Medical Research Archives

ESMED
European Society of Medicine

OPEN ACCESS

Published: January 31, 2023

Citation: Kishida Y., 2022. Induction Therapy with Natural Interferon-Beta and a Protease Inhibitor Restores Innate-Immune Responses and Suppresses Chronic Hepatitis C Infection, Medical Research Archives, [online] 11(1). https://doi.org/10.18103/mra.v11i1.3488

DOI
https://doi.org/10.18103/mra.v11i1.3488

ISSN: 2375-1924

RESEARCH ARTICLE

Induction Therapy with Natural Interferon-Beta and a Protease Inhibitor Restores Innate-Immune Responses and Suppresses Chronic Hepatitis C Infection

Yutaka Kishida M.D., Ph.D.

Division of Gastroenterology and Hepatology Department of Internal Medicine Osaka Kaisei Hospital, Osaka, Japan

Email: y-kishida@muse.ocn.ne.jp

ABSTRACT

Background: Persistent hepatitis C virus (HCV) infection results from inefficient innate and adaptive immune responses and exhausted virus-specific T-cell responses. Host cytokines and innate immune responses play important roles in controlling HCV infection. Innate immune responses modulate adaptive immune responses. These responses have recently been shown to have roles in antiviral therapy for chronic HCV infection. My previous study has indicated that viral clearance early in the course of therapy is associated with the restoration of innate and adaptive immune responses, and thus has potential as a novel therapeutic strategy for chronic hepatitis C (CHC).

Methods: The efficacy and safety of induction therapy (IT) with natural (n)-interferon (IFN)-beta followed by pegylated-IFN-alpha and ribavirin (PR) alone (group A, n = 30) were compared with those of IT with a protease inhibitor (PI) (Simeprevir or Vaniprevir) plus PR (group B, n = 13) in patients with CHC with genotype 1b and high viral load.

Results: During IT with n-IFN-beta, the virologic response rates in group A and group B were 10% and 8% (p = 0.6792) at week 4; 30% and 16% (p = 0.6989) at week 12; and 47% and 20% (p = 0.0887) at week 24. During and after treatment with PR alone, or PI plus PR, the virologic response rates in groups A and B were 50% and 82% (p = 0.01535) at week 4: 53% and 91% (p = 0.006745) at week 8; 57% and 91% (p = 0.001126) at week 12; 57% and 100% (p = 0.001845) at the end of the treatment; and 57% and 80% (p = 0.005166) after treatment cessation.

Conclusion: IT with PI plus PR restored the innate immune response, was tolerated well, overcame virological breakthrough, enhanced early virologic responses, and resulted in a sustained virologic response in patients with intractable CHC. Thus, IT with PI plus PR is beneficial for patients with intractable CHC. Steps for augmenting immune responses must be identified.

Introduction

Globally, an estimated 58 million people are chronically infected with hepatitis C virus (HCV), which can lead to liver fibrosis, liver cirrhosis, and hepatocellular carcinoma (HCC) [1].

The inability of the immune system to eliminate pathogens often results in the development of persistent viral infection. Persistent HCV infection leads to chronic hepatitis C (CHC) and eventually causes cirrhosis and HCC. The human immune system has developed two arms (innate and adaptive immunity) that co-operatively protecting against infection and limit the damage caused by invading pathogens. Persistent HCV infection results from inefficient innate and adaptive immunity, and exhausted virus-specific T-cell responses. Spontaneous clearance of HCV infection is associated with rapid induction of innate immunity. Innate immune responses modulate adaptive immune responses through direct interactions and signaling between immune cells. Although the host innate immune system senses and responds to eliminate viral infections, HCV evades immune attack and establishes persistent infections within the liver.

Because a sustained virologic response (SVR) has been shown to be more likely if favorable early viral kinetics (i.e., a rapid and profound decrease in HCV RNA levels) is achieved, the rapidity of the initial viral clearance augmented by induction therapy [IT, involving high-dose, more frequent IFN administrations, or combination with pegylated (Peg)-interferon (IFN)-alpha, ribavirin (RBV) and Telaprevir for the first several months] has been postulated to provide approach for achieving SVR. However, how to increase the initial virologic response rate has not been resolved. HCV exists as a genetically heterogeneous viral population, termed a quasispecies. Thus, the clinical success of HCV therapies depends on the ability to suppress all viral variants and to prevent the emergence of resistant viruses [2].

A prolonged period of replication and the delay in host responses can enable further introduction of mutations that can lead to immune escape or exhaustion of the induced response, owing to higher numbers of infected cells [3]. If viral replication can be suppressed for a sufficient length of time, the viral load should decline to a point at which the continued production of quasispecies with the potential to resist drug treatment no longer occurs. Resolution of HCV infection may restore the down-regulation of innate and adaptive immunity. HCV nonstructural (NS) 3/4A protein blocks IFN-beta induction through the RIG-I pathway via the proteolytic cleavage of IFN-beta promoter stimulator-1 (IPS-1; also known as MAVS, VISA, or Cardif) [4, 5].

IFN-beta, compared with IFN-alpha, has different signaling and biological activities, and achieves a higher rate of viral clearance [6, 7, 8]. In contrast to IFN-alpha signaling, IFN-beta and IFN-lambda signaling in the liver do not become refractory during repeated stimulation of the IFN signaling transduction pathway. The sustained efficacy of IFN-beta and IFN-lambda may be important for the treatment of patients who do not respond to Peg-IFN-alpha through a pre-activated endogenous IFN system. Marked differences in the levels of IFN stimulated gene (ISG) induction by type I and III IFNs show a clearly detectable hierarchy (IFN-beta > IFN-alpha > IFN-lambda-3 > IFN-lambda-1 > IFN-lambda-2) (Figure 1). In the case of IFN-alpha, the induction of an antiviral state appears to be transient. In contrast, IFN-beta and all three IFN-lambdas induce long-lasting gene expression. Transcriptomic analysis in human hepatoma Huh7 cells or primary human hepatocytes stimulated with similar concentrations of IFN-alpha, IFN-beta, IFN-lambda-1, IFN-lambda-2, or lambda-3 has revealed a hierarchy of gene expression, with IFN-beta as the most biologically active cytokine, followed by IFN-alpha and IFN-lambdas. Both IFN-beta and IFN-lambda trigger a sustained ISG response. In contrast, IFN-alpha produces a kinetic profile of genes that peaks early in treatment and then rapidly decreases. No significant differences has been observed in STAT1 tyrosine phosphorylation levels between IFN-beta and IFN-alpha, whereas serine phosphorylation at position 727 is stimulated more strongly by IFN-beta than by IFN-alpha. The finding that IFN-beta invariably has the highest activity among type I and type III IFNs suggests that IFN-beta and IFN-lambda-3 may have superior clinical activity [9].

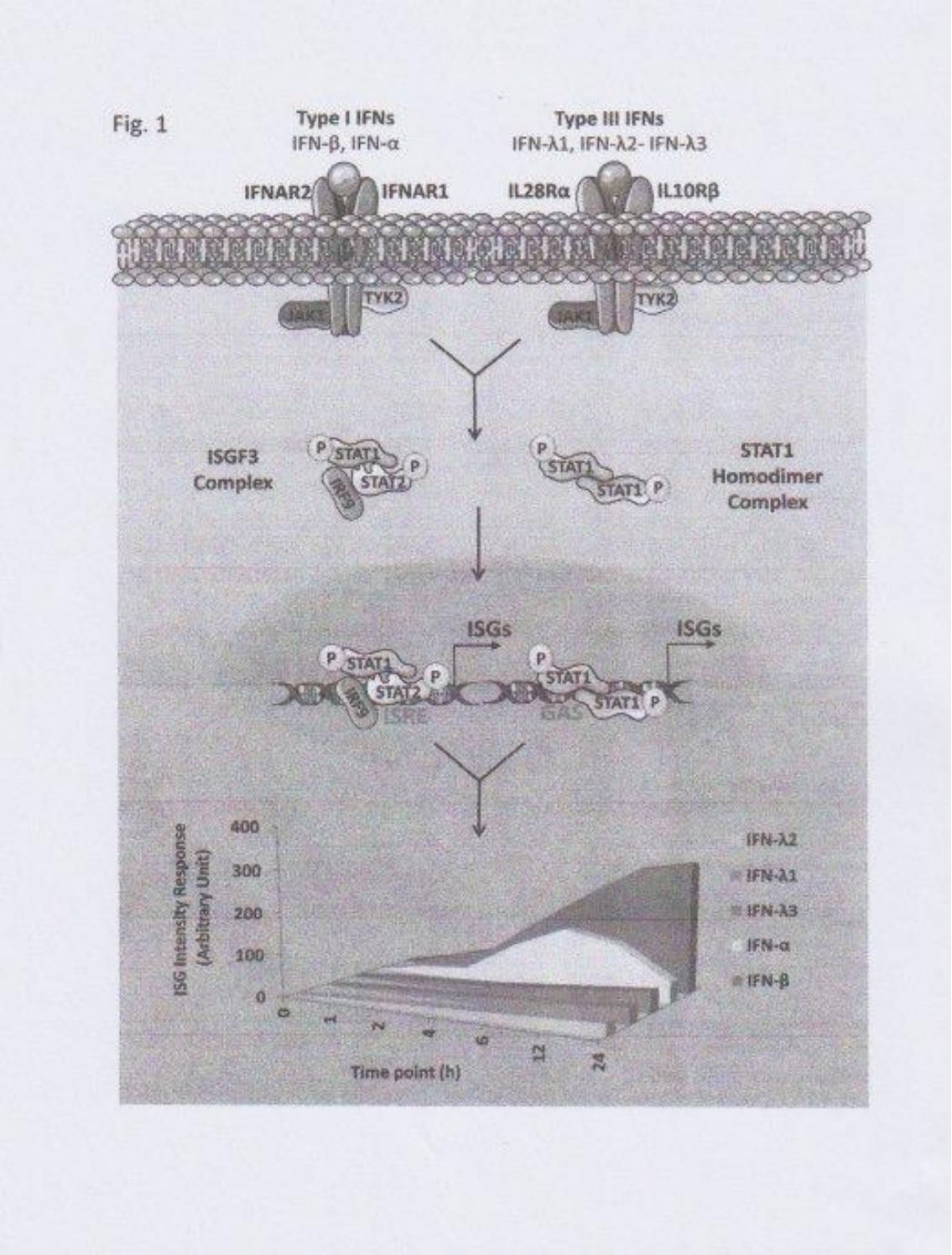

Figure 1. Type I and III IFN-signaling pathways lead to different kinetics and strengths of stimulation of the ISG response. Both type I and III IFNs induce STAT phosphorylation through the JAK-TYK kinases associated with the respective receptor subunits. Receptor engagement results in the activation of STAT1 and STAT2, which complexed with IRF 9 and form the transcription factor, ISGF3. STAT1 also homodimerizes after being phosphorylated. These complexes induced the expression of genes with ISRE or GAS in their promoters, thus leading to different kinetics and magnitudes of the ISG stimulation. The graph above indicates the relative abundance, kinetics, and magnitudes of the resulting ISG response to type I and III IFN. (Olagenier D and Hiscott J. Type I and Type III interferon Induced Immune Response: It's a Matter of Kinetics and Magnitude. Hepatology. 2014; 59 (4): 1225–1228.)

My previous study has indicated that cyclic and periodic IFN treatment (CPIT) consisting of induction treatment (IT) with natural (n)-IFN-beta (Feron®,Toray, Tokyo, Japan) and subsequent maintenance treatment (MT) with n-IFN-alpha (Sumiferon®, Sumitomo, Osaka, Japan) restores the innate immune responses, as shown by a significant decrease in CXCL-10, CXCL-8, and CCL-4, and a significant increase in interleukin (IL)-12 and IL-15 [10]. The findings indicated that early virologic clearance by IT with n-IFN-beta restored innate immune responses associated with adaptive immune responses, thus achieving SVR. HCV viral titers significantly decreased ($p < 0.05$) with respect to baseline after IT followed by Peg-IFN-alpha (Pegintron®, Schering Plough, Kenilworth, USA; 60-120 μg/day, percutaneously injection, once weekly) and RBV (Rebetol®: Schering Plough, Kenilworth, NJ, USA; 200−800 mg/day, per os, daily) [standard of care (SOC)] [novel combination treatment (NCT)] and SOC after the beginning of treatment. HCV RNA levels decreased more after SOC plus IT than SOC alone (Figure 2). The rate of rapid virologic response (RVR) in week 4, partial EVR in week 12, complete early virologic response (EVR) (extended RVR) in week 12, virologic response in week 24, end-of-treatment virologic response (ETVR) and SVR among patients with CHC with genotype (GT) 1b and high viral load receiving SOC, or SOC plus IT were 87.5% vs. 100%, 50% vs. 25%, 50% vs. 75%, 50% vs. 75%, 50% vs. 100% ($p = 0.0764$), and 37.5% vs. 75% ($p = 0.0435$), respectively. SOC plus IT (NCT) ($n = 8$) achieved a higher SVR rate than SOC ($n = 8$) in patients with intractable CHC (Figure 3). The results indicated the safety of the n-IFN-beta treatment and supported the use of n-IFN-beta as a safe and alternative option. SOC plus IT is more effective and has less adverse effects than SOC alone in patients with intractable CHC with GT 1b and a high viral load [11].

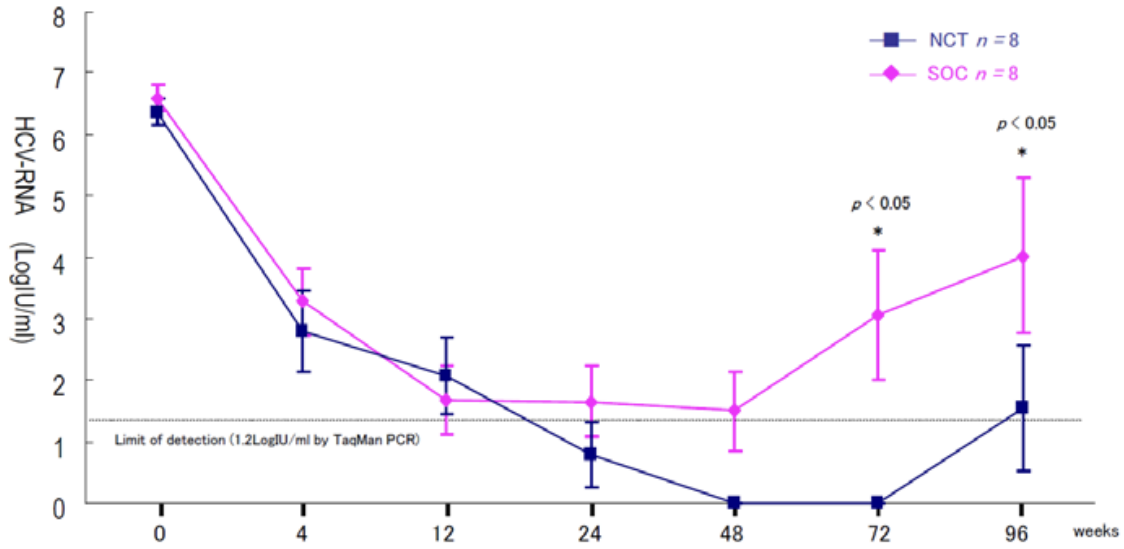

Figure 2. Changes in levels of serum HCV RNA during and after NCT and SOC in patients with CHC with GT1b and high viral load. NCT: novel combination treatment consisting of induction therapy with n-IFN-beta followed by SOC. SOC: standard of care, consisting of Peg-IFN-alpha 2b plus ribavirin (RBV).

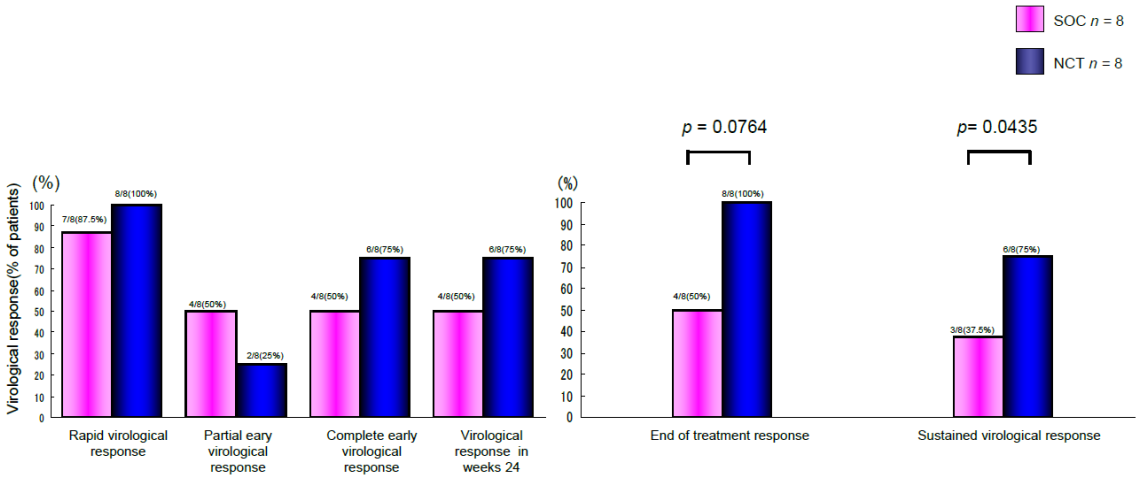

Figure 3. Rates of early virologic responses after 4, 12, and 24 weeks (left panel), and end-of–treatment virologic response, and sustained virologic response (right panel) in patients with CHC with GT 1b and high viral load treated with NCT or SOC according to intention-to-treatment, NCT: novel combination treatment consisting of induction therapy with n-IFN-beta followed by SOC. SOC: standard of care (SOC) consisting of Peg-IFN-alpha 2b plus RBV.

My previous study supports that viral clearance early in the course of therapy is associated with the restoration of innate and adaptive immune responses [10]. The restoration of the innate immune responses has potential as a novel therapeutic strategy for CHC. The efficacy and safety of IT with n-IFN-beta followed by Peg-IFN-alpha and RBV (PR) alone were compared with those of IT plus protease inhibitor (PI) plus PR (NCT with a PI) in patients with CHC with GT 1b and high viral load. IT plus PI and PR is beneficial for treating patients with intractable CHC [12]. CPIT with n-IFN-beta as IT, followed by triple therapy with PI and PR is more effective than IT with n-IFN-beta followed by PR alone in the treatment of patients with intractable CHC with GT 1b and high viral load.

Early virologic clearance by IT with n-IFN-beta induced the restoration of innate immune responses associated with adaptive immune responses, thereby resulting in SVR. HCV perturbs the activation of innate immune responses. The NS proteins of HCV, particularly NS3−4A, have been found to interfere with type I IFN induction pathways [13]. IFNs are the first line of defense against viruses and act directly on viral replication and indirectly via the activation of immune responses. The clearance of HCV may lead to the restoration of innate and adaptive immune responses. However, many challenges are associated with addressing the induction of persistent viral suppression associated with the restoration of innate immune responses resulting in SVR. Furthermore, IFNs, in contrast to direct-acting-antiviral agents (DAAs), have no resistance to HCV [14]. The presence of resistance associated viruses (RAVs) with resistance to NS5A inhibitors does not attenuate the efficacy of NS3 inhibitors or PR, and the combination of Simeprevir and PR may provide an alternative option to combination therapy with DAAs for treating Y93H RAV. Therapeutic options for the past two decades have consisted of treatments with IFN-alpha and RBV. HCV therapeutic approaches have rapidly evolved since the approval of the first DAAs in 2011. The development of DAAs represents a substantial improvement in the treatment of HCV infection. However, the emergence of RAVs with resistance to DAAs in the subset of patients who do not respond to DAAs represents a new issue [15, 16, 17]. Successful antiviral treatments result in a rapid decrease in serum levels of HCV RNA to undetectable levels that remain negative throughout therapy and thereafter. The faster the virus becomes undetectable during therapy, the better the chance of achieving SVR. Response rates with DAAs regimens are generally lower in patients who did not respond to previous treatments containing Peg-IFN-alpha than in treatment-naïve patients, and are markedly lower among prior null responders who are considered difficult to cure. Despite the enthusiasm for all-oral, IFN-free therapy, higher rates of virologic failure have been observed in early evaluation of real-world data. The mechanism of relapse after therapy with DAAs remains poorly understood. New treatments are not sufficient to eliminate HCV. Thus, more effective, tolerable, and/or tailored therapies are required for patients with a prior null response to PR therapy [18]. In addition to the restoration of innate immune responses due to viral suppression with CPIT during

the initial early course of therapy, persistent virologic clearance with CPIT followed by triple therapy consisting of PI (Simeprevir or Vaniprevir) with PR is more likely to result in higher RVR, EVR, ETVR, and SVR than those with NCT alone [12].

On the basis of these findings, I treated a patient with CHC with GT 1b, a high viral load, a prior null response to IFN treatments and chronic active hepatitis with advanced fibrosis by using IT with n-IFN-beta followed by triple therapy with Simeprevir (Sovriad®, Janssen, Titusville, NJ, USA, 100 mg/day per os, daily), Peg-IFN-alpha and RBV, which resulted in SVR and SBR. The levels of serum HCV RNA decreased after 4 weeks of CPIT. CPIT markedly decreased the levels of serum HCV RNA from 6.7 log IU/ml before treatment to less than 1.2 log IU/ml 12 weeks after CPIT with complete EVR. The CPIT was tolerated well, and only mild AEs developed. Subsequent Simeprevir, Peg-IFN-alpha-2b, and RBV treatment caused persistent virologic clearance without virologic breakthrough and relapse, thus resulting in an ETVR and a SVR 24 weeks after cessation of the treatment. Levels of serum alanine amino transferase (ALT) decreased within 3 weeks after the initiation of CPIT and were only slightly elevated during the CPIT. After the initiation of triple therapy, levels of serum ALT decreased to a normal range and subsequently remained normal (SBR) (Figure 4) [12].

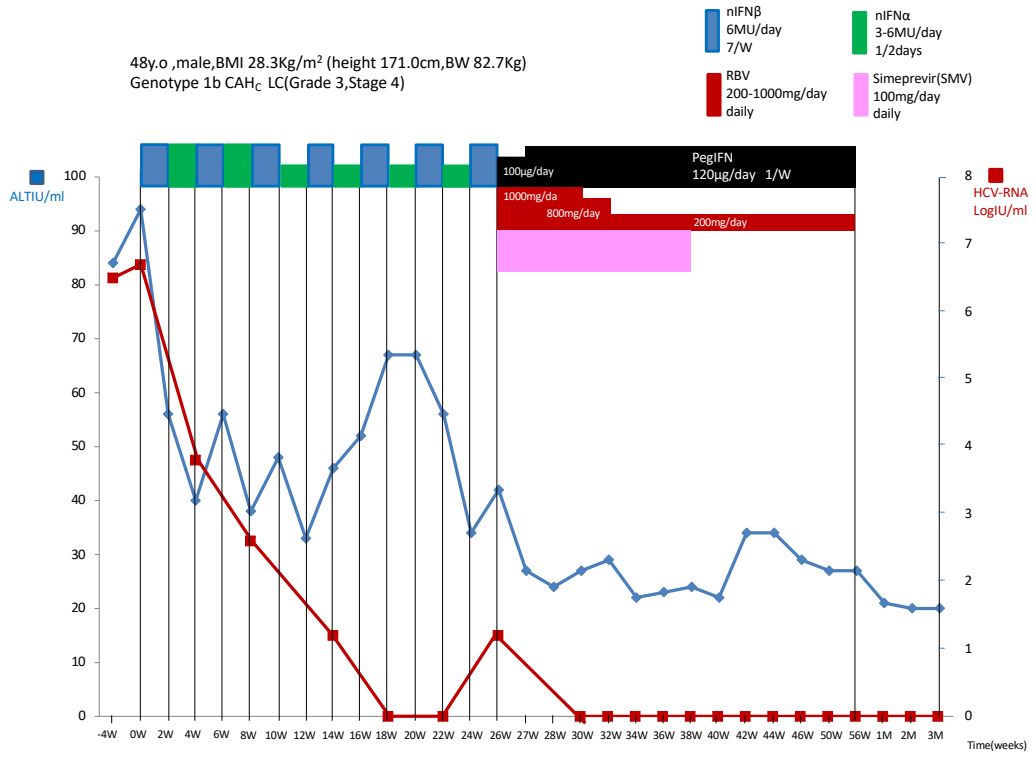

Figure 4. Serial determinations of serum levels of HCV RNA and alanine amino transferase (ALT) in a patients with CHC [a 48 year old man, BMI 28.3 kg/m^2, serotype 1 (GT 1b), level of serum HCV RNA 6.7 log IU/ml, chronic active hepatitis with advanced fibrosis (grade 3, Stage 3−4) who was treated with CPIT for 26 weeks and subsequently with Peg-IFN-alpha and RBV for 18 weeks. CPIT: cyclic and periodic IFN treatment consisting of an induction treatment with n-IFN beta followed by a maintenance treatment with n-IFN-alpha.

On the basis of these findings, the efficacy and safety of CPIT with n-IFN-beta as IT followed by triple therapy with PI plus PR (NCT with PI) versus CPIT followed by PR (NCT) alone were compared in patients with CHC with GT 1b and high viral load. The SVR rates were significantly higher among patients receiving CPIT followed by triple therapy with PI (Simeprevir or Vaniprevir) with PR than patients receiving CPIT followed by PR alone. CPIT followed by triple therapy with PI plus PR was more effective than CPIT followed by PR alone in treating patients with intractable CHC with GT 1b and a high viral load. The restoration of innate immune responses has potential as a novel therapeutic strategy for CHC.

Patients and Methods:

The efficacy and safety of IT with n-IFN-beta followed by PR (NCT) alone (group A, n = 30) versus IT followed by PI (Simeprevir or Vaniprevir) with PR (NCT with PI) (group B, n = 13) were compared in patients with CHC with GT 1b and high viral load.

Group A: standard treatment with IT (NCT) (n=30): 30 patients with CHC were treated with CPIT with n-IFN-beta for 24 weeks followed by PR for 48 weeks (NCT). The outcomes of previous treatments indicated 26 naïve, 4 relapsers and 0 null responders in group A.

Group B: PI treatment with IT (NCT with PI) (n = 13): CPIT for 24 weeks as IT, followed by PI (Simeprevir or Vniprevir) with Peg-IFN-alpha-2b and RBV for 24−36 weeks (a total of 48−60 weeks). Ten patients with CHC were treated with Simeprevir for 12 weeks. Three patients with CHC were treated with Vaniprevir (Vanihep®, MSD, Whitehouse Station, NJ, USA, 400 mg/day, peroral, daily) for 24 weeks. The outcomes of previous treatments indicated four naïve, five relapsers, and four null responders in group B.

The efficacy and safety was compared between group A (n = 30) and group B (n = 13) in patients with CHC with GT 1b and high viral load. Patients had undergone liver biopsy within 6 months before treatment initiation. In one patient in group B, hepatic fibrosis was assessed with FibroScan (Fibroscan®, Echosens SA, Paris, France) and showed 5.3 kPa (F0–1). All patients were monitored with clinical and biochemical assessments. Virologic responses were determined before and every 1−4 weeks during the 48−72-week treatment period, and were followed for at least an additional 24 weeks after treatment cessation. Levels of serum HCV RNA were determined with quantitative TaqMan real time polymerase chain reaction. Direct sequencing of the NS3 and NS5A regions of HCV RNA was successfully performed for two patients in group B. The IL28B genotype had been determined by the rs8099917, rs1188122, and rs8103142 single-nucleotide polymorphisms for the polymerase chain reaction (PCR) amplification and sequencing of patients in group B.

Written informed consent was obtained from all study patients. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee in Japan.

Assessments and Efficacy Endpoints

The primary efficacy endpoint was SVR, defined as undetectable HCV RNA serum levels 12–24 weeks after treatment completion. Secondary endpoints included RVR, defined as undetectable HCV RNA serum levels at the end of 4 weeks of treatment, and EVR, defined as undetectable HCV RNA serum levels at the end of 12 weeks of treatment.

Measurements to Improve Treatment Adherence

Full adherence to all drugs regimens is associated with high SVR rates. In contrast, suboptimal exposure to therapy is associated with virologic breakthrough or post-treatment relapse and the emergence of RAVs, particularly during the early phases of treatment. Before the initiation of antiviral therapy, patients were informed of the schedule and AEs to be expected during the treatment. Patients were instructed on preventive and therapeutic measures to ameliorate these AEs.

Assessments of Safety

AEs were monitored every 1−4 weeks during and after the end of the treatment. Laboratory (hematology and biochemistry) tests, vital signs, symptoms of fatigue, flu-like symptoms, electrocardiography assessments, and physical examinations were performed at screening and thereafter at regular intervals thereafter throughout the treatment. The Peg-IFN-alpha-2b dose was decreased from 60−120μg to 50−100 μg/day for the management of AEs or laboratory abnormalities that reached the predetermined thresholds of severity. The RBV dose was decreased from 1000 to 200 mg per day for the management of AEs or laboratory abnormalities that had reached the predetermined thresholds of severity. If AEs were resolved or improved, a return to initial dosing levels was permitted.

Results

Table 1 summarizes the baseline characteristics of the study population.

Table 1.Characteristics in chronic hepatitis C patients with serotype 1 and high viral loads

Characteristics	Standard Treatment with Induction Therapy (n=30)	Protease Inhibitor Treatment with Induction Therapy (n=13)
Sex(M/F)	10/20	2/11
Age(yr),mean±SD	58.9±10.1	64.0±8.7
Weight(kg),mean±SD	58.2±10.1	58.7±10.4
Body mass index(kg/m^2),mean±SD	23.2±3.9	24.0±3.3
Alanine amoninotransferase;ALT(IU/l),mean±SD	65.2±51.6	44.3±23.7
HCV RNA(LogIU/ml),mean±SD	6.5±0.4	6.6±0.4
Liver histology(Stage;F),n(%)		
F0	2(7)	1 (8)
F1	15(50)	4(31)
F2	7(23)	3(23)
F3~4	3(10)	3(23)
missing	3(10)	2(15)
Hb(g/dl),mean±SD	13.3±1.3	13.0±1.8
Platelets(10^4/μl)	18.2±6.8	16.0±5.0
Outcome of Previous Treatment Naive/relapser/null responder	26/4/0	4/5/4

Table 1. Standard Treatment with Induction Therapy; induction therapy with n-IFN-beta followed by Peg-IFN-alpha and RBV (PR), PI Treatment with Induction Therapy; standard treatment with induction therapy with PI (Simeprevir or Vaniprevir), Induction Therapy (IT); treatment with cyclic and periodic IFN treatment (CPIT) which consisting of induction treatment with natural IFN-beta and maintenance treatment with natural IFN-alpha, Hb; hemoglobin in the peripheral blood

The levels of serum HCV RNA were 6.5 ± 0.4 log IU/mL in group A and 6.6 ± 0.4 log IU/mL in group B at baseline. The levels of HCV RNA significantly decreased ($p < 0.05$) from the baseline in groups A and B after the beginning of the treatment. During IT with CPIT, the virologic response rates in groups A and B were 10% and 8% ($p = 0.6792$) at week 4 (RVR); 30% and 16% ($p = 0.6989$) at week 12 (EVR); and 47% and 20% ($p = 0.0887$) at week 24 (at the end of IT) (Figure 5).

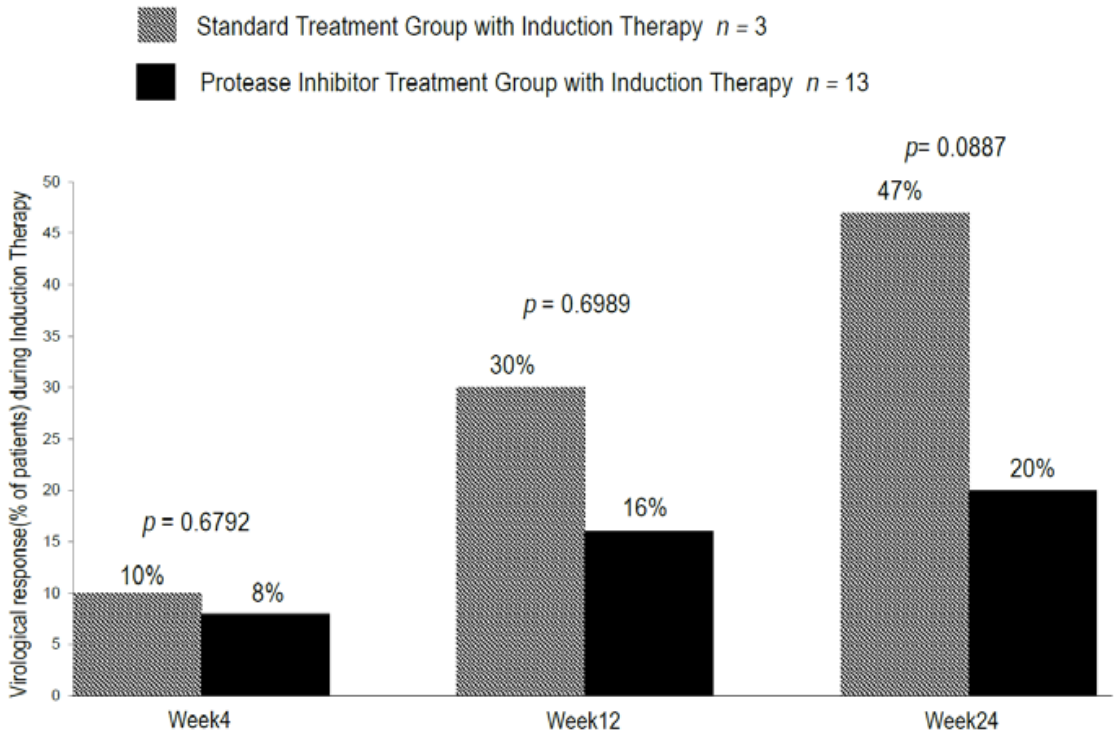

Figure 5. Rates of early virologic responses in the initial 4, 12, and 24 weeks in patients with CHC with GT 1b and high viral loads during IT in standard treatment with IT with n-IFN-beta (NCT) (n = 30) and PI treatment with IT with n-IFN-beta (NCT with PI) (n = 13). Virologic response was defined by undetectable serum levels of HCV RNA (< 15 IU/mL). Paired-t test was used to evaluate the differences in the means between two groups; a p-value < 0.05 was considered significant.

During and after treatment with PR alone, or with PI plus PR, the virologic response rates in groups A and B were 50% and 82% (p = 0.01535) at week 4; 53% and 91% (p = 0.006745) at week 8; 57% and 91% (p = 0.001126) at week 12 (Figure 6); 57% and 100% (p < 0.001845) at the end of the treatment; and 57% and 80% (p < 0.005166) 12 weeks after the cessation of treatments (SVR) (Figure 7).

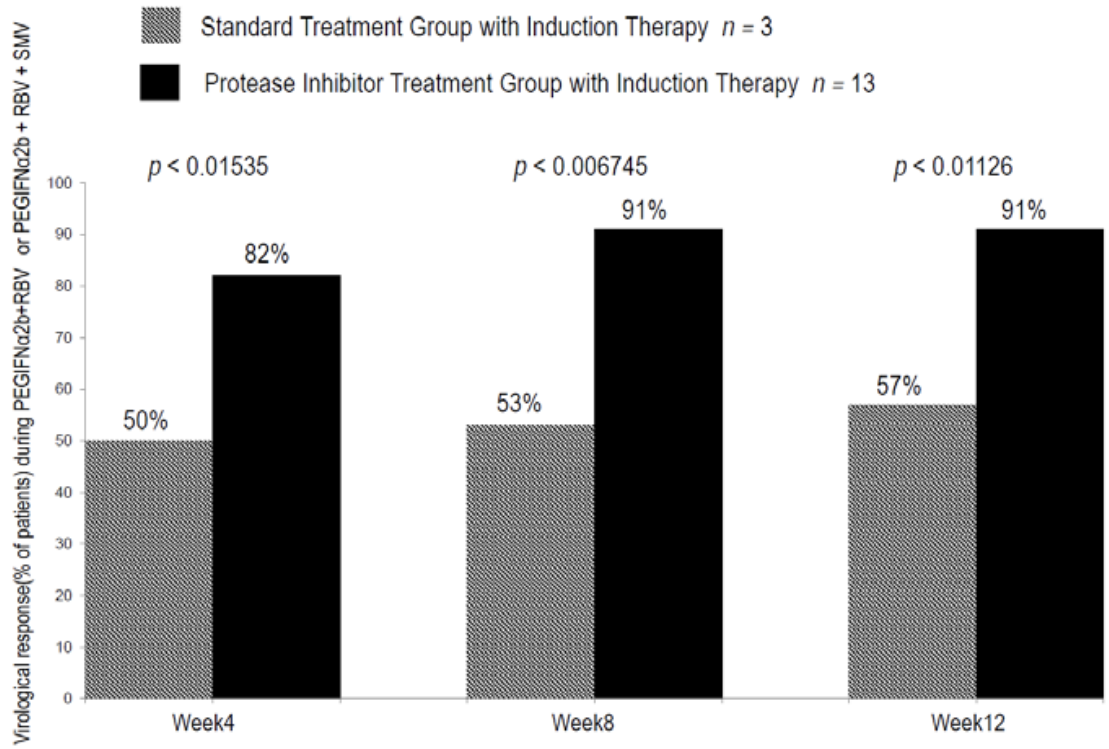

Figure 6. Rates of early virologic responses in the initial 4, 8, and 12 weeks in patients with CHC with GT 1b and high viral load during standard treatment with IT with n-IFN-beta (NCT) (n = 30), and PI treatment with IT with n-IFN-beta (NCT with PI) (n = 13). Virologic response was defined by an undetectable serum level of HCV RNA (<15 IU/mL). Paired-t test was used to evaluate the differences in the means between two groups; a p-value of <0.05 was considered significant.

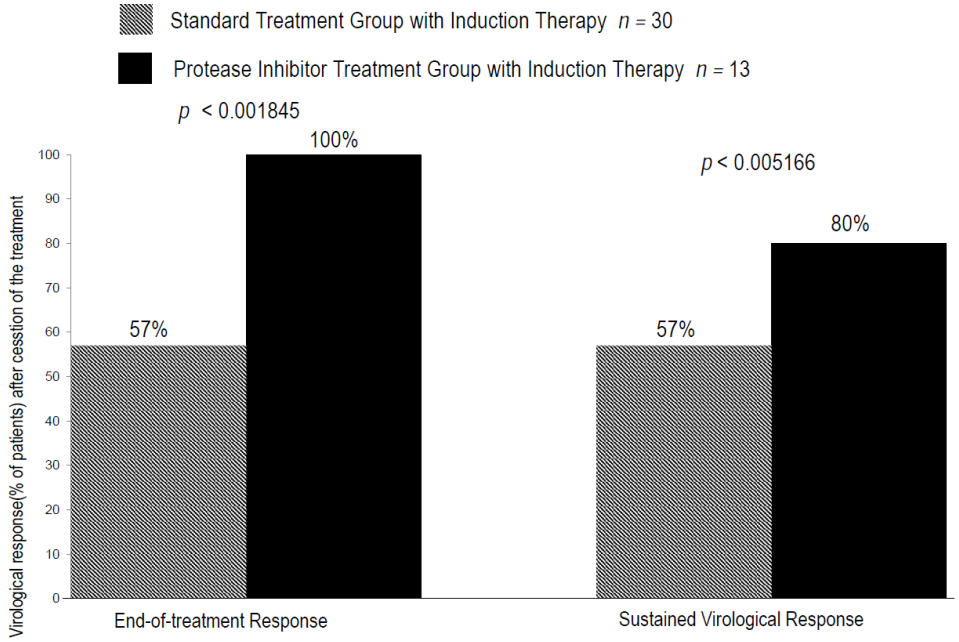

Figure 7. Rates of virologic responses at the end-of treatment and rates of SVR in patients with CHC with GT 1b and high viral load with standard treatment with induction therapy with n-IFN-beta (NCT) (n = 30) and PI treatment with IT with n-IFN-beta (NCT with PI) (n = 13).

During IT with CPIT, the virologic response rates were higher in group A than in group B. However, during the treatment with PI plus PR or PR after the cessation of IT, virologic response rates were significantly higher in group B than in group A. No patients exhibited virologic breakthrough. Two

patients in group B showed virologic relapse. One of the two patients with an HCV variant with a mutation at NS3 aa position D168, which was an HCV resistant to NS3A PI, and the major type of the IL 28B genotype (rs8099917, rs1188122, rs8103142), relapsed 4 weeks after the treatment with PI plus PR, was discontinued. Another patient with an HCV variant with a mutation at NS3A aa position D168A/V, which was resistant to NS3A PI; the HCV variant with a mutation at NS5A aa position Y93H, which was resistant to NS5A; and the hetero-type of the IL28B genotype (rs8099917, rs1188122, rs8103142) relapsed 4 weeks after the treatment with PI plus PR was discontinued. These patients were considered virologic relapsers (DAA failure) and difficult to cure. These two virologic relapsers were successfully treated with DAAs [NS5A inhibitor (Sofosbuvir) and an NS5B polymerase inhibitor (Ledipasvir)] as a retreatment strategy. The overall safety profile was similar in the two groups. The levels of serum ALT in groups A and B (65.2±51.6 vs. 44.3 ± 23.7 IU/l at baseline) decreased to 29.3±18.2 ($p = 0.0010$) in group A and to 29.0 ± 21.2 ($p = 0.2262$) in group B 12 weeks after treatment cessation.

Discussion

A low baseline HCV viral load has been identified as an independent predictor of SVR [19]. Viral kinetics in response to anti-HCV treatments is considered an important factor during treatments. Successful antiviral treatments result in rapidly decrease serum HCV RNA to undetectable levels that remain negative throughout therapy and thereafter. Early virologic clearance by IT with n-IFN-beta induces the restoration of innate immune responses associated with adaptive immune responses, thus resulting in SVR [10]. My previous study has indicated higher SVR rates in patients with GT 1b HCV infection receiving IT with n-IFN-beta followed by PR than receiving with PR alone [11].

In addition to the restoration of innate immune responses due to viral suppression with CPIT during the initial early course of therapy, persistent virologic clearance with triple therapy consisting of PI (Simeprevir or Vaniprevir) with PR is associated with higher RVR, EVR, ETVR, and SVR [12]. The findings of my previous study support the concept that viral clearance early in the course of therapy is associated with the restoration of innate and adaptive immune responses, thereby suggesting that agents providing viral suppression leading to RVR and EVR, including n-IFN-beta, IFN-lambda, DAAs, and newly developed agents, may be preferable in initial early IT. Initial viral clearance induced by a CPIT in combination with these agents may lead to the restoration of innate and adaptive immune responses, thus resulting in SVR in patients with intractable CHC. CPIT consisting of IT with n-IFN-beta and subsequent maintenance treatment with n-IFN-alpha prevents viral breakthrough, achieves RVR and EVR, and induces restoration of innate immune responses, as shown by a significant decrease in CXCL-10, CXCL-8, and CCL-4, and a significant increase in IL-12 and IL-15 in CHC [10].

Response rates with DAA regimens are generally lower in patients who did not respond to previous treatments containing Peg-IFN-alpha than in treatment-naïve patients, and are markedly lower among prior null responders who are considered difficult to cure. Despite the enthusiasm for all-oral, IFN-free therapy, higher rates of virologic failure have been observed in early evaluation of real-world data. The mechanism of relapse after therapy with DAAs remains poorly understood. New treatments are not sufficient to eliminate HCV infection. Thus, more effective, tolerable, and/or tailored therapies are required for patients with a prior null response to PR therapy.

NCT induced the restoration of innate immune responses associated with adaptive immune responses, thus resulting in SVR. I have previously reported treatment of a patient with intractable CHC with GT 1b, a high viral load, null response to previous IFN treatments, and advanced hepatic fibrosis with IT with n-IFN-beta, followed by triple therapy with Simeprevir with PR, which resulted in SVR and SBR. In addition to the restoration of innate immune responses due to viral suppression with CPIT during the initial early course of therapy, persistent virologic clearance with triple therapy consisting of PI (Simeprevir or Vaniprevir) plus PR is more likely to result in higher RVR, EVR, ETVR, and SVR than those in NCT alone [12].

On the basis of these findings, this study compared the efficacy and safety of CPIT with n-IFN-beta as IT, followed by triple therapy with PI plus PR versus those of CPIT with n-IFN-beta followed by PR alone, in patients with CHC with GT 1b and high viral load. Viral suppression was associated with the restoration of innate immune responses with IT with n-IFN-beta followed by PI and Peg-IFN-alpha and RBV, and the treatment was tolerated well without discontinuation, overcame viral breakthrough, and induced persistent viral clearance, thus leading to an enhanced early virologic response, and resulting in SVR and SBR in patients with intractable CHC with GT 1b and high viral load.

CPIT with n-IFN-beta followed by triple therapy with PI (Simeprevir or Vaniprevir) and PR showed significantly higher SVR rates than patients receiving CPIT followed by PR alone, and also

showed effective, eradication of HCV infection and mild AEs. The restoration of innate immune responses may provide a novel therapeutic strategy for chronic HCV infection.

IFN-free, DAA-based antiviral strategies and the understanding of their future clinical use in patients with CHC have markedly improved. High SVR rates are achievable with IFN free, DAA-based regimens in patients with CHC.

In clinical trials with new IFN-free, DAA-based therapies, treatment failure occurs in 5%–7% of patients on average. Most treatment failures are post-treatment relapses and, and at the time of failure, these patients harbor viral populations resistant to more than one of the DAAs administered [20, 21, 22, 23, 24, 25]. Patients in whom DAA-containing regimens fail to eradicate HCV are likely to select viral populations that are resistant to the DAAs administered. Retreatment strategies must be assessed in patients who still harbor resistant viruses at the time of retreatment. HCV variants resistant to NS3A PI and to non-nucleoside inhibitors of the HCV RNA polymerase are currently selected in patients who do not respond to therapies based on these drugs. However, RAVs decrease progressively after treatment interruption and are no longer detectable within 12−16 months of the treatment. This aspect is particularly important in patients exposed to NS5A inhibitors, because these variants appear to persist as dominant species for many months to years, and potentially for life, after treatment failure [14]. HCV resistance, particularly resistance to NS5A inhibitors, is emerging as a novel issue in patients who do not to achieve SVR with IFN-free, DAA-based therapy. Sequential treatments with non-curative prior DAAs therapies may generate complex resistant variants, thus limiting future treatment options. For example, NS5B resistance has not been observed in treatment-naïve patients in whom, Sofosbuvir (SOF)-containing regimens have failed; however, SOF-associated NS5B resistant variants have emerged in patients who relapsed after receiving SOF-containing regimens and retreatment with SOF-Ledipasvir.

Patients have achieved viral clearance with IFN-based treatments [14], which are considered more useful than IFN-free DAA treatment for patients with CHC with the major type of the IL28B genotype. The mechanism underlying the IFN susceptibility of Y93H RAV remains unclear. Previous studies have shown that Y93H RAV is relatively less frequently detected in patients with the unfavorable IL28B genotype non-TT (rs8099917); that is. Y93H RAV may have a greater tendency to replicate in the liver in IL28B TT patients than in non-TT patients. The most prominent difference in the liver environment between IL28B TT and non-TT is the hepatic expression of ISGs, which is higher in non-TT patients, thus potentially reflecting the basal activation of intrinsic IFN system. One possible hypothesis is that Y93 RAV is susceptible to externally administered IFN [14]. Furthermore, RBV is a useful therapy adjunct. HCV directly impairs immune function by interfering with IFN production and signaling [16].

Therefore, the suppression of HCV by DAAs may restore innate immune function, thus augmenting the benefits of therapy [21, 22, 23]. The benefits of IT with CPIT in enhancing RVR and EVR rates may be relevant in treatment strategies involving a combination of IT with CPIT with n-IFN-beta followed by PR with DAAs in patients with intractable CHC. In my previous study [12], I reported that a patient with intractable CHC with GT 1b, who was a prior null responder to IFN treatments and had chronic active hepatitis with advanced fibrosis was successfully treated with IT with CPIT with nIFN-beta followed by PI (Simeprevir) and PR; these treatments were tolerated well and were associated with only mild AEs. The present study showed that the virologic responses rates for IT with CPIT with nIFN-beta followed by PI plus PR were significantly higher than those for IT with CPIT with n-IFN-beta followed by PR alone in patients infected with HCV GT 1b and high viral load. During IT with CPIT, the virologic response rates in group B were lower than those in group A. These lower virologic response rates might be attributable to the outcomes of previous treatments; group A had zero null responders [0/30 (0.0%)], whereas group B had four [4/13 (30.7%)]. However, during and after the treatment with PR alone, or PI plus PR, the virologic response rates were significantly higher in group B than in group A. CPIT with n-IFN-beta followed by PI plus PR was tolerated well; enhanced the RVR, EVR, ETVR and SVR rates in patients with intractable CHC with GT 1b and high viral load; and was associated with only mild AEs. Higher virologic response rates highlight the benefit of PI plus PR with IT with n-IFN-beta in patients with CHC with GT 1b and high viral load. Early virologic clearance due to CPIT with n-IFN-beta for 24 weeks before the beginning of PI plus PR induced the restoration of innate immune responses associated with adaptive immune responses, thus resulting in SVR and SBR. SVR rates were significantly higher in patients with CHC patients with GT 1b and high viral load receiving CPIT followed by PI plus PR than receiving CPIT with n-IFN-beta followed by PR alone. CPIT followed by PI plus PR was beneficial for treating patients with intractable CHC with GT 1b and high viral load.

Persistent HCV clearance continued in my patients, and HCV RNA levels decreased after IT followed by triple therapy. These results suggested that IT is associated with decreased HCV RNA levels before the beginning of triple therapy with PI plus PR, and may be used to treat patients with intractable CHC with GT 1b and a high viral load.

Two patients in group B showed virologic relapse. One of the two patients, who had an HCV variant with a mutation at NS3A aa position D168 which was resistant to NS3A PI, and the major type of the IL28B genotype (rs8099917, rs11881222, rs8103142), relapsed 4 weeks after the treatment with PI plus PR was discontinued. The other patient, who showed the HCV double variant with a mutation at NS3A aa position D168, which was resistant to NS3A PI; the HCV variant with a mutation at NS5A aa position Y 93H, which was resistant to NS5A; and the hetero-type of the IL28B genotype (rs8099917, rs1188122, rs8133142) relapsed 4 weeks after the treatment with PI plus PR was discontinued. These patients were considered virologic relapsers (DAA failure) and difficult to cure. How to treat patients with RAVs in whom eradication fails on DAA-containing regimens remains unresolved. SOF plus PR for 12 weeks is effective and safe in patients who have not achieved SVR with earlier regimens of one or more DAAs plus PR. One potential reason for this result is that SOF is in a different therapeutic class and has been demonstrated to have a very high barrier to resistance [24]. Virologic relapsers may be successfully treated with IT with CPIT with n-IFN-beta followed by DAAs, such as the NS5B polymerase inhibitor, with PR as a retreatment strategy.

Strategy for Treatment Failure in Intractable HCV

Resistance-associated substitutions (RASs) in HCV genome are a major cause of failed treatment. RASs in the genome of HCV are 1 of the major causes for failed treatment [15]. RASs have been reported after failure of various treatments for CHC, and more complicated RASs have been found to accumulate in the viral genome with successive failed treatments. The highly resistant P32 at the NS5A region is uniquely found in patients in whom DAAs treatment has failed, and is associated with the presence or absence of specific RASs [26]. The prevalence of patients with RASs in NS3A, or NS5A, or both, increases significantly with increasing numbers of failed regimens. Among DAA-naive GT1b-infected patients, the baseline prevalence of NS3-D168E is 1.2%, that of NS5A-L31M is 3.6%, and that of NS5A-Y93H is 17.6%. Baseline polymorphisms in NS3 or NS5A are less prevalent in GT2, with the exception of the common L/M31 polymorphism in NS5A. The baseline prevalence of the NS5A P 32 deletion is 6.3% [27].

Failure of multiple DAAs regimens can lead to the generation of multiple RASs in the NS3 and NS5A regions of the HCV 1b genome. These mutations contribute to viral resistance to multiple treatment regimens and, therefore, should be considered during decision-making for treatment of chronic HCV infection. No FDA-approved treatments are currently available for prior DAA failures. IFNs, in contrast to DAAs, have no resistance to HCV. IFNs exert board antiviral effects and contribute to the clearance of resistant HCV. Therefore, IFNs may play a role in the treatment of patients with DAA resistance and enhance the success of retreatment with NCT with DAAs [14]. CPIT with n-IFN-beta as IT, followed by triple therapy with DAAs [NS3/4A PI (Glecaprevir) plus NS5A inhibitor (Piblentasvir) [28] or NS5B polymerase inhibitor (Sofosbuvir) plus NS5A inhibitor (Velpatasvir or Daclatasvir)] with Peg-IFN-alpha and RBV might potentially play a role in treatment and enhance treatment success in patients with CHC with DAA resistance and mutations at NS3A aa position D168 and/or at NS5A aa position Y93H, L31F, L31 I, L31M, L31V, or highly resistant P32, A92 K regions of the HCV 1b genome which are found in patients in whom DAA treatment has failed.

Summary

The findings of my previous study have indicated that CPIT consisting of IT with n-IFN-beta and subsequent maintenance treatment with n-IFN-alpha prevents viral breakthrough and achieves RVR and EVR, and restores innate immune responses, as shown by a significant decrease in CXCL-10, CXCL-8 and CCL-4, and a significant increase in IL-12 and IL-15 in CHC [10]. Early virologic clearance by IT with n-IFN-β induced the restoration of innate immune responses associated with adaptive immune responses, thereby resulting in SVR. My previous study revealed higher SVR rates in patients with GT 1b HCV infection receiving IT with n-IFN-beta followed by PR than receiving PR alone [11]. In addition to the restoration of innate immune responses due to viral suppression with CPIT during the initial early course of therapy, persistent virologic clearance with triple therapy consisting of PI (Simeprevir or Vaniprevir) plus PR is more likely to result in higher RVR, EVR, ETVR, and SVR than those with NCT alone. I have previously reported that a patient with intractable CHC, GT 1b, a high viral load, null response to previous IFN treatments, and advanced hepatic fibrosis was treated by IT with n-IFN-beta followed by triple therapy with

Simeprevir with PR, which resulted in SVR and SBR [12]. On the basis of these findings, this study compared the efficacy and safety of CPIT with n-IFN-beta as IT followed by triple therapy with PI plus PR versus CPIT with n-IFN-beta followed by PR alone in patients with CHC with GT 1b and high viral load. Viral suppression was associated with the restoration of innate immune responses with IT with n-IFN-beta followed by PI with Peg-IFN-alpha and RBV, and the treatment was tolerated well without discontinuation, overcame viral breakthrough, and induced persistent viral clearance, thus leading to an enhanced early virologic response, and resulting in SVR and SBR in patients with intractable CHC with GT 1b and high viral load. Patients with intractable CHC receiving CPIT with n-IFN-beta followed by triple therapy with PI (Simeprevir or Vaniprevir) plus PR, compared with those receiving CPIT followed by PR alone, showed significantly higher SVR rates, effective eradication of HCV infection, and only mild AEs.

Conclusion

CPIT with n-IFN-beta as IT followed by triple therapy with PI and PR was more effective for the treatment of patients with intractable CHC with GT 1b and high viral load than IT with n-IFN-beta followed by PR alone. The restoration of innate immune responses induced by clearance of HCV has potential as a novel therapeutic strategy for CHC. In strategies for treatment failure for intractable HCV infection, IFNs may play a role in the treatment of patients with DAAs resistance and enhance the success of retreatment with CPIT with PR (NCT) with DAAs in intractable CHC.

Conflict of Interest Statement

The author has no conflicts of interest to declare.

References

1. World Health Organization (WHO). Global Hepatitis Report 2017. World Health Organization; 2017. [Accessed December 9,2021] https://www.who.int/publications/i/item/global-hepatitis-report-2017.
2. Yutaka Kishida. Immunological aspects controlling hepatitis C virus infection. Research Trends; Current Topics in Virology. 2019;16:75-93.
3. Major EM, Dahari H, Mihalik K, Puig M, Rice CM, Neuman AU, Feinstone SM. Hepatitis C virus kinetics and host responses associated with disease and outcome of infection in Chimpanzees. Hepatology. 2004;39:1709-1720. DOI: 10.1002/hep.20239
4. Li XD, Sun L, Seth RB, Pineda G, Chen ZJ. Hepatitis C virus protease NS3/4A cleaves mitochondrial antiviral signaling protein off the mitochondria to evade innate immunity. Proc Natl Acad Sci USA. 2005; 102: 17717-17722. DOI: 10.1073/pnas.0508531102
5. Loo YM, Owen DM, Li K, Erickson AK, Johnson CL, Fish PM, Carney DS, Wang T, Ishida H, Yoneyama M, Fujita T, Saito T, Lee WM, Hagedorn CH, Lau DT, Weinman SA, Lemon SM, Gale M Jr. Viral and therapeutic control of IFN-beta promoter stimulator and during hepatitis C virus infection. Proc Natl Acad Sci USA. 2006; 103 (15): 6001-6006. doi: 10.1073/pnas.0601523103.
6. Platanias LC, Shahab US and Colamonics OR. Tyrosine phosphorylation of the alpha and beta Subunit of the Type 1 interferon Receptor. J Biol Chem. 1994; 269 (27): 17761-17764.
7. Domarski P, Nadeu OW, Platanias LC, Fish E, Kellum M, Pitha P, Colamonici OR. Differential use of the betaL subunit of the type 1 inteferon (IFN) receptor determines signaling specificity for IFN-alpha 2 and beta. J Biol Chem. 1998; 273: 3144-3147. doi: 10.1074/jbc.273.6.3144.
8. Da Silvia AJ, Brickelmaier M, Majeau GM, Lukashin AV, Peyman J, Whitety A, Hochman PS. Comparison of gene expression patterns induced by treatment of human umbilical vein endothelial cells with IFN-alpha 2b vs IFN-beta 1a understanding the functional relationship between distinct type 1 interferons that act through a common receptor. J interferon Cytokine Research. 2002; 22: 173-188. DOI: 10.1089/10799902753536149
9. Olagenier D and Hiscott J. Interferon (IFN)-beta has different signaling and biological activities from IFN-alpha, and achieves a higher rate of viral clearance than IFN-alpha. Hepatology. 2014; 59:1225-1228.
10. Yutaka Kishida, Yoshimichi Haruna, Masahumi Naitoh, Kazuhiro Katayama, Toru Kashiwagu. Multiple Cytokine Profiling of the Therapeutic Responses to Ribavirin and Pegylated Interferon-alpha2 Using "Induction" Approach With Natural Interferon-bet in Difficult-to-Treat Chronic Hepatitis C. Journal of Interferon and Cytokine Research. 2009; 29 (6): 353-368.
11. Yutaka Kishida, Naohiko Imaizumi, Hirihisa Tanimura, Toru Kashiwagi. Restoration of innate and adaptive immune responses by HCV viral inhibition with an induction approach using natural IFN-beta in chronic hepatitis C. Clinical and Developmental Immunology. 2012; Article ID 582716: 1-15. doi: 10.1155/2012/582716
12. Yutaka Kishida, Naohiko Imaizumi, Hirohisa Tanimura, Shinichiro Kashiwamura, Toru Kashiwagi. Treatment of Chronic Hepatitis C with Viral-Suppression linked to Restoration of Innate-Immune Responses with Induction-Therapy with n-IFN-beta followed by Simprevir. MOJ Immunology. 2015; 2(2): 1-7.
13. Liang Y, Cao X, Ding Q, Zhao, He Z, Zhong J. Hepatitis C virus NS4B induces the degradation of TRIF to inhibit TLR3-mediated interferon signaling pathway. PLoS Pathog. 2018; 14 (5), e1007075. doi: 10.1371/journal. Ppat. 1007075.
14. Itakura J, Kurosaki M, Higuchi M, Takada H, Nakakuki N, Itakura Y, Tamaki N, Yasui Y, Suzuki S, Tsuchiya K, Nakanishi H, Takahashi Y, Maekawa S, Enomoto N, Izumi N. Resistance-associated NS5A variants of hepatitis C Virus are susceptible to interferon-based therapy. PLos One. 2015; 10(9). Doi: 10.1371/journal.pone.0138060
15. Itakura J, Kurosaki M, Kakizaki S, Amano K, Nakayama N, Inoue J, Endo T, Marusawa H, Hasebe C, Joko K, Wada S, Akahane T, Koushima Y, Ogawa C, Kanto T, Mizokami M, Izumi N. Features of resistance-associated substitutions after failure of multiple direct-acting antiviral regimens for hepatitis C. JHEP Rep. 2020; 2 (5): 100138. doi: 10.1016/j-jhepr. 2020.100138.
16. Gozlan Y, Bucris E, Shirazi R, Rakovsky A, Ben-Ari Z, Davidov Y, Veizman E, Saadi T, Braun M, Cohen-Naftaly M, Shlomai A, Shibolet O, Zigmond E, Katchman H, Menachem Y, Safadi R, Galun E, Zuckerman E, Nimer A, Hazzan R, Maor Y, Saif AB, Etzion O, Lurie Y, Mendelson E, Mor O.. High frequency of multiclass HCV

resistance-associated mutations in patients failing direct-acting antivirals: real-life data. Antiviral Ther. 2019; 24 (3): 221-228. doi: 10.3851/MP3301.

17. Ceccherin-Silberstein F, Cento V, Di Mario VC, Perno CF, Craxi A. Viral resistance in HCV infection. Curr. Opin. Virol. 2018; 32: 115-127. doi: 10.1016/j.coviro.2018.10.005.
18. Yutaka Kishida, Naohiko Imaizumi, Hirohisa Tanimura, Shinichiro Kashiwamura, Toru Kashiwagi. A Protease Inhibitor with Induction Therapy with Natural Interferon-beta in Patients with HCV Genotype 1b Infection. International Journal of Molecular Sciences. 2016; 17 (3): 350. 1-12. doi: 10.3390/ijms17030350
19. Lawitz E, Sulkowski MS, Ghalib R, Rodriguez-Torres M, Younossi ZM, Cooregidor A, DeJesus E, Pearlman B, Rabinovitz M, Gitlin N, Lin JK, Pockros PJ, Scott JD, Fevery B, Lambrecht T, Ouwerkerk-Mahadevan S, Callewaert K, Symonds WT, Picchio G, Lindsay K, Beumont M, Jacobson IM. Simeprevir plus sofosbuvir with or without ribavirin, to treat chronic infection with hepatitis C virus genotype 1 in non-responders to pegylated interferon and ribavirin and treatment-naïve patients: the COSMOS randomized study. Lancet. 2014; 384: 1756-1765.
DOI: 10.1016/S0140-6736(14)61036-9
20. Sulkowski MS, Gardiner DF, Roriguez-Torres M, Reddy KR, Hassanein T, Jacobson I, Lawitz E, Lok AS, Hinesrosa F, Thuluvath PJ, Schwartz H, Nelson DR, Everson GT, Eley T, Wind-Rotolo M, Huang SP, Gao M, Hernandez D, McPhee F, Sherman D, Hindes R, Symonds W, Pasquinelli C, Grasela DM; A1444040 Study Group. Daclatasvir plus sofosbuvir for previously treated or untreated chronic HCV infection. N Engl J Med. 2014; 370: 211-221. DOI: 10.1056/NEJMoa1306218
21. Poordad F, Hezode C, Trinh R, Kowdley K, Zeuzem S, Agarwal K, Shiffman ML, Wedemeyer H, Berg T, Yoshida EM, Forms X, Lovell SS, Silva-Tillmann BD, Collins CA, Campbell AL, Podsadecki T, Bernstein B. ABT-450/r-ombitasvir and dasabuvir with ribavirin for hepatitis C with cirrhosis. N Engl J Med. 2014; 370: 1973-1982.
DOI: 10.1056/NEJMoa1402869
22. Kowdley KV, Gordon SC, Reddy KR, Rossaro L, Bernstein D, Lawitz E, Shiffman ML, Schiff E, Ghalib R, Ryan M, Rustgi V, Chojkier M, Herring R, Bisceglie AM, Pockros PJ, Subramanian GM, An D, Svarovskaia E, Hyland RH, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Pound D, Fried MW; ION-3 investigators. Ledipasvir and sofosbuvir for 8 or 12 weeks for chronic HCV without cirrhosis. N Engl J Med. 2014; 370: 1879-1888.
DOI: 10.1056/NEJMoa1402355
23. Afdhal N, Reddy KR, Nelson DR, Lawitz E, Gordon SC, Schiff E, Nahass R, Ghalib R, Gitlin N, Herring R, Lalezari J, Younes ZH, Pockros PJ, Bisceglie AM, Arora S, Subramanian GM, Zhu Y, Dvory-Sobol H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Sulkowski M, Kwo P; ION-2 investigators. Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection. N Engl J Med. 2014; 370: 1483-1493. DOI: 10.1056/NEJMoa1316366
24. Afdhal N, Zeuzem S, Kwo P, Chojkier M, Gitlin N, Puoti M, Romero-Gomez M, Zarski JP, Agarwal K, Buggisch P, Foster G, Brau N, Buti M, Jacobson IM, Subramanian GM, Ding X, Mo H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Mangia A, Marcellin P; ION-1 investigators. Ledipasvir and Sofosbuvir for untreated HCV genotype 1 infection. N Engl J Med. 2014; 370: 1889-1898.
DOI: 10.1056/NEJMoa1402454
25. Pawlotsky JM. Hepatitis C Treatment: The data flood goes on-An update from the liver meeting 2014. Gastroenterology. 2015; 148: 15 468-479. DOI: 10.1053/j.gastro.2015.01.002
26. Uemura H, Uchida Y, Kouyama J, Niki K, Tsuji S, Sugawara K, Nakao M, Motoya D, Nakayama N, Imai Y, Tomiya T, Mochida S. NS5A-P32 deletion as a factor involved in virologic failure in patients receiving glecaplevir and pibrentasvir. J Gastroenterology. 2019; 54 (5): 459-470. doi: 10.1007/s00535-018-01543-9.
27. Chayama K and Hayes CN. HCV drug resistance challenges in Japan: The role of pre-existing variants and emerging resistant strains in direct acting antiviral therapy. Viruses. 2015; 7: 5328-42. Doi: 10.10.3390/v7102876.
28. Krishnan P, Schnell G, Tripathi R, Beyer J, Reisch T, Dekhtya T, Irvin M, Xie W, Fu B, Burroughs M, Redman R, Kumada H, Chayama K, Collins C, Pilot-Matias T. Integrated resistance analysis of CERTAIN-1 and CERTAIN-2 studies in hepatitis C virus-Infected patients receiving Glecaprevir and Pibrentasvir. Antimicrob Agents Chemother. 2018; 62 (2): e02217-17. doi: 10.1128/AAC.02217-17.