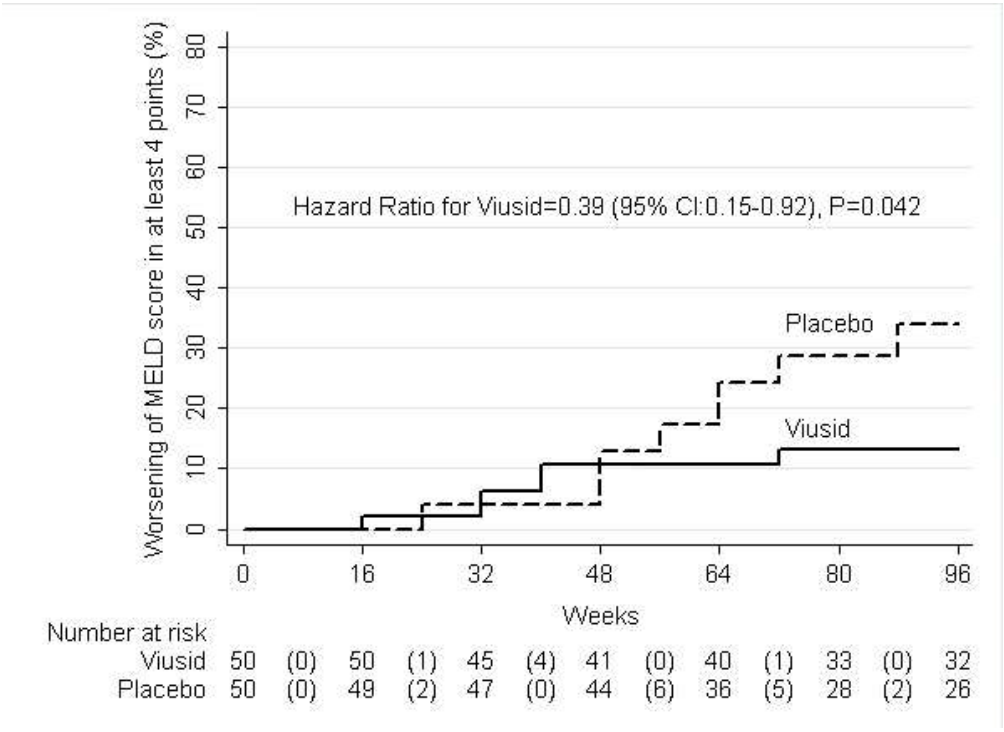

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