Global Health & Medicine. 2021; 3(5):321-334.
DOI: 10.35772/ghm.2021.01083

REVIEW

Treatment progress and expansion in Japan: From interferon to direct-acting antiviral

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Abstract: Hepatitis C virus (HCV) was first discovered in 1989, and patients infected with HCV were initially treated with interferon (IFN) monotherapy. In the 2000s, pegylated IFN combined with ribavirin was the mainstay of therapy for infected patients, but the sustained virologic response (SVR) rate was less than 50% for patients with HCV genotype 1. To further improve the therapeutic effect, direct-acting antiviral (DAA) was developed, and combination therapy with DAA and IFN has been available since 2011. In addition, IFN-free DAA therapy became available in 2014, and SVR was achieved in more than 95% of patients with chronic hepatitis and compensated cirrhosis. Thus, in just 30 years since the discovery of HCV, we aim to eliminate HCV in almost all patients. However, there are remaining issues to be addressed. Many of the patients who achieved SVR with DAA therapy had advanced liver fibrosis, and it is necessary to verify to what extent DAA therapy improves their prognosis in terms of liver function, hepatocellular carcinoma occurrence, and mortality. Resistance-associated substitutions can cause failure of DAA therapy, and the search for an effective therapy for high-level resistant viruses such as P32 deletion is particularly important. DAA therapy was approved for use in patients with decompensated cirrhosis in Japan in 2019, which is an unmet need so far. It is also important to verify the efficacy and safety in real-world settings. The World Health Organization aims to eliminate HCV by 2030, and Japan must tackle its remaining issues to achieve this goal.

***Keywords*:** hepatitis C virus, interferon, direct-acting antiviral, elimination

Introduction

Hepatitis C virus (HCV) was discovered in 1989 (*1*), with more than 90% of patients previously diagnosed with non-A and non-B hepatitis having HCV infection. Once HCV infection is established, approximately 30% of patients are cured during the acute infection stage, while approximately 70% remain infected with HCV, leading to chronic hepatitis C. In patients with chronic hepatitis C, spontaneous elimination of HCV is rare (annual rate of approximately 0.2%), and persistent inflammation caused by HCV infection induces liver fibrosis, which progresses to cirrhosis and hepatocellular carcinoma (HCC) (*2*). Antiviral therapy has been administered to reduce the progression of liver pathologies.

Initially, interferon (IFN) therapy was administered as antiviral therapy, but the therapeutic effect was unsatisfactory. Thereafter, the structure of viral protein which is essential for HCV replication was elucidated, and a culture system for HCV replication was created, leading to the development of direct-acting antivirals (DAAs). DAA was initially administered in combination with and greatly improved the therapeutic effect of IFN, but problems such as IFN intolerance persisted. IFN-free DAA therapy has been available since 2014, and has enabled us to eliminate HCV safely and at a high rate in elderly patients and patients with cirrhosis, who were difficult to treat in the IFN era. In addition, since February 2019, DAA therapy has been available for patients with decompensated cirrhosis, and it is now possible to consider the indication for DAA therapy in all HCV patients. However, new problems have emerged. Since many patients with cirrhosis are included in those who achieved sustained virologic response (SVR) with DAA therapy, it is necessary to verify the degree of improvement in liver fibrosis, suppression of HCC occurrence, and improvement in mortality. Furthermore, there are issues that need to be clarified, such as the problem of resistance-associated substitutions (RASs) in patients for whom previous DAA therapy failed. Real-world data on the efficacy and safety of DAA therapy in patients with decompensated cirrhosis also requires clarification. This review article describes the progress and the remaining issues of HCV therapy in Japan.

Changes in antiviral therapy for HCV

IFN-based therapy

The beginning of IFN therapy

IFN therapy for patients with HCV began in 1986, when Hoonfnagle *et al.* reported that elevated serum aminotransferase levels in patients with chronic non-A and non-B hepatitis decreased after administration of recombinant human interferon-alpha (*3*). Thereafter, with the development of a highly sensitive polymerase chain reaction technique, serum HCV-RNA was found to be undetectable in patients whose serum aminotransferase levels were improved by IFN therapy (*4*). Thus, the antiviral and anti-inflammatory effects of IFN therapy for patients with HCV were confirmed, and the clinical use of IFN therapy for patients with HCV was approved in Japan in 1992.

IFN monotherapy

Therapeutic effects of IFN were classified into four types: SVR (serum HCV-RNA undetectable at 24 weeks after the cessation of therapy), relapse (serum HCV-RNA undetectable during the therapy but detectable after the cessation of therapy), breakthrough (serum HCV-RNA detectable during therapy after once being undetectable), and nonresponse (serum HCV-RNA detectable throughout the course of antiviral therapy). The SVR rate of IFN monotherapy has been reported to vary depending on the viral genotype and load (*5,6*). Namely, patients with HCV genotype 1 have been shown to be more resistant to IFN monotherapy than those with HCV genotype 2, and patients with a high viral load are less responsive than those with a low viral load. Of the patients with HCV genotype 1 (which accounts for approximately 70% of Japanese HCV patients), those with a high viral load are particularly difficult to treat, and the SVR rate of IFN monotherapy for these patients was approximately 5% (*7*).

Various efforts have been made to improve the limited therapeutic effect of IFN monotherapy, especially in patients with HCV genotype 1 (Figure 1). Kasahara *et al.* reported that the SVR rate in patients with HCV genotype 1 who received IFN therapy for 12 months was higher than that of those who received IFN therapy for 6 months (*8*). The meta-analysis also showed the superiority of IFN therapy for 12 months over that for 6 months (*9*). Based on these results, long-term IFN administration became available in Japan in 2002. In addition, self-injection of IFN was approved in 2005, and long-term IFN administration has become widely used in clinical practice.

Development of Peg-IFN

Pegylated interferon (Peg-IFN) is an IFN to which polyethylene glycol has been added, and the advent of Peg-IFN has brought dramatic advances in IFN therapy. Pegylation is performed to delay drug elimination, reduce immunogenicity and regulate the effects of the drug. Conventional IFN has a short half-life and needs to be administered three times a week. Since Peg-IFN has a slower drug clearance rate and a longer half-life than conventional IFN, it makes it possible to maintain an effective blood concentration with once-weekly administration. Another advantage is that it has milder adverse effects than conventional IFN, including flu-like symptoms such as fever, joint pain, and fatigue.

Combination therapy with ribavirin

Development of ribavirin

Ribavirin (RBV), which was developed in 1972, is an oral nucleic acid analog with broad antiviral activity against RNA and DNA viruses. RBV monotherapy did

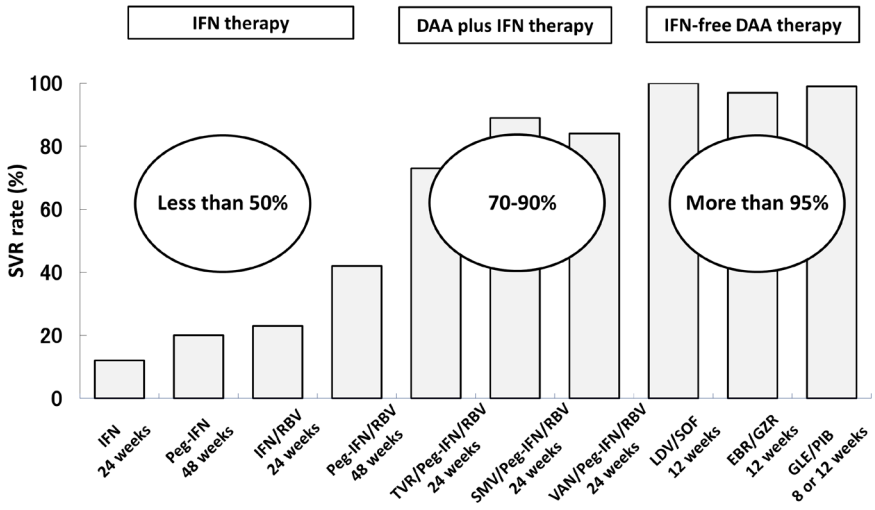

Figure 1. Changes in HCV therapy for patients with genotype 1. Abbreviations: DAA, direct-acting antiviral; EBR, elbasvir; GLE, glecaprevir; GZR, grazoprevir; IFN, interferon; LDV, ledipasvir; Peg-IFN, pegylated interferon; PIB, pibrentasvir; RBV, ribavirin; SMV, simeprevir; SOF, sofosbuvir; SVR, sustained virologic response; TVR, telaprevir; VAN, vaniprevir.

not show antiviral effects against HCV (*10*); however, several studies reported that IFN and RBV combination therapy was more effective than IFN monotherapy for patients with HCV (*11-13*). In 2001, IFN and RBV combination therapy was approved in Japan. Although the exact antiviral mechanism of RBV is unknown, the possible mechanisms include immune induction from Th2 to Th1, reduction of glutamyl transpeptidase pool in hepatocytes, inhibition of HCV-RNA polymerase activity, and induction of HCV mutation (*14*). The adverse effects of RBV include hemolytic anemia and therefore, caution is needed when administering it to patients with coexisting anemia or cardiac disease. Since RBV is excreted by the kidney and is not removed by dialysis, it is contraindicated in patients with renal impairment and dialysis. Ochi *et al.* reported that single nucleotide polymorphisms (SNPs) near the gene inosinetriphosphatase (ITPA) on chromosome 20 were strongly associated with hemolytic anemia during Peg-IFN and RBV combination therapy (*15*).

Peg-IFN and RBV combination therapy

Large-scale clinical studies of Peg-IFN and RBV combination therapy from Europe and the United States reported that SVR rates in patients with HCV genotype 1 ranged from 42% to 52% (48 weeks administration) and that in patients with HCV genotype 2 or 3 ranged from 81% to 84% (24 weeks administration); moreover, they showed favorable therapeutic effects (*16,17*). In Japan, Peg-IFN and RBV combination therapy was approved in 2004. Peg-IFN and RBV combination therapy for 48 weeks in patients with HCV genotype 1 and for 24 weeks in patients with HCV genotype 2 had SVR rates of 40-50% and 80%, respectively (*7*).

Factors associated with therapeutic effect of Peg-IFN and RBV combination therapy

Peg-IFN and RBV combination therapy for 48 weeks for patients with HCV genotype 1 and for 24 weeks for patients with HCV genotype 2 or 3 became standard care and was widely used in clinical practice. Thereafter, with the accumulation of real-world data, various studies investigating the factors associated with the therapeutic effects of IFN-based therapy have been published. The following are reports addressing patients with HCV genotype 1, which is difficult to treat.

Host factors

Host factors such as age, sex and race have traditionally been reported to be associated with the therapeutic effects of Peg-IFN and RBV combination therapy. Younger, males and Asian patients show higher therapeutic effects, and older, females and African-American patients show lower therapeutic effects. On the other hand, associations between therapeutic effects and genetic polymorphisms have also been reported. Tanaka *et al.* reported that SNPs near the gene interleukin (IL) 28B on chromosome 19 were strongly associated with the effect of Peg-IFN and RBV combination therapy in 2009 (*18*). Patients with a minor allele of rs8099917, the G allele, showed resistance to Peg-IFN and RBV combination therapy.

Viral factors

As for viral factors that contribute to the therapeutic effect, amino acid sequence mutations in the nonstructural protein (NS) 5A and HCV core regions have been reported. Enomoto *et al.* reported that the number of amino acid mutations in NS5A is significantly associated with the response to IFN therapy (*19*). Since an increase in the number of mutations in the region that spans amino acid residues 2209 to 2248 of NS5A affected the effect of IFN monotherapy, they named this region the interferon sensitivity determining region.

El-Shamy *et al.* reported that the number of mutations in the variable region 3 (V3, amino acids 2356 to 2379) and pre V3 (amino acids 2334 to 2355) of NS5A were associated with IFN and RBV combination therapy, and they named this region IFN/RBV resistance-determining region (IRRDR) (*20*). Patients with six or more mutations in IRRDR showed a better response to Peg-IFN and RBV combination therapy compared to those with five or fewer mutations in IRRDR.

Akuta *et al.* reported that the SVR rate of Peg-IFN and RBV combination therapy in patients with substitutions of amino acids 70 and/or 91 in the HCV core region was lower than that in patients with double wild type in the core region (*21*).

These host and viral factors could be used to predict therapeutic effects before starting antiviral therapy and are useful for identifying patients who are likely and unlikely to respond to antiviral therapy.

Drug dose and treatment response

McHutchison *et al.* reported that patients who were able to maintain at least 80% of the scheduled dose of IFN or Peg-IFN and RBV combination therapy were more likely to achieve SVR (*22*). Thereafter, various studies investigating the relationship between drug adherence and the therapeutic effects of Peg-IFN and RBV have been reported.

In Peg-IFN and RBV combination therapy, the timing of HCV-RNA negativity was associated with SVR. Oze *et al.* reported that the dose of Peg-IFN was associated with complete early virologic response (c-EVR) defined as HCV-RNA negativity at week 12 of therapy (*23*). Hiramatsu *et al.* reported that the dose of RBV was associated with relapse after 48 weeks of Peg-IFN and RBV combination therapy (*24*). Maintaining a high dose of Peg-IFN and RBV as long as possible may lead to higher SVR rates.

In patients who did not achieve c-EVR, the SVR rate was very low even after 48 weeks of Peg-IFN and RBV combination therapy. Berg *et al.* and Pearlman *et al.* reported that 72 weeks of Peg-IFN and RBV combination therapy improved the SVR rate in patients with late virologic response, defined as HCV-RNA detectable at week 12 and undetectable at week 24, compared to 48 weeks of Peg-IFN and RBV combination therapy (*25,26*). Based on these results, response-guided therapy was administered.

Advent of DAA

Peg-IFN and RBV combination therapy was revolutionary; however, the SVR rate is approximately 50%, especially in difficult to treat patients with HCV genotype 1 and high viral load; the therapeutic effect of this regimen was not sufficient. Therefore, the development of DAAs, which directly inhibit the replication and proliferation of HCV, was promoted to improve the therapeutic effect. Among the NS protein regions of HCV, the NS3/4A, NS5A, and NS5B regions were the main targets for drug development (*27*), and DAAs were divided into three types: NS3/4A protease inhibitors, NS5A inhibitors, and NS5B polymerase inhibitors (Figure 2). The establishment of an in vitro cell culture system for viral replication and viral lifecycle contributed largely to the development of DAA (*28*).

DAA and IFN combination therapy

DAA monotherapy is prone to viral mutations, and combination therapy with IFN is initially administered. Among the DAA, the NS3/4A protease inhibitor was the first to be developed. In Japan, the first-wave, first-generation NS3/4A protease inhibitor, telaprevir (TVR), for 12 weeks plus Peg-IFN and RBV combination therapy for 24 weeks was first developed. Although serious skin disorders including Stevens-Johnson syndrome, drug rashes, and serious anemia were more frequently observed in patients who were treated with TVR plus Peg-IFN and RBV combination therapy than in those who were treated with Peg-IFN and RBV combination therapy, SVR rates in treatment-naïve patients, relapsers and non-responders to previous therapy were 73%, 88% and 34%, respectively, and good therapeutic effects were observed (*29,30*). Based on these results, TVR plus Peg-IFN and RBV combination therapy was approved in Japan in 2011.

The next step in development was the second-wave, first-generation NS3/4A protease inhibitor, simeprevir (SMV) for 12 weeks plus Peg-IFN and RBV combination therapy for 24 weeks. SVR rates in treatment-naïve patients, relapsers and non-responders to previous therapy were 89%, 96% and 53%, respectively, and good therapeutic effects were observed (*31,32*). Based on these results, SMV plus Peg-IFN and RBV combination therapy was approved in Japan in 2013. Regarding the profile of adverse events, the frequency of hemoglobin decrease and drug eruption did not differ between patients who were treated with SMV plus Peg-IFN and RBV combination therapy and those who were treated with Peg-IFN and RBV combination therapy, but transient bilirubin increase after 1-2 weeks of therapy was more frequently observed in patients who were treated with SMV plus Peg-IFN and RBV combination therapy (*33*). Tahata *et al.* reported that the ITPA genotype was associated with hyperbilirubinemia during SMV plus Peg-IFN and RBV combination therapy (*34*).

Next came the second-wave, first-generation NS3/4A

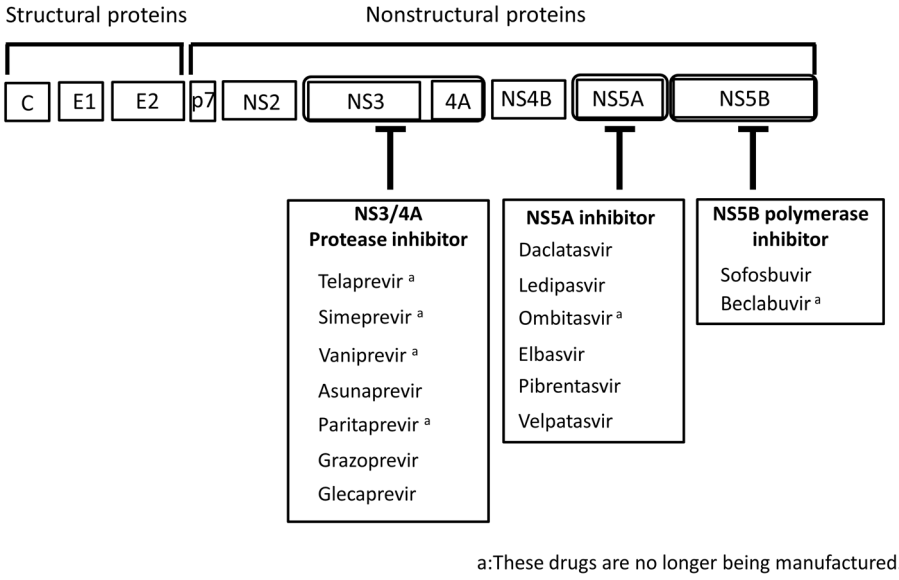

Figure 2. The structure of HCV and the action point of DAA. Abbreviations: DAA, direct-acting antiviral; HCV, hepatitis C virus; NS, nonstructural.

protease inhibitor, vaniprevir (VAN), for 12 weeks plus Peg-IFN and RBV combination therapy for 24 weeks was developed. SVR rates for treatment-naïve patients and relapsers to previous therapy were 84% and 92%, respectively, and the profile of adverse events in patients who were treated with this combination was comparable to those who were treated with Peg-IFN and RBV combination (*35,36*). In this triple therapy, patients with partial (detectable but decreased by more than 2 $\log_{10}$ in HCV-RNA after 12 weeks of therapy) or null response (detectable and decreased by less than 2 $\log_{10}$ in HCV-RNA after 12 weeks of therapy) to previous therapy were treated with VAN plus Peg-IFN and RBV combination therapy for 24 weeks. SVR rates were 62% in patients with partial or null responses to previous therapy and 55% in patients only with a null response to previous therapy (*36*).

IFN-free DAA therapy

The aforementioned NS3/4A protease inhibitor plus Peg-IFN and RBV combination therapy showed a high therapeutic effect, but had some problems; these triple therapies were not sufficiently effective for patients such as the elderly or those with poor response to IFN therapy, who had difficulty receiving IFN therapy due to adverse effects. In addition, patients with cirrhosis were not eligible for this therapy because of concerns about hepatotoxicity due to the combination of NS3/4A protease inhibitor plus IFN. Following the development of NS 3/4A protease inhibitors, drugs that inhibit the function of NS5A or NS5B proteins have been developed, and combination therapy with multiple DAAs targeting different proteins is now being used.

In Japan, combination therapy with daclatasvir (DCV, NS5A inhibitor) and asunaprevir (ASV, the second-wave, first-generation NS3/4A inhibitor), was the first to be available for clinical use in patients with HCV genotype 1. In a phase 3 study of DCV plus ASV therapy for 24 weeks, SVR rates were 87% in patients with IFN-ineligibility and intolerance, 81% in patients with non-responder to previous IFN-based therapy, and 91% in patients with cirrhosis (*37*). Favorable therapeutic effects were observed, and DCV plus ASV therapy for 24 weeks was approved in Japan in 2014. However, the SVR rate was approximately 40% in patients with NS5A L31 and/or Y93 RASs. Therefore, it was strongly recommended that NS5A RAS be investigated before starting DCV plus ASV therapy and not to use this regimen in patients with NS5A RAS.

In Europe and the United States, several phase 3 studies have shown the efficacy and safety of sofosbuvir (SOF, nucleotide polymerase inhibitor of NS5B), plus RBV therapy for patients with HCV genotype 2, 3 (*38-40*), and SOF plus ledipasvir (LDV, NS5A inhibitor), therapy for those with HCV genotype 1 (*41,42*). SOF plus RBV therapy for patients with HCV genotype 2 and 3 was approved in 2013, and SOF plus LDV therapy for patients with HCV genotype 1 was approved in 2014. In Japan, a phase 3 study of SOF plus RBV therapy for patients with HCV genotype 2 showed that the overall SVR rate was 97% and that 98% of treatment-naïve patients and 95% of previously treated patients achieved SVR (*43*). A phase 3 study of SOF plus LDV therapy for patients with HCV genotype 1 showed 100% SVR regardless of the presence or absence of cirrhosis, previous history of antiviral therapy, or IL28B genotype (*44*). Based on these results, SOF plus RBV therapy for patients with HCV genotype 2 and SOF plus LDV therapy for patients with HCV genotype 1 were approved in 2015. In real-world data of SOF plus RBV therapy for patients with HCV genotype 2, SVR rates ranged from 94% to 97%, which was equivalent to a phase 3 study (*45-47*). Because this regimen included RBV, a decrease in hemoglobin was observed during therapy, and the ITPA genotype was reported to be associated with this decrease in hemoglobin levels (*45,46*). Since the SVR rate was 100% in a phase 3 study of SOF plus LDV therapy, drug development for patients with HCV genotype 1 seemed to be solved. However, in real-world data of SOF plus LDV therapy for patients with HCV genotype 1, SVR rates ranged from 96% to 99% (*48-50*), which is slightly lower than the SVR rates in the phase 3 study. Some studies have reported that baseline NS5A RASs are involved in attenuating therapeutic effects (*48,49*). In another study, Akuta *et al.* reported that the SVR rate of SOF plus LDV therapy for patients in whom therapy with DCV plus ASV failed, was 71% (*51*). Developing effective DAAs for patients (particularly, those with HCV genotype 1) with RASs due to failed DAA therapy was desired. On the other hand, for patients with HCV genotype 2, a phase 3 study of SOF plus LDV therapy was conducted, with SOF plus RBV therapy as the control group (*52*). SVR rates were 96% in SOF plus LDV therapy and 95% in SOF plus RBV therapy, and SOF plus LDV therapy proved to be non-inferior to SOF plus RBV therapy. Regarding safety, few patients experienced grade 3 or 4 adverse effects in both groups, although anemia was more frequently observed in patients treated with SOF plus RBV than in those treated with SOF plus LDV. Based on these results, SOF plus LDV therapy for patients with HCV genotype 2 was approved in Japan in 2018. SOF plus LDV therapy for 12 weeks has become one of the first-line therapies for patients with HCV genotype 1 and 2; SOF plus RBV therapy for 12 weeks has become one of the first-line therapies for patients with HCV genotype 2 (*53*). Doi *et al.* reported that hepatitis B virus (HBV) reactivation was observed in 3.4% of HCV patients who are seropositive for the HBV core antibody during SOF-based therapy (*54*). Takayama *et al.* reported a case of HBV/HCV coinfection in a patient who experienced HBV reactivation during therapy with DCV plus ASV (*55*). The Japanese guidelines state that

careful monitoring of HBV reactivation during DAA therapy is necessary for patients with HBV coinfection or with a history of HBV infection (*53*).

The next DAA to be developed in Japan was ombitasvir (OBV, NS5A inhibitor) plus paritaprevir (PTV, second-wave, first-generation NS3/4A protease inhibitor that is administered with low-dose ritonavir (r) to improve the activity of PTV) for 12 weeks for patients with HCV genotype 1. A Japanese phase 3 study showed that SVR rates ranged from 94.9% (for patients without cirrhosis) to 90.5% (for patients with cirrhosis) (*56*). The aforementioned SOF is mainly excreted by the kidney and is not removed by dialysis, and it is contraindicated in patients with severe renal impairment (estimated glomerular filtration rate [eGFR] less than 30 mL/min/1.73m^2) and those on dialysis. On the other hand, OBV plus PTV/r is mainly metabolized in the liver and excreted in the feces, and it can be used for patients with severe renal impairment and those on dialysis. Japanese real-world data showed that the SVR rate of OBV plus PTV/r therapy for patients on dialysis was 97%. A good therapeutic effect was observed in them (57). This regimen was approved in 2015, but was discontinued in 2018.

Next, elbasvir, (EBR, NS5A inhibitor) plus grazoprevir, (GZR, second-generation NS3/4A protease inhibitor) therapy for 12 weeks for patients with HCV genotype 1 was developed in Japan. A Japanese phase 2/3 study showed that SVR rates were 96.5% in patients without liver cirrhosis and 97.1% in patients with cirrhosis (*58*). As for the influence of baseline NS3 or NS5A RASs on SVR, SVR rates were 100% and 96.8% in patients with and without NS3 RASs and 93.1% and 98.9% in patients with and without NS5A RASs, respectively; and preferable therapeutic effects were observed in patients with NS3 or NS5A RASs. Although patients with a creatinine clearance rate of < 50 mL/min were excluded from the Japanese phase 2/3 study, real-world data on patients undergoing dialysis reported that all patients completed EBR plus GZR therapy and that the SVR rate was 96.7% (*59*). EBR plus GZR therapy for 12 weeks was approved in Japan in 2016 and has become one of the first-line therapies for patients with HCV genotype 1 (*53*).

The next DAAs to be developed in Japan were the first triple combination therapy with DCV plus ASV plus beclabuvir, (BCV, non-nucleoside polymerase inhibitor of NS5B) for 12 weeks. A Japanese phase 3 study showed that the SVR rate was 96%, which was comparable in subgroup analyses of age, sex, IL28B genotype, and the presence or absence of cirrhosis (*60*). BCV is a non-nucleoside polymerase inhibitor of NS5B, and SOF is a nucleotide polymerase inhibitor of NS5B. While SOF is incorporated into viral genes and inhibits RNA replication, BCV binds NS5B polymerase protein and inhibits its enzymatic activity. This triple therapy was approved in 2016 but was discontinued in 2018. As another triple DAA combination therapy, adafosbuvir (nucleotide polymerase inhibitor of NS5B) plus odalasvir (NS5A inhibitor) plus SMV (NS3/4A protease inhibitor), was being tested in clinical trials (*61*). SVR rates of this regimen for 8 weeks in patients with chronic hepatitis and for 12 weeks in those with cirrhosis were both 100%; however, this regimen was never used clinically.

Next, glecaprevir, (GLE, second-generation NS3/4A protease inhibitor) plus pibrentasvir (PIB, NS5A inhibitor) therapy was developed in Japan. For patients with HCV genotype 1 (without cirrhosis), a phase 3 study of GLE plus PIB therapy for 8 weeks was conducted, with OBV plus PTV/r therapy for 12 weeks as the control group (*62*). In this study, all patients with HCV genotype 1 and cirrhosis were treated with GLE plus PIB for 12 weeks. SVR rates were 99.1%, 100% and 100% in GLE plus PIB therapy for 8 weeks, OBV plus PTV/r therapy for 12 weeks, and GLE plus PIB therapy for 12 weeks, respectively. For patients with HCV genotype 2 and without cirrhosis, a phase 3 study of GLE plus PIB therapy for 8 weeks was conducted, with SOF plus RBV therapy for 12 weeks as the control group (*63*). In this study, all patients with HCV genotype 2 and cirrhosis were treated with GLE plus PIB for 12 weeks. SVR rates were 97.6%, 93.5% and 100% in GLE plus PIB therapy for 8 weeks, SOF plus RBV therapy for 12 weeks, and GLE plus PIB therapy for 12 weeks, respectively. As for difficult to treat patients, three groups were established in a phase 3 study: patients with HCV genotype 1 or 2 and failure to previous DAA therapy, patients with HCV genotype 1 or 2 and renal impairment (eGFR less than 30 mL/min/1.73m^2), and patients with HCV genotype 3 (*64*). DAA-naïve patients with HCV genotype 1 or 2, renal impairment, and without cirrhosis were treated with GLE plus PIB for 8 weeks; all other patients were treated for 12 weeks. SVR rates were 93.9%, 100% and 83.3% in patients with HCV genotype 1 or 2 in whom previous DAA therapy failed, in those with renal impairment, and in patients with HCV genotype 3, respectively. Based on these results, GLE plus PIB therapy was approved in Japan in 2017; and GLE plus PIB therapy for 8 weeks became one of the first-line therapies for DAA-naïve patients with HCV genotype 1 or 2 without cirrhosis, and GLE plus PIB therapy for 12 weeks became one of the first-line therapies for patients with cirrhosis, failure to respond to previous DAA therapy, and HCV genotype 3 (*53*). GLE plus PIB therapy shortened the therapeutic period from 12 weeks to 8 weeks for patients with HCV genotype 1 or 2, those who were DAA-naïve, and without cirrhosis. Although GLE plus PIB therapy had a high SVR rate and good tolerability regardless of HCV genotype, renal impairment, or the presence of cirrhosis, it was not a panacea. Of 33 patients who failed to respond to previous DAA therapy and who were treated with GLE plus PIB, two experienced virologic failures.

Both patients were previously treated with DCV plus ASV and had NS5A P32 deletion at baseline. Research on effective therapies for NS5A 32 deletion has become a new challenge.

Next, SOF plus velpatasvir, (VEL, NS5A inhibitor) therapy was developed in Japan. Two phase 3 studies were conducted; one targeting patients with failure to respond to previous DAA therapy and the other targeting patients with decompensated cirrhosis. For the former study group, SOF plus VEL plus RBV therapy was administered for 12 weeks or 24 weeks (*65*). SVR rates were 85% for 12 weeks administration and 98% for 24 weeks administration in patients with HCV genotype 1; they were 70% for 12 weeks and 92% for 24 weeks in patients with HCV genotype 2. For patients with NS5A RASs at baseline, SVR rates were 85% and 96% after 12 and 24 weeks, respectively. On the other hand, for patients with NS5A P32 deletion at baseline, SVR rates were 100% (2/2) after 12 weeks of administration and 67% (2/3) for 24 weeks. In response to this result, SOF plus VEL plus RBV therapy for 24 weeks was approved for patients who failed to respond to previous DAA therapy in 2019 and became one of the therapeutic options for such patients.

DAAs described above have been targeted for patients with chronic hepatitis or compensated cirrhosis, and patients with decompensated cirrhosis have long been an unmet need. SOF plus VEL therapy was finally available in Japan in 2019 for patients with decompensated cirrhosis. In a Japanese phase 3 study, patients with decompensated cirrhosis were randomly treated with SOF plus VEL with or without RBV for 12 weeks (*66*). SVR rates were 92% in both regimens, and four patients in the group without RBV and seven in the group with RBV experienced severe adverse effects. In addition, three patients died during the study, and all three patients were part of the RBV group. In response to this result, SOF plus VEL therapy for 12 weeks was approved for patients with decompensated cirrhosis in 2019.

Future tasks

Long term prognosis after SVR in patients with cirrhosis

HCC occurrence

In the early days after IFN-free DAA therapy was introduced, some European study groups reported that early HCC occurrence and recurrence were observed after starting DAA therapy (*67,68*), and it was discussed whether or not DAA therapy could inhibit the occurrence of HCC. However, there were some problems with these reports. Namely, the number of patients was small, the control group was not established, and the observation period was short. Thereafter, various study groups reported that DAA therapy suppresses the development of HCC in a larger number of patients (*69-72*). Ioannou *et al.* compared the HCC occurrence rates between patients with and without SVR among 21,948 U. S. veterans treated with DAA. During the 1.5 years observation period, 280 out of 19,909 patients experienced HCC occurrence in patients with SVR and 165 out of 2,039 patients experienced HCC occurrence in patients without SVR, and a 71% reduction in the risk of HCC occurrence was observed in patients with SVR compared to those without SVR. Similar results were observed in a subgroup analysis limited to patients with cirrhosis (*69*). Nahon *et al.* reported that there was no difference in HCC occurrence rates between patients who were treated with DAA and those who achieved SVR by IFN therapy in 1,270 patients with histologically diagnosed cirrhosis, adjusting for patients' background (*70*). Kanwal *et al.* reported that in 18,076 U. S. veterans who achieved SVR with DAA therapy, HCC occurrence was observed in 544 patients over an observation period of approximately 2.9 years, and the cumulative HCC occurrence rates at 1 year, 2 years, and 3 years were 1.1%, 1.9%, and 2.8%, respectively (*73*). The cumulative HCC occurrence rates at 1, 2, and 3 years were 2.2%, 3.8%, and 5.6%, respectively, in patients with cirrhosis, and cirrhosis was a significant risk factor associated with HCC occurrence (hazard ratio: 4.13). Thus, even in patients with advanced liver fibrosis, DAA therapy is expected to reduce the risk of HCC. However, the HCC occurrence rate after SVR in patients with cirrhosis is higher than that in patients without cirrhosis, and careful surveillance for HCC is recommended after SVR.

The elimination of HCV by DAA therapy is generally thought to suppress HCC occurrence but does not completely eliminate the risk of HCC occurrence. The risk of HCC occurrence is particularly high in patients with cirrhosis, and it is important to identify the risk factors for its occurrence. Age, male sex, diabetes mellitus, liver stiffness measurement (LSM) at baseline, model for end stage liver disease (MELD) score (≥ 10), albumin levels at baseline (< 3.5 g/dL), platelet counts at baseline (< 12.0 $\times 10^4/\mu L$) and alpha-fetoprotein levels at the end of therapy (EOT) have been reported as risk factors for HCC after DAA therapy in patients with cirrhosis (*73-76*) (Table 1). In the future, it will be important to validate effective HCC surveillance using these risk factors.

Mortality

The ultimate goal of HCV therapy is to suppress the progression of liver fibrosis and the development of HCC by eliminating HCV, thereby improving prognosis. In the era of IFN-based therapy, overall and liver-related mortality was significantly reduced in patients with SVR compared to those without SVR (*77,78*). In recent years, there have been some reports of improved prognosis in patients treated with DAA (*71,79*). Backus *et al.* compared the overall survival rates among 15,059 patients (with advanced liver fibrosis) with and without

SVR. During the 1.6 years observation period, 598 out of 13,992 patients with SVR and 195 out of 1,067 patients without SVR died; a 78.9% reduction in the risk of death was observed in patients with SVR compared to those without SVR (*79*). Carrat *et al.* compared mortality between 6,320 patients with cirrhosis who were treated with DAA and 1,578 patients with cirrhosis who were not treated within the 33.4 months observation period and reported that DAA therapy reduced all-cause mortality by 66%, liver-related mortality by 72%, and non-liver-related mortality by 60% (*71*). In a Japanese study comparing IFN-based therapy with IFN-free DAA therapy, Tahata *et al.* reported that overall survival rates among patients with a previous history of HCC treatment did not differ between patients with SVR by IFN-based therapy and those with SVR by IFN-free DAA therapy after adjusting for patients' characteristics by propensity score matching (*80*). Thus, in patients with cirrhosis, the achievement of SVR with DAA therapy as well as IFN therapy can be expected to improve mortality, but the observation periods in these previous studies were short; thus, studies with a longer observation period are necessary.

Improvement in liver fibrosis

The gold standard for the diagnosis of liver fibrosis is pathological evaluation of liver biopsy specimens, but liver biopsy has some limitations such as invasiveness, sampling error and inconsistency of diagnosis among pathologists. Furthermore, it is difficult to perform repeated evaluations of liver fibrosis, especially in patients with SVR. As such, there have been some reports in recent years on the use of non-invasive diagnostic methods such as elastography to evaluate liver fibrosis over time, instead of liver biopsy (*81,82*). Bachofner *et al.* examined the changes in liver stiffness at baseline and at 12 weeks after end of therapy (EOT) by using transient elastography in 392 patients who were treated with DAA; they reported that there was a significant decrease in liver stiffness measurement (LSM) in patients with SVR, whereas there was no significant decrease in patients without SVR (*82*). Knop *et al.* reported that in 54 patients with cirrhosis who achieved SVR after DAA therapy, liver stiffness was measured over time, and LSM at 24 weeks after the EOT was significantly lower compared to baseline LSM (*81*). In addition, in 46% of patients, there was a reduction in LSM of 30% or more at 24 weeks after EOT compared to baseline. Thus, although the observation periods in these studies were relatively short, improvement in liver fibrosis was expected in patients with SVR by DAA therapy. The advantage of liver fibrosis assessment by elastography is that it is non-invasive and repeatable. A disadvantage is that it is affected by liver inflammation. Since the degree of liver fibrosis improvement may be overestimated due to the elimination of HCV by DAA therapy and suppression of liver inflammation, caution should be exercised when assessing liver fibrosis by elastography.

Portal hypertension

In patients with cirrhosis, esophagogastric varices, splenomegaly, and pancytopenia are observed in association with increased portal pressure. The gold standard for the measurement of portal pressure is the measurement of hepatic venous pressure gradient (HVPG), but it is an invasive method and not widely used in Japan. Several studies in Europe and the United States have evaluated portal pressure by measuring the HVPG before and after DAA therapy (*83-85*). Lens *et al.* compared HVPG before and after DAA therapy in 226 cirrhotic patients with HVPG ≥ 10 mmHg before DAA therapy (*83*). At the time of SVR24, 140 (62%) of patients had a 10% or more reduction in HVPG compared with baseline, and 176 (78%) of patients had a HVPG ≥ 10 mmHg. A baseline albumin level of 3.5 g/dL or less was a significant factor associated with HVPG not decreasing by more than 10% compared to baseline. Lens *et al.* reported a study of HVPG over a longer period (SVR96) (*84*). Of the 176 patients with a HVPG of 10 mmHg or more at the time of SVR24, HVPG at SVR96 was measurable in 117 patients, and 55 (47%) of patients had a HVPG of less than 10 mmHg at SVR96. Mandorfer *et al.* measured HVPG before and after DAA

Table 1. Risk factors for HCC occurrence after SVR in patients with cirrhosis

Author (*Ref.*)	Patients	Observation period (months)	Risk factor	Hazard ratio
Calvaruso *et al.* 2018 (*75*)	2,249	14	Platelet count at baseline, < 12.0 ×10^4/μL	3.97
			Albumin at baseline, < 3.5 g/dL	1.77
Ogawa *et al.* 2018 (*76*)	271	17	AFP at EOT (every 1 ng/mL)	1.1
Degaspei *et al.* 2019 (*74*)	505	25	Male	6.17
			DM	2.52
			LSM at baseline (every 1 kPa)	1.03
Kanwal *et al.* 2020 (*73*)	6,938	35	Age (every 1 year)	1.02
			MELD score (< 10)	1
			10 - 15	1.74
			> 15	1.78

Abbreviations: AFP, alpha-fetoprotein; DM, diabetes mellitus; EOT, end of therapy; HCC, hepatocellular carcinoma; LSM, liver stiffness measurement; MELD, model for end-stage liver disease; SVR, sustained virologic response.

therapy in 60 patients with baseline HVPG of 6 mmHg or more who achieved SVR and reported that patients with Child-Pugh class B might have less improvement in HVPG than those with Child-Pugh class A (*85*). These results suggest that SVR may not be expected to improve portal pressure in patients with decompensated cirrhosis, suggesting the existence of a point of no return. Further studies are needed to determine which patient conditions lead to portal pressure improvements and which conditions do not.

Retreatment of patients with failure to respond to previous DAA

DAA therapy has revolutionized HCV therapy, with SVR being achieved in more than 95% of patients; however, in some patients HCV was not eliminated. In patients with failure to DAA therapy, RASs at NS3, NS5A and NS5B regions are generated (*86,87*) and may attenuate the therapeutic effect of retreatment. These RASs have become a problem in HCV therapy. Itakura *et al.* reported that dual RASs at NS5A L31 plus Y93 were observed in 63% of patients with failure to respond to DCV plus ASV therapy, co-existence of RASs at NS3 D168 plus NS5A L31 or Y93 was observed in 62% of patients, and NS5A P32 deletion was observed in approximately 5% of patients (*86*). Even in DAA therapy that appeared after DCV plus ASV therapy, various RASs were observed in patients who failed to respond to DAA therapy, and as the number of failures with DAA regimens increased, the prevalence of multiple RASs at NS3 or NS5A increased (*87*). Therefore, it is important to reliably eliminate HCV using a small number of DAA regimens.

NS5A inhibitors are key drugs in DAA therapy, and RASs at NS5A region are clinically important. In particular, NS5A P32 deletion, which is not detected in DAA-naïve patients, can be detected in patients with failed previous antiviral therapy involving NS5A inhibitors and has high-level resistance to all NS5A inhibitors (*88,89*). In a Japanese phase 3 study of patients with failed DAA therapy, the SVR rate of GLE plus PIB therapy for 12 weeks was 93.9% (31/33) and a good therapeutic effect was observed, but two patients with failure to respond to GLE plus PIB therapy both had NS5A P32 deletion. In contrast, the SVR rate of SOF plus VEL plus RBV therapy for 24 weeks was 96.7% (58/60), and the SVR rate of patients with NS5A P32 deletion at baseline was 66.6% (2/3). Of the two patients who had failed SOF plus VEL plus RBV therapy, one had HCV genotype 1 and NS5A P32 deletion at baseline and the other patient had HCV genotype 2 and NS5A L31 RAS at baseline. Several Japanese studies have reported the real-world efficacy of GLE plus PIB therapy for patients with failed DAA therapy, and SVR rates ranged from 92.9% to 97.7% (*90-92*). In these studies, seven patients did not achieve SVR, all patients had a previous history of DCV plus ASV therapy, four had NS5A P32 deletion and the other three had multiple RASs at NS5A other than P32 deletion at baseline. All four patients with NS5A P32 deletion at baseline did not achieve SVR with GLE plus PIB therapy. There are still few reports on the real-world efficacy of SOF plus VEL plus RBV therapy in patients with failed DAA therapy. One case report stated that SOF plus VEL plus RBV for 24 weeks was administered to three patients with HCV genotype 1b and NS5A P32 deletion; all patients had a history of DCV plus ASV therapy, and two out of three patients achieved SVR (*93*). Another case report stated that SOF plus VEL plus RBV for 24 weeks was administered to three patients with failed GLE plus PIB therapy, no patients had NS5A P32 deletion, and all three patients achieved SVR (*94*). Thus, although GLE plus PIB therapy for patients with failed DAA therapy shows good therapeutic efficacy, it is unlikely to be effective, especially in patients with NS5A P32 deletion at baseline. The Japanese guidelines also recommend the measurement of RASs prior to retreatment for patients with failure to respond to DAA therapy (*53*). There are few reports on the efficacy of SOF plus VEL plus RBV therapy in patients with NS5A P32 deletion at baseline, and further accumulation of these patients in clinical practice is desirable. It should be noted that GLE plus PIB therapy cannot be used for patients with decompensated cirrhosis, and SOF plus VEL plus RBV therapy cannot be used for patients with decompensated cirrhosis or severe renal impairment.

Patients with decompensated cirrhosis

Patients with decompensated cirrhosis have the worst prognosis among patients with liver disease, and Maesaka *et al.* reported that the overall survival rates of patients with HCV-related decompensated cirrhosis who were not treated with antiviral therapy at 1 and 2 years were 82.9% and 64.8%, respectively (*95*). However, antiviral therapy for patients with decompensated cirrhosis has long been an unmet need. In Japan, SOF plus VEL therapy for patients with decompensated cirrhosis was approved in 2019, and several studies on its short-term therapeutic effects have been reported (*96-98*). In these studies, SVR rates ranged from 90.2% to 95.8%, and treatment completion rates ranged from 96.3% to 97.2%. Although good therapeutic effects and tolerability were observed, most reasons for treatment discontinuation were due to worsening of hepatic encephalopathy, or ascites, or variceal bleeding, and clinicians should pay attention to the occurrence of decompensated cirrhotic events during treatment. With regard to changes in liver function, Tahata *et al.* reported that among patients with SVR, 50% of patients with CP class B at baseline improved to CP class A at SVR and 9% and 27% of patients with CP class C at baseline improved to CP class A and B, respectively, at SVR; and improvement of albumin levels had a

significant effect on the improvement of CP class (*96*). Thus, in the short-term, patients with SVR show improvement in liver function, mainly in albumin levels. However, the long-term effect of antiviral therapy on the prognosis of patients with decompensated cirrhosis is controversial. Verna *et al.* reported that MELD score decreased by 0.30 points, total bilirubin levels increased by 0.23 mg/dL and albumin levels increased by 0.36 g/dL in long-term follow-up of approximately four years; but improvement in liver function was limited (*99*). Krassenburg *et al.* reported that SVR was significantly associated with improvement in event-free survival in patients with CP class A, but not in those with CP class B or C (*100*). In patients with CP class B and C, a decrease in the MELD score by 2 points or more (considered clinically significant) was not associated with event-free survival. As shown above, some studies in Europe and the United States reported negative results regarding the impact of DAA therapy on the prognosis of patients with decompensated cirrhosis, and further studies are needed.

Conclusion: Expectations for HCV elimination

Approximately 30 years have passed since the discovery of HCV in 1989, and it is now possible to achieve an SVR of more than 95% in patients with chronic hepatitis and compensated cirrhosis by oral medications only. In recent years, patients with decompensated cirrhosis have become a new target for antiviral therapy, and their therapeutic outcomes are comparable to those of compensated cirrhosis. However, no matter how good a drug is, it is useless if it cannot be administered to patients, and it is important to detect and guide untreated HCV patients to adequate therapy. In Japan new infections from blood transfusions have almost disappeared, except among people who inject drugs, which may lead to the spread of new infections and the emergence of multidrug-resistant viruses. SVR is a surrogate indicator for HCV therapy, and its true goal is to reduce liver-related deaths. Since many patients with cirrhosis are included in those who achieve SVR with DAA therapy, proper surveillance for liver disease is also important even after SVR. The World Health Organization's goal for HCV elimination is a 65% reduction in mortality and an 80% reduction in incidence by 2030, using 2015 as a baseline. Japan also needs to address the remaining issues so that HCV can be eliminated by 2030.

Funding: None.

Conflict of Interest: Professor Tetsuo Takehara received grants from Gilead Sciences, Inc., MSD K. K. and Abbie Inc. and is on the speakers' bureau for Gilead Sciences, Inc., MSD K. K. and Abbie Inc. All other authors declare that they have no conflicts of interest.

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Received July 6, 2021; Revised August 24, 2021; Accepted September 10, 2021.

Released online in J-STAGE as advance publication September 21, 2021.

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