7 Treatment Effects and Resistance-Associated Variants of Sofosbuvir Regimen for Japanese Patients with Chronic Hepatitis C Virus Genotypes 1 and 2

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Abstract

More than 40,000 people have already been treated in Japan since September 2014 when the first interferon-free all-oral therapy with daclatasvir (DCV), an NS5A inhibitor, and asunaprevir (ASV), an NS3/4A protease inhibitor (PI), was approved for treatment of chronic hepatitis C virus genotype 1. On the other hand, regimens containing the nucleic acid-type NS5B polymerase inhibitor sofosbuvir in combination with a PI, NS5A inhibitor, and/or ribavirin are now becoming standard throughout the world and achieving greater than 95% sustained virological response (SVR) rates against multiple HCV genotypes. On March 23, 2015, sofosbuvir was approved for treatment of genotype 2 in Japan, priced at 61,700 yen per pill. Furthermore, Harvoni, a combination drug containing sofosbuvir co-formulated with the NS5A inhibitor ledipasvir, was approved in Japan on July 3, 2015, for treatment of genotype 1 with pricing set at 81,171 yen per pill. While the 6,800,000 yen price tag of 12 weeks of Harvoni is higher than that of DCV/ASV therapy, the therapy is more effective, and the one pill per day dosing and 12-week duration provides simpler and shorter therapy. Similarly, sofosbuvir is approved for and effective against both genotypes 1 and 2.

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This review discusses the mechanism and characteristics of sofosbuvir and summarizes results of phase III clinical trials for genotypes 1 and 2 and reports on the effects of antiviral resistance.

Keywords
Chronic hepatitis C • Sofosbuvir • Ledipasvir • Chain termination • Resistance-associated variants

Abbreviations

ASV	Asunaprevir
DAA	Direct-acting antiviral
DCV	Daclatasvir
HCV	Hepatitis C virus
LDV	Ledipasvir
PI	Protease inhibitor
RBV	Ribavirin
SOF	Sofosbuvir
SVR	Sustained virological response

7.1 The Mechanism of Action of Sofosbuvir and a Note of Caution

Direct-acting antiviral (DAA) agents for treatment of chronic hepatitis C virus (HCV) infection act by directly targeting viral structures. As noted in previous reports, monotherapy with a protease inhibitor or NS5A inhibitors rapidly induces antiviral resistance. Two types of agents that target the virally encoded NS5B-dependent RNA polymerase have also been developed, including nucleotide and non-nucleotide analogs. Sofosbuvir (SOF) is a nucleotide analog NS5B polymerase inhibitor that interferes with HCV replication by terminating strand RNA synthesis when incorporated as a defective substrate (chain termination). A key advantage of nucleotide analogs is that they are less vulnerable to resistance than non-nucleotide analogs.

7.2 Characteristics of Sofosbuvir

Gilead Sciences acquired the drug candidate PSI-7977 when it purchased the pharmaceutical company Pharmasset for $11 billion in 2011 [1]. In 2013, Gane et al. reported a 100 % (10/10) SVR rate among treatment-naive patients with HCV genotype 2 or 3 after 12 weeks of SOF plus ribavirin (RBV), and 60 % of patients achieved SVR with SOF monotherapy (Fig. 7.1). While patients with genotype 2 or

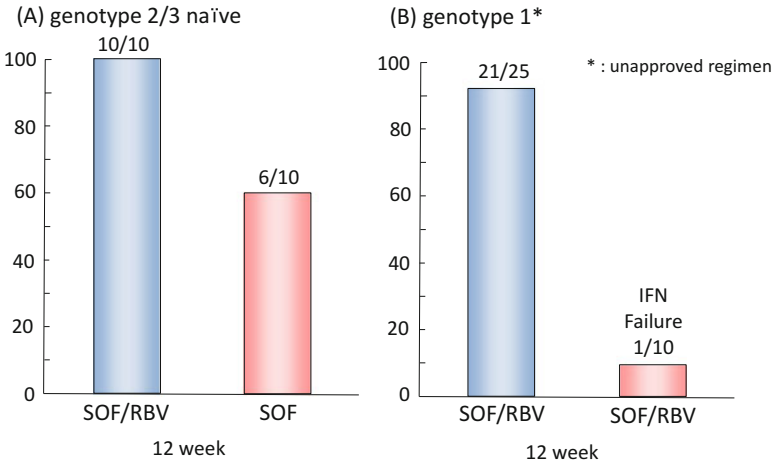


Fig. 7.1 SVR rate following sofosbuvir plus ribavirin therapy in (**a**) treatment-naive patients with genotype 2 or 3 (**b**) and in patients with genotype 1 [7]. Note that SOF+RBV therapy showed high response rates

3 can be successfully treated with SOF plus RBV, SVR rates in patients with genotype 1 are not as high. While 84% (21/25) of genotype 1 patients achieved SVR with 12 weeks of SOF plus RBV therapy, only 10% (1/10) of HCV genotype 1 patients who had failed prior IFN therapy achieved SVR. Therefore, treatment in combination with other DAAs, such as ledipasvir (LDV), is suggested for treatment of genotype 1. Nonetheless the success of these trials demonstrates the effectiveness of SOF against HCV.

SOF exhibits antiviral activity against multiple HCV genotypes, and the potential for drug interactions is low because SOF metabolism bypasses cytochrome P450. With over 300,000 people having already been treated with SOF since 2014, the drug's safety and tolerability have been well demonstrated, even among patients with renal dysfunction.

Development of many NS5B inhibitors has been abandoned due to cardiac toxicity. Although extremely rare in the case of SOF, bradycardia has been reported in nine patients treated in combination with amiodarone, and one patient experienced cardiac arrest following treatment with SOF in combination with a protease inhibitor. No causal relationship has been established, but caution should be exercised in clinical practice.

Harvoni is a co-formulated preparation of LDV and SOF for treatment of HCV genotype 1. Although the characteristics of LDV have been described in detail elsewhere, LDV shows stronger and more potent inhibitory activity against genotype 1 NS5A complex at picomolar concentrations compared to daclatasvir (DCV), another NS5A inhibitor already approved in Japan. Although in vitro studies suggest that the effect is somewhat reduced against genotypes 2 and 3, at the International

Symposium on Viral Hepatitis and Liver Disease in 2015, Gane et al. reported that Harvoni is effective against genotype 2.

Although activity of LDV is reduced in the presence of NS5A resistance mutations, which is also a problem in DCV/ASV therapy, LDV is effective against SOF-resistant NS5B S282T mutant strains. An additive effect of SOF and LDV has been confirmed, and no important drug interactions between SOF and LDV have been identified. LDV is also excreted in the bile, whereas SOF is excreted in the kidney.

In addition to the NS5B S282T substitution, L159F and C316N variants have been reported, but SOF-associated resistance-associated variants (RAVs) are rarely detected prior to treatment with direct sequencing analysis.

7.3 Sofosbuvir/Ribavirin Therapy for Genotype 2

7.3.1 Study Design and Patients Characteristics

A multicenter, randomized, open-label study was performed with the following inclusion criteria: (1) at least 20 years of age, (2) HCV RNA titer of at least 4 logIU/mL, (3) creatinine clearance of at least 1.0 mL/s [Cockcroft-Gault formula: men: (140−age)*weight/(72*serum creatinine); women: 0.85*(140−age)*weight/(72*serum creatinine)], and (4) platelet count of at least 50,000/mm^3. In total, 153 patients were enrolled, and all patients received 400 mg SOF once per day and RBV twice daily. RBV dosage was determined by body weight as follows: patients weighing less than 60 kg received 600 mg RBV, patients weighing 60–80 kg received 800 mg, and patients weighing more than 80 kg received 1000 mg. In addition, patients were classified according to prior treatment history. Ninety-three patients had no prior history of interferon treatment (naive), whereas 63 patients had failed to achieve SVR during prior treatment with IFN (treatment experienced). Patients were treated for 12 weeks with daily hospital visits for the first 6 weeks followed by hospital visits at 2-week intervals. The primary end point was attainment of SVR 12 weeks after the end of treatment (SVR12) (Fig. 7.2) [1, 2].

Patients with increased risk of HCC were also included in the study: 22 % of patients were over age 65, 46 % were male, and 11 % had liver cirrhosis. Cirrhosis was determined by liver biopsy or on the basis of a FibroScan score greater than 12.5 kPa within the previous 6 months. Patients with Child A decompensated cirrhosis or HCC were not included in the study.

7.3.2 Efficacy and Safety

All 153 patients successfully completed 12 weeks of SOF/RBV therapy without discontinuation of either drug, and all patients completed follow-up until 24 weeks after the end of treatment. The HCV RNA-negative (<25 IU/mL) rates during the course of therapy are shown in Fig. 7.3.

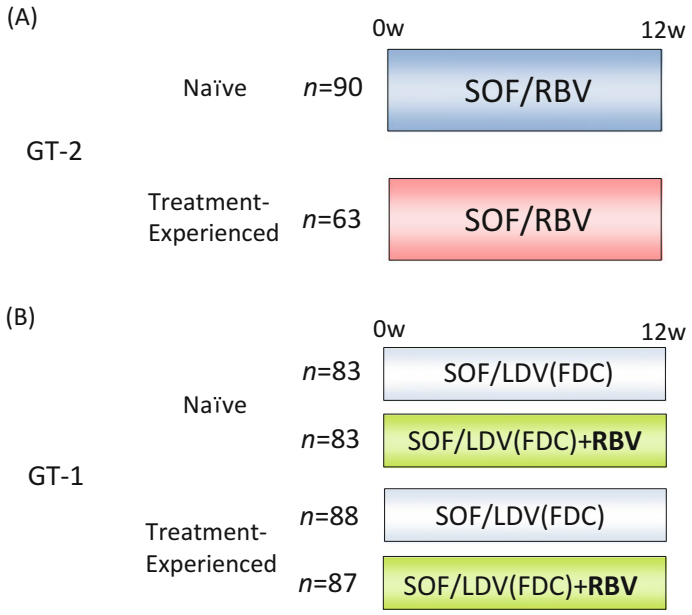


Fig. 7.2 Study design of SOF-based therapy for patients with genotype 2 (**a**) and those with genotype 1b (**b**). *FDC* fixed dose combination

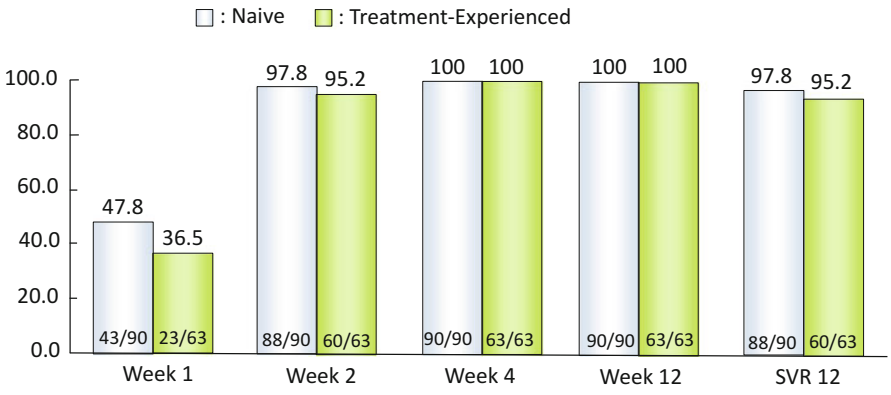


Fig. 7.3 HCV RNA-negative rate at key time points during and after therapy

All patients became HCV RNA negative by week 4, and all except five patients (two naive and three treatment experienced) remained negative until 12 weeks after the end of treatment (SVR12). It should be noted that all 148 patients who achieved SVR12 also achieved SVR24. About 5 % of patients experienced mild to moderate adverse events, including nasopharyngitis (30 %), headache (10 %), and RBV-induced anemia (10 %).

Table 7.1 Profiles of patients who failed to achieve SVR by SOF/RBV [1]

IFN history	LC	Age/sex	RNA	<25 KIU/mL/not detected	Adherence (SOF/RBV)
1. Naive	No	46/F	5.9	Week 1/week 2	98.5 %/98.5 %
2. Naive	No	**69**/F	6.1	Week 1/week 3	100 %/**93.7 %**
3. Experienced	**LC**	**63**/M	**7**	**Week 2/week 4**	98.5 %/98.2 %
4. Experienced	No	**70**/F	**6.8**	**Week 2/week 5**	100 %/99.4 %
5. Experienced	No	59/F	**7.3**	Week 2/week 3	100 %/100 %

HCV RNA-negative rate at each time points from the beginning of the therapy
LC liver cirrhosis

7.3.3 NS5B RAVs and Sofosbuvir/Ribavirin Therapy

Five patients experienced relapse following treatment (Table 7.1). In treatment-experienced patients, multiple factors such as age, sex, initial viral load, and time until becoming HCV RNA negative are thought to influence outcome of therapy. However, even one young treatment-naive patient with relatively low initial viral titer who became HCV RNA negative early relapsed, making it difficult to determine the cause. Although the NS5B S282T mutation reduces sensitivity to SOF, the presence of the S282T mutation before and after therapy has not been confirmed in these five patients, and it is unclear whether extension of therapy could improve the SVR in these patients.

7.3.4 Real-World Study of Sofosbuvir/Ribavirin Therapy

Beginning with the 2014 meeting of the American Association for the Study of Liver Diseases, real-world data on the safety and efficacy of SOF therapy in patients undergoing dialysis as well as liver reserve improvement in patients awaiting liver transplantation have begun to be reported. As mentioned above, SOF therapy is contraindicated in patients with decompensated liver cirrhosis or renal insufficiency (eGFR <30 mL/min/1.73 m^2). On the basis of creatinine clearance (Cockcroft-Gault method, in which the score is reduced by 15 % for women), some patients were not eligible for entry into the phase III clinical trial, including some older women with normal renal function, due to higher risk of RBV-induced anemia and SOF-related renal failure because the body weight of Japanese patients tends to be lower and patients tend to be older compared to western patients.

There was a 65-year-old male patient with HCV genotype 2b (7.8 log IU/mL HCV RNA) with compensated cirrhosis and type 2 diabetes with diabetic kidney disease (eGFR of 40 mL/min/1.73 m^2) who had failed to respond to prior PEG-IFN/RBV therapy. The patient was denied entry into the phase III clinical trial for genotype 2 due to anemia (Hb <12.0 g/dL) and renal dysfunction (creatinine clearance <1.0 mL/s). After FDA approval of SOF, the patient was able to begin self-financed

SOF/RBV therapy, but RBV dose reduction was necessary due to marked progression of anemia (ITPA: rs1127354 CC genotype). Furthermore, HCV RNA became undetectable at 8 weeks, showing a different clinical course than in the study patients, but the patient ultimately achieved SVR. Overseas guidelines recommend treatment of more than 16 weeks in GT-2 patients with cirrhosis, and the patient achieved SVR24 after a 4-week extension of the therapy to 16 weeks. In real-world data, in the case of a delay in HCV RNA decline due to RBV dose reduction, for example, it should be considered that 12 weeks of treatment may not be sufficient and longer treatment may benefit the patient.

7.4 Sofosbuvir/Ledipasvir Therapy for Genotype 1

7.4.1 Study Design and Patients Characteristics

The study design of the multicentre phase 3 clinical trial for SOF/LDV therapy for genotype 1 was similar to the genotype 2 study, except that selection criteria included HCV RNA ≥5 log IU/ml. The 341 enrolled patients were divided into four groups based on prior interferon treatment history (naive vs treatment experienced) and treatment with 400 mg SOF and 90 mg LDV with or without RBV (Fig. 7.2). SOF and LDV were administered for 12 weeks as a once daily co-formulated tablet. RBV dosage, treatment and follow-up timing, and study end points are the same as in the genotype 2 clinical trial.

About twice as many patients with cirrhosis (22 %) were enrolled compared to in the genotype 2 study. In addition, 33 % of the patients were at least 65 years old, and 28 patients over age 65 with cirrhosis were enrolled. There were 175 patients with prior interferon treatment history, about 60 % of whom had previously been treated with PEG-IFN/RBV and 25 % of whom had been treated with triple therapy that included an NS3/4A protease inhibitor. *As in the genotype 2 study*, *patients with Child A decompensated cirrhosis or HCC were ineligible for the study.*

7.4.2 Efficacy and Safety

The proportion of HCV-negative patients in each group and the time until HCV RNA became undetectable (<25 IU/mL) are shown in Table 7.2 and Fig. 7.4. All patients became HCV RNA negative by week 4 and remained negative until the end of treatment at week 12. There was no difference in the HCV RNA-negative rate between the RBV and RBV-free treatment arms.

Three hundred thirty-eight (99 %) patients achieved SVR12, all of whom also achieved SVR24. All three patients who failed to clear the virus were in the treatment-naive group treated with RBV. Two patients discontinued treatment due to (1) skin rash at day 6 and (2) and severe infection symptoms followed by cardiac arrest during week 8, respectively. Only one patient relapsed after completing the

Table 7.2 HCV RNA-negative rate at each time point during the therapy [2]

	IFN history (−)		IFN history (+)		
Time point and HCV RNA	SOF/LDV	SOF/LDV/RBV	SOF/LDV	SOF/LDV/RBV	Total
Negative rate (number of negative patients/number of measurements (%))	83	83	88	87	**341**
1 week	26/83 (31)	21/82 (25)	26/88 (30)	26/87 (22)	99/340 (29)
2 weeks	67/83 (81)	64/82 (78)	69/88 (78)	72/87 (83)	272/340 (79)
4 weeks	83/83 (100)	82/82 (100)	88/88 (100)	87/87 (100)	340/340 (100)
End of therapy	83/83 (100)	81/81 (100)	88/88 (100)	87/87 (100)	339/339 (100)

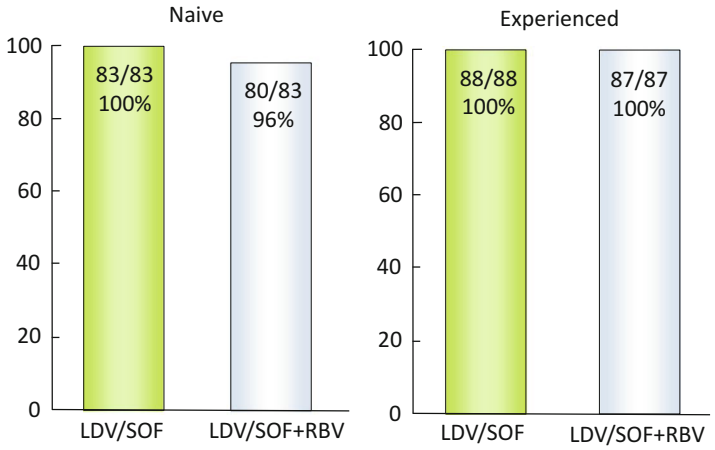


Fig. 7.4 SVR12 rate of patients in a phase III clinical trial of LDV/SOF. Patients who had never been treated with IFN-based therapy (naive, *left*) and patients with a prior history of IFN therapy (experienced, *right*) in total, 338/341 (99 %) patients achieved SVR12 [2]

12-week therapy. Adverse events were similar to that in the genotype 2 study and included nasopharyngitis, anemia, and headache. In addition, a 71-year-old male with cirrhosis in the treatment-experienced RBV arm experienced myocardial infarction 9 days after the end of therapy.

For these reasons, the use of RBV with LDV/SOF therapy is not approved in Japan.

7.4.3 NS5B Resistance-Associated Variants and Sofosbuvir/Ledipasvir Therapy

Figure 7.5 shows the effect of substitutions in the NS5A region on the outcome of LDV/SOF therapy. It should be noted that the presence of drug-resistant variants was determined using the Illumina MiSeq platform using a minimum frequency of 1 % as a cutoff. No resistance mutations were detected in 265 patients, 99 % of whom achieved SVR, except for two patients who discontinued therapy early. Pretreatment substitutions in the NS5A region were present in 76 patients (22 %), 58 of whom harbored NS5A Y93H mutations, but all patients achieved SVR except for one patient who relapsed. The presence of single NS5A substitutions does not appear to affect outcome of therapy as it does in DCV/ASV therapy.

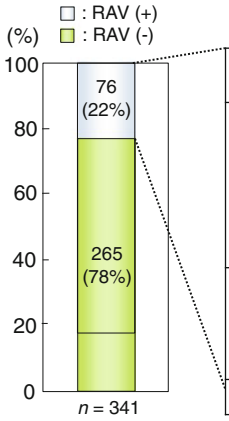
Although the Y93H mutation was detected at a frequency of >99 % in one patient who relapsed, a similar pattern was detected in ten other patients who did not. Analysis of next-generation sequencing data is complicated by differences among sequencing hardware and methods that can lead to differences in reported values among institutions. In addition, at the time of relapse (4 weeks after the end of treatment), no mutations in the NS5A or NS5B regions other than NS5A Y93H could be detected. As in the case with genotype 2, extension of therapy may help to eliminate HCV in such patients.

7.4.4 Sofosbuvir/Ledipasvir Therapy in the Real World

Although SOF-based regimens are on the way to becoming the standard of care, IFN-free therapy with DCV/ASV arrived on the scene in Japan first. Although many patients successfully achieved SVR with this therapy, a downside of early adoption of this NS5A inhibitor-containing therapy is the high frequency of NS5A L31/Y93 double mutations [3] and P32 deletions [4, 5] among the several thousand patients who failed to respond to DCV/ASV therapy. Re-treatment of these patients with an NS5A inhibitor is likely to be less effective, presenting a major challenge for re-treatment with Harvoni.

Although one patient with a NS5A L31I+Y93H double mutation achieved SVR in the phase III clinical trial in Japan (Fig. 7.5), NS5A L31M/V+Y93H strains are strongly resistant to NS5A inhibitors in vitro, and the therapeutic effect in humans is unclear. In fact, while about 1 % of patients had NS5A L31+Y93 double mutants prior to oral DAA therapy, the emergent NS5A L31+Y93 substitutions can be detected even by direct sequencing in non-SVR DCV/ASV patients.

Strains from patients who have experienced DCV/ASV treatment failure due to the presence of resistance-associated variants have been reported to be suppressed in vitro with the addition of DCV/SOF or LDV/SOF (Harvoni) [6]. However, as described above, DCV and LDV share the same mechanism of action, and LDV is ineffective against DCV-resistant strains. Therefore, such treatment is effectively equivalent to SOF monotherapy, and the therapeutic effect is likely to be insufficient.



	Base line RAVs	genotype	Numberof patients	SVR24% (n/n)
Single RAV	L31M	1b	9	100(9/9)
	L31I	1b	1	100(1/1)
	L31F	1b	1	100(1/1)
	L31V	1b	1	100(1/1)
	Y93H	**1b**	**58**	**98(57/58)**
	Q30R	1a	5	100(1/1)
Multiple RAV	**L31I, Y93H**	1b	1	100(1/1)
	Y93S, Y93N, Y93H	1b	1	100(1/1)
	Y93F, Y93H	1b	1	100(2/2)
	L31I, Y93N, Y93C	1a	2	100(1/1)
		total	76	99(75/76)

Fig. 7.5 Outcome of therapy with respect to frequencies of single and multiple resistant-associated variants (RAVs) at baseline

At the 2015 meeting of the European Society of Hepatology, Lawits et al. reported results of re-treatment with 24 weeks of Harvoni for patients who failed to achieve SVR after 8 or 12 weeks of Harvoni therapy. The SVR rate for patients with NS5A resistance variants prior to therapy was 60 % (18/30). In addition, *emergent NS5B resistance mutations* (*S282T*, *L159F*) *were detected in 33 % of patients*. Even if a variety of novel oral antiviral agents are developed in the future, the emergence of multidrug-resistant strains should be avoided as much as possible, and caution should be used in re-treatment of patients who experience DAA treatment failure.

7.5 Conclusion and Future Perspectives

This review described the results of the first SOF phase III trial conducted in Japan as well as real-world treatment effects of SOF. The 100 % SVR rate of ribavirin-free LDV/SOF therapy for genotype 1 is surprising, especially since more than 30 % of the patients were aged 65 or older and more than 20 % of the patients had cirrhosis. Achieving a high therapeutic effect without IFN or ribavirin following a shorter 12-week therapy even in elderly patients is indeed very good news.

On the other hand, although the majority of patients respond to the therapy, 12 weeks of therapy is insufficient to clear the virus for some patients. As with patients who failed to respond to DCV/ASV, re-treatment in the case of DAA failure is an urgent challenge in order to suppress liver carcinogenesis and improve prognosis. Remaining challenges also include improved treatment options for (1) patients with genotypes other than genotypes 1 and 2, (2) patients with renal failure or who are undergoing dialysis, (3) patients with decompensated cirrhosis, and (4) genotype 2 patients who are intolerant of ribavirin.

As we are entering an era of high rates of treatment, failure to clear the virus must be avoided as much as possible.

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