



NS5A-P32 Deletion in Hepatitis C Genotype 1b Infection is the Most Refractory Treatment-Mediated Amino Acid Change Exhibiting Resistance to all NS5A Inhibitors

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Abstract

NS5A-P32 deletion (P32del) is a resistance-associated amino acid change that has recently gained popularity in direct-acting antiviral treatment for chronic hepatitis C. Although not yet detected in naive patients, it appears in 5 to 10% of hepatitis C genotype 1b patients who fail to respond to daclatasvir/asunaprevir and sofosbuvir/ledipasvir treatments. In contrast to signature resistance-associated substitutions, such as substitutions at the NS5A-L31 and NS5A-Y93 positions, it shows complete resistance to all NS5A inhibitors in replicon and cell culture. Studies of humanized liver mice suggest that P32del retains good replication fitness and requires two classes of antivirals, except NS5A inhibitors, to be suppressed effectively. Patients with the P32del virus do not respond to glecaprevir/pibrentasvir but do respond to sofosbuvir/velpatasvir/voxilaprevir, presumably to sofosbuvir + glecaprevir/pibrentasvir, and at least partially to sofosbuvir/velpatasvir + ribavirin. Attention should be given to P32del in patients who experience failure with any NS5A inhibitor, especially those with genotype 1b infection.

Keywords

- ▶ chronic hepatitis C (CHC)
- ▶ resistance-associated substitutions (RAS)
- ▶ cross-resistance
- ▶ P32del
- ▶ mutation

Antiviral treatments for chronic hepatitis C patients have advanced remarkably over the past 5 years. In the past, antiviral treatments were based on interferon (IFN), which sometimes caused severe adverse events, including flu-like symptoms and thrombocytopenia, and their therapeutic effects were not satisfactory. Currently, approximately 95% of patients can achieve virologic cure by combination treatment with direct-acting antiviral agents (DAAs) without any severe adverse events.^{1–8} Conversely, the genetic barrier of DAAs is lower than that of IFNs, and resistance-associated amino acid changes are a new issue for patients who experience virologic failure to DAA therapy.^{9–11}

In particular, NS5A inhibitors, which are key drugs in DAA therapy, have extensive cross-resistance across NS5A inhibitors. First-generation NS5A inhibitors, such as daclatasvir and ledipasvir, are not effective for signature resistance-associated substitution, NS5A-Y93H ± L31M/I/V.¹² Second-generation NS5A inhibitors, including pibrentasvir and velpatasvir, are more effective for these signature resistance-associated substitutions than first-generation inhibitors.^{12,13}

NS5A P32 deletion (P32del), which has never been reported in DAA-naive patients, has been reported in patients with hepatitis C virus (HCV) genotype 1b who have failed DAA therapy, including daclatasvir-containing and sofosbuvir/ledipasvir treatments. HCV with NS5A P32del shows high-level resistance to all commercial NS5A inhibitors and is persistent for a long time in patients with virologic failure to DAA therapy. The treatment for HCV with NS5A P32del has not yet been established. Thus, in the present review, we summarize the data that have been reported for NS5A P32del HCV.

NS5A P32del HCV was Selected in Patients Who Fail to Respond to NS5A Inhibitor-Containing Therapies

Although NS5A P32del HCV was not found in *in vitro* experiments using HCV replicon treated with daclatasvir,^{14–17} it was discovered in clinical patients treated with daclatasvir-containing therapy (▶ **Table 1**). In a clinical trial of daclatasvir

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Table 1 Patients who carried HCV with P32del

Tx	Number of analyzed patients who experienced VF	Sequencing method	Number of patients who carried NS5A P32del HCVI	Genotype	Age	Sex	Pre-Tx	NS5A P32del at baseline	Tx effect	Frequency of NS5A P32del clone after Tx	NS5A L31 at baseline	NS5A L31 at VF	Ref		
DCV	17 Pts; genotype 1a 7 Pts; genotype 1b	Population	1 Pt	1b	-	-	Naive	N.D.	No-response	55% (post-2 wk), 25% (post-24 wk)	L	L/F	19		
DCV + PegIFN + RBV	19 Pts: genotype 1b	Population	2 Pts	1b	-	-	IFN + RBV	N.D.	Breakthrough	Major clone (8 wk on Tx), 0% (12 k on Tx)	L	L	20		
				1b	-	-	IFN + RBV	N.D.	Breakthrough	Major clone	L	F			
DCV + ASV	21 Pts: genotype 1b	Population	2 Pts	1b	71	M	SMV + IFN + RBV	N.D.	No-response	Major clone (at VF), 0% (post-12 wk)	L	L	21		
				1b	66	M	SMV + IFN + RBV	N.D.	No-response	Minor clone	-	-			
DCV + ASV	11 Pts: genotype 1b	Deep	1 Pt	1b	-	-	IFN	N.D.	Breakthrough	99.7%	L	L	22		
DCV + ASV	68 Pts: genotype 1b	Population	3 Pts	-	-	-	NS5A naive	-	-	-	-	F	23		
				-	-	-	NS5A naive	-	-	-	-	-		F	
				-	-	-	NS5A naive	-	-	-	-	-		-	L
DCV + ASV	110 Pts: genotype 1b	Population	6 Pts	1b	74	F	IFN	N.D.	Breakthrough	-	L	F	24		
				1b	64	F	IFN	N.D.	Breakthrough	-	-	L		L	
				1b	56	M	IFN + RBV	N.D.	No-response	-	-	F		F	
				1b	71	F	IFN + RBV	N.D.	Relapse	-	-	L		L	
				1b	72	M	SMV + IFN + RBV	N.D.	No-response	-	-	L		L	
				1b	54	F	IFN + RBV	N.D.	No-response	-	-	L		L	
				1b	49	F	DAA naive	N.D.	-	-	-	-		100% (post-2 mo), 100% (post-13 mo)	L
DCV + ASV	71 Pts: genotype 1b	Population	6 Pts	1b	76	M	DAA naive	N.D.	-	100% (post-3 mo), 77% (post-28 mo)	L	L	25		
				1b	46	F	SMV + IFN + RBV	-	-	-	-	50% (post-1 mo), 67% (post-25 mo)		-	W/L
				1b	65	F	SMV + IFN + RBV	N.D.	-	-	-	0% (post-1 mo), 50% (post-6 mo)		L	L/I
DCV + ASV	23 Pts: genotype 1b	Population	1 Pt	1b	67	F	SMV + IFN + RBV	N.D.	-	-	L	F	26		
				1b	69	M	DAA naive	-	-	-	-	-		-	L/V
SOF/LDV	10 Pts: genotype 1b	Deep	1 Pt	1b	-	-	DAA naive	-	Relapse	Major clone	-	L	27		
				1b	75	M	Naive	N.D.	Relapse	85.4% (post-4 wk), 99.9% (post-52 wk)	M	M			

Abbreviations: ASV, asunaprevir; DCV, daclatasvir; IFN, interferon; LDV, ledipasvir; M, male; N.D., not detected; Pt, patient; RBV, ribavirin; SMV, simeprevir; SOF, sofosbuvir; Tx, treatment; VF, virologic failure.

monotherapy, a total of 17 patients with genotype 1a HCV infection and 7 patients with genotype 1b HCV infection in the U.S. were treated with daclatasvir for 14 days.^{16,18} Various resistance-associated amino acid changes were detected after treatment. In one patient with genotype 1b HCV infection, NS5A P32del was detected as a major clone after treatment.¹⁹ In another clinical trial of daclatasvir + peg-IFN + ribavirin treatment in Japan, 19 prior IFN + ribavirin nonresponders with genotype 1b HCV infection developed virologic failure.²⁰ Among them, NS5A P32del was detected in two patients. One patient experienced viral breakthrough at 6 weeks after treatment but continued the treatment for 36 weeks. NS5A P32del was detected at 8 weeks after treatment, but it was no longer detected at 12 and 24 weeks after treatment or 4 weeks posttreatment. The other patient experienced viral breakthrough at 8 weeks after treatment but continued the treatment for 40 weeks. NS5A P32del was detected at 24 weeks after the initial treatment and at 4 weeks posttreatment. In both patients, NS5A P32del was not detected at baseline.

After the introduction of IFN-free DAA treatment for clinical use, Uchida et al reported that among 21 patients with genotype 1b HCV infection who experienced virologic failure by daclatasvir + asunaprevir, NS5A P32del was detected in two patients at the time of virologic failure.²¹ Both patients were prior simeprevir + IFN + ribavirin nonresponders. In one patient, HCV with NS5A P32del was detected as a minor clone and coexisted with a major clone of HCV carrying the NS5A P29 deletion at the time of virologic failure. In the other patient, it existed as a major clone at the time of virologic failure. In both patients, NS5A P32del was not detected by deep sequencing at baseline prior to daclatasvir + asunaprevir treatment or simeprevir + IFN + ribavirin treatment. Kai et al reported that among 11 patients with genotype 1b HCV infection who experienced virologic failure by daclatasvir + asunaprevir, NS5A P32del was detected in one patient as a major clone at the time of virologic failure analyzed by deep sequencing.²² The patient had never received any DAAs, including simeprevir, prior to daclatasvir + asunaprevir treatment. By population sequencing analysis, Itakura et al,²³ Kobayashi et al,²⁴ and Iio et al²⁵ reported that among 68, 110, and 74 patients with genotype 1b HCV infection, respectively, who experienced virologic failure to daclatasvir + asunaprevir treatment, NS5A P32del was detected in 3, 6, and 7 patients at the time of virologic failure, respectively. Teraoka et al also reported that among 23 patients with genotype 1b HCV infection who experienced virologic failure to daclatasvir + asunaprevir ± beclabuvir treatment, NS5A P32del was detected in one patient treated with daclatasvir + asunaprevir at the time of virologic failure.²⁶ These reports indicated that NS5A P32del was detected in 5 to 10% of patients with genotype 1b HCV infection who failed to daclatasvir-containing therapies (► **Table 1**).

NS5A P32del was also reported after sofosbuvir/ledipasvir treatment.²⁷ Doi et al reported that among 10 patients with genotype 1b HCV infection who experienced virologic failure to sofosbuvir/ledipasvir treatment, NS5A P32del was detected as a major clone in one patient at the time of virologic failure.²⁷

Phylogenetic tree analysis clarified that the NS5A P32del HCV clones in this case were generated by the complete deletion of three bases, which encode NS5A P32 from the original HCV clones. The patient had not received any anti-HCV therapy and had liver cirrhosis with a history of hepatocellular carcinoma treatment. Conversely, there has not been a reported case of naturally occurring NS5A P32del without DAA treatment or a case infected with NS5A P32del HCV that was not genotype 1b until now.

NS5A P32del HCV Shows High-Level Resistance to all NS5A Inhibitors

NS5A protein, an active component of HCV replicase, consists of domains I, II, and III, and domain I consists of the N-terminal membrane-anchoring helix, domain Ia and domain Ib (► **Fig. 1**).^{28–30} Domain I forms dimers, and domain Ib binds ribonucleic acid (RNA) for HCV replication.³¹ NS5A inhibitors bind NS5A domain I, leading to inhibition of RNA binding.^{31–33} Recently, NS5A domain I was reported to play a role not only in viral replication but also in viral assembly.³⁴ P32 is located at the linker between the membrane-anchoring helix and domain Ia (► **Fig. 1**). NS5A inhibitors bind a space formed by the linker, domain I and the helix (► **Fig. 1**).^{32,33}

Using the genotype 1b HCV replicon system (Con1 replicon), NS5A P32del HCV showed extremely high-level resistance against daclatasvir and ledipasvir (► **Table 2**).^{13,19} The mean EC₅₀ of daclatasvir or ledipasvir against NS5A P32del HCV is approximately 0.3 to 0.4 million times higher than that against wild-type HCV.^{13,19} In regard to NS5A L31M/V-Y93H double mutation HCV, which is most frequently selected in patients who failed to respond to daclatasvir + asunaprevir^{21–23,26} or sofosbuvir/ledipasvir,²⁷ the mean EC₅₀ of daclatasvir or ledipasvir against the double mutant HCV is approximately 7,000 to 75,000 times higher than that against wild-type HCV^{15–17,35} (► **Table 2**). NS5A P32del HCV shows higher-level resistance to daclatasvir or ledipasvir than the NS5A L31M/V-Y93H double mutation HCV. While HCV with the NS5A L31M/V-Y93H double mutation HCV is sensitive to a second-generation NS5A inhibitor, such as pibrentasvir¹³ and velpatasvir,³⁶ NS5A P32del HCV shows high-level resistance against all NS5A inhibitors used in clinical practice, including ombitasvir, elbasvir, pibrentasvir, and velpatasvir.¹³ The mean EC₅₀s of second-generation NS5A inhibitors against NS5A P32del HCV are approximately 1,000 to 1.7 million times higher than those against wild-type HCV (► **Table 2**).¹³

The JFH-1 HCV clone, genotype 2a HCV, can infect hepatoma cells and is useful as an in vitro infection model. Using JFH-1-based recombinant HCV with NS5A from genotype 1b HCV, HCV with NS5A P32del demonstrated high-level resistance to daclatasvir or ledipasvir, which is consistent with replicon studies (► **Table 2**).^{12,37,38} Importantly, while the NS5A L31M/V-Y93H double mutation HCV is relatively sensitive to second-generation NS5A inhibitors, including velpatasvir and pibrentasvir, NS5A P32del HCV still remains highly resistant to all second-generation NS5A inhibitors.^{12,37–39} The EC₅₀ of velpatasvir against NS5A P32del HCV is 1,000 to 40,000 times higher than that against wild-type HCV, while that

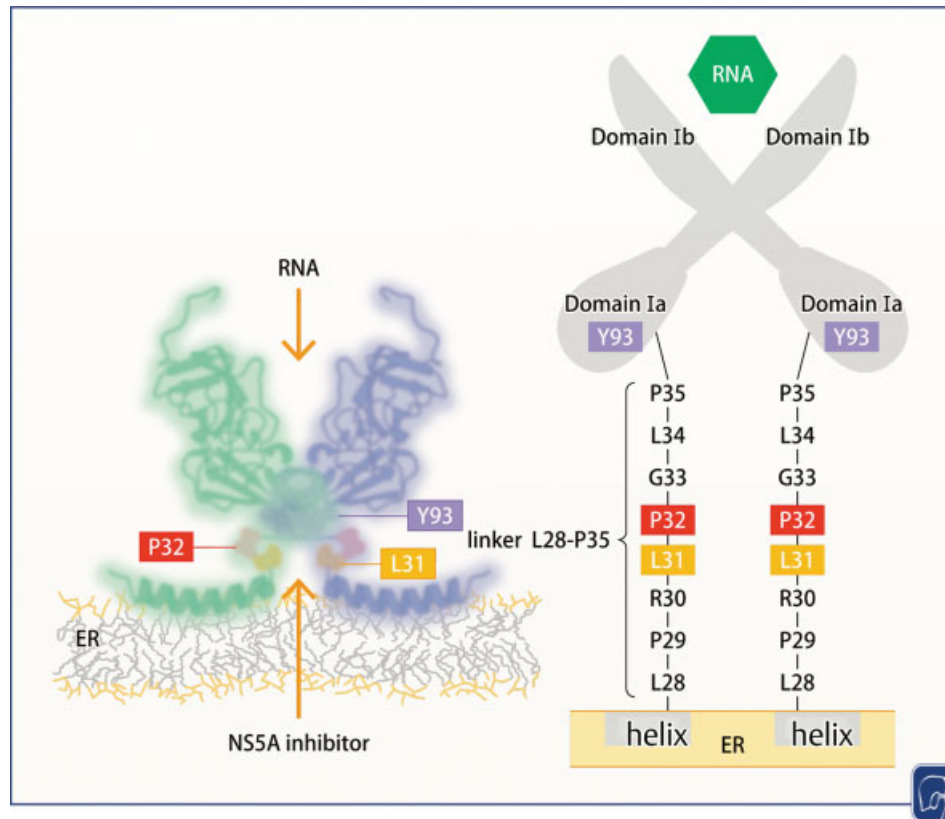


Fig. 1 Structure of NS5A domain I.

against NS5A L31M-Y93H HCV is 44 to 999 times higher than that against wild-type HCV (►Table 2).^{12,13} The EC_{50} of pibrentasvir against NS5A P32del HCV is 1,000 times higher than that against wild-type HCV, while that against NS5A L31M-Y93H HCV is 0.7 to 79 times higher than that against wild-type HCV (►Table 2).^{12,13} These data suggest that HCV with NS5A P32del, which is selected in patients with a failed response to an NS5A inhibitor, acquires cross-resistance to all other NS5A inhibitors. Conversely, the EC_{50} 's of other class antivirals, including NS3/4A inhibitors, NS5B inhibitors, ribavirin, and IFN, against NS5A P32del HCV are similar to those of wild-type HCV.^{37,38}

NS5A P32del HCV Replicates at a Low Level In Vitro but Persists in Patients

In contrast to the extremely high-level resistance of NS5A P32del HCV to all NS5A inhibitors, its replication levels decrease to 30% when compared with replication levels of wild-type HCV in replicon systems.¹⁹ Similarly, the replication levels of NS5A P32del HCV are lower than those of wild-type HCV in the JFH-1-based recombinant HCV infection system (►Table 2).^{37,38} Despite the low replication data of NS5A P32del HCV in vitro, clinical data have shown that selected NS5A P32del HCV by DAA treatment failure remains in patients for more than 1 year.^{25,27} Amino acid substitutions of NS5A L31F and L31M are sometimes codetected in patients with NS5A P32del HCV (►Table 1). The results of in vitro experiments indicated that these substitutions increased the

replication capacity of P32del HCV only slightly; however, the replication levels of NS5A L31F/M + P32del HCV were still lower than those of wild-type HCV (►Table 2).^{37,38} Doi et al reported that no other compensatory amino acid change was detected in NS5A domain 1 lesions in patients with P32del HCV.²⁷ We cannot exclude the possibility that some other amino acid changes outside of the NS5A domain 1 contribute to replication fitness.

Examining HCV infection in vivo is difficult because HCV does not typically infect experimental small animals. However, mice whose hepatocytes are replaced by human hepatocytes have been used to establish an experimental model with several immune-deficient mouse strains.^{40–43} After sera from HCV-infected patients or a chimpanzee are inoculated into them, the mice develop persistent viremia of HCV.^{43–47} Doi et al reported that after patient serum containing over 99% of NS5A P32del HCV was inoculated into 25 humanized liver mice, all of them developed persistent viremia, with over 99% carrying NS5A P32del HCV; no compensatory amino acid change was detected in NS5A domain 1 lesions.³⁷ Their serum HCV RNA levels were similar to those of mice inoculated with patient serum containing wild-type HCV.³⁷ Osawa et al also reported that after patient serum containing NS5A P32del HCV as the major clone was inoculated into humanized liver mice, they developed persistent viremia carrying NS5A P32del HCV as the major clone.⁴⁸ These findings support the idea that the NS5A P32del that occurs selectively in patients who fail to respond to DAAs already obtained replication fitness.

Table 2 Replication level and fold resistance calculated by EC₅₀ in 1b replicon system and JFH-1-based recombinant HCV with NS5A from genotype 1b HCV

System	NS5A resistance-associated amino acid change	Replication level	Fold resistance							Ref	
			Daclatasvir	Ledipasvir	Ombitasvir	Elbasvir	Ruzasvir	Velpatasvir	Pibrentasvir		
Replicon	L31V	144%	61								14
	Y93H	20%	49								
Replicon	L31F	146%	5								15
	P32L	18%	17								
	P58S	121%	1								
	Y93N	21%	28								
	L23F + L31F	65%	13								
	R30Q + L31F	224%	90								
	L31V + Y93H	30%	8,336								
	F37L + Y93H	49%	19								
	Q54H-Y93H	22%	10								
	L31M	99%	3								
Replicon	L31V	158%	28								
	Q54H	83%	1								
	Q54N	83%	1								
	Y93H	27%	24								
	L31M-Y93H	70%	7,105								
	L31V-Y93H	50%	14,789								
	Q54H-Y93H	22%	9								
	L31V-Q54H-Y93H	189%	19,000								
	L31V	157.9%	81								17
	Y93H	26.9%	145								
Replicon	L31V + Y93H	49.9%	80,645								19
	L31I	54%	1.4								
	L31I + Y93H	43%	2,526								
	P32del	29.1%	> 3.9 × 10 ⁵								
	L31M-Y93H	-	24,500	65,500							
Replicon	L28M	81%	1.3	1.3					1.3		36

(Continued)

Table 2 (Continued)

System	NS5A resistance-associated amino acid change	Replication level	Fold resistance							Ref	
			Daclatasvir	Ledipasvir	Ombitasvir	Elbasvir	Ruzasvir	Velpatasvir	Pibrentasvir		
	L31F	173%	9.1	7.8					3.9		
	L31M	274%	3.8	3.4					1.0		
	L31V	113%	8.9	43					1.7		
	Q54H	98%	-	1.3					1.1		
	Y93H	55%	38	3,310					3.3		
	L28M + Y93H	334%	-	14,467					7.0		
	L31F + Y93H	-	-	12,273					51		
	L31M + Y93H	104%	-	20,270					44		
	L31V + Y93H	195%	-	75,483					510		
	L31V + Q54H ⁺ Y93H	-	-	40,116					215		
Replicon	Q24K	-								1.6	
	L28M	-								1.0	
	L28T	-								0.9	
	R30Q	-								0.5	
	L31F	-								1.2	
	L31M	-								1.5	
	L31V	-								0.8	
	P58S	-								1.2	
	A92E	-								0.5	
	A92V	-								0.5	
	Y93H	-								0.6	
	Y93S	-								0.4	
	Q24K + R30Q	-								1.6	
L28M + R30Q	-								0.4		
L28M + Y93H	-								1.2		
R30Q + Y93H	-								1.2		
L31F + A92E	-								0.6		
L31F + Y93H	-								1.5		

Table 2 (Continued)

System	NS5A resistance-associated amino acid change	Replication level	Fold resistance								Ref	
			Daclatasvir	Ledipasvir	Ombitasvir	Elbasvir	Ruzasvir	Velpatasvir	Pibrentasvir			
JFH1-based	L31M + Y93H	-									0.7	
	L31V + A92K	-									2.6	
	L31V + Y93H	-									0.9	
	P58S + Y93H	-									0.8	
	L28M + R30Q + Y93H	-									0.5	
	P32del	-		528,271	1,726,401	115,281				40,875	1,036	
	L32F P32del	-		389,340	452,479	1,363,165	100,594			62,944	20,461	
	L28M	130%		4-79								12
	Y93H	100%		80-999	≥ 1,000	≥ 1,000	4-79	4-79	4-79	≤ 3	≤ 3	
	Y93N	80%		80-999	≥ 1,000	≥ 1,000	4-79	4-79	4-79	4-79	≤ 3	
	L28M + Y93N	100%		≥ 1,000	≥ 1,000	≥ 1,000	≥ 1,000	80-999	80-999	80-999	≤ 3	
	L31V	-		4-79	≥ 1,000	80-999	≤ 3	≤ 3	≤ 3	≤ 3	≤ 3	
L31M + Y93H	-		≥ 1,000	≥ 1,000	≥ 1,000	≥ 1,000	≥ 1,000	≥ 1,000	80-999	4-79		
P32del	-		≥ 1,000	≥ 1,000	≥ 1,000	≥ 1,000	≥ 1,000	≥ 1,000	≥ 1,000	≥ 1,000		
JFH1-based	L31M	155%	3.90									38,39
	L31V	80%	27.1									
	L31I	100%	1.65									
	Y93H	85%	74		5.75 × 10 ³		16.5			2.3		
	L31M + Y93H	95%	1.33 × 10 ⁴		3.96 × 10 ⁵							
	L31V + Y93H	55%	4.27 × 10 ⁴		5.42 × 10 ⁵		2.9 × 10 ⁴			2.1 × 10 ³		
	L31I + Y93H	70%	3.64 × 10 ³		2.40 × 10 ⁵							
	A92K	45%			1.7 × 10 ⁷		8.6 × 10 ³			13.7		
	R30Q + A92K	240%			2.0 × 10 ⁷		3.1 × 10 ⁴			6.2 × 10 ²		
	Q24K + L28M + R30Q + A92K	15%			1.7 × 10 ⁷		6.2 × 10 ⁴			1.9 × 10 ⁴		
	P32del	10%			2.6 × 10 ⁷		2.5 × 10 ⁶			1.1 × 10 ⁵		
	L32F + P32del	20%			5.3 × 10 ⁷		9.1 × 10 ⁶			5.9 × 10 ⁶		
JFH1-based	P32del	15%	NA		1.67 × 10 ⁷	NA	NA			2.56 × 10 ⁵		37
	L31M P32del	20%	NA		6.40 × 10 ⁶	NA	NA			3.44 × 10 ⁵		

Abbreviations: HCV, hepatitis C virus; NA, not available/too high.

Identifying Treatments that can Eliminate HCV with NS5A P32del

The effects of some treatments against NS5A P32del HCV were examined using NS5A P32del HCV-infected humanized liver chimeric mice. Osawa et al reported that serum HCV RNA levels of NS5A P32del HCV-infected mice did not decrease to below the lower limit of detection levels with glecaprevir/pibrentasvir treatment, while both wild-type HCV-infected mice and L31M/Y93H HCV-infected mice achieved sustained virologic response (SVR) with glecaprevir/pibrentasvir.⁴⁸ Teraoka et al reported that NS5A P32del HCV-infected mice showed high-level resistance to glecaprevir/pibrentasvir treatment but were sensitive to the combination treatment of sofosbuvir + glecaprevir/pibrentasvir.²⁶ Doi et al reported that NS5A P32del HCV-infected mice showed high-level resistance against the combination treatment ledipasvir + GS-558093, a nucleotide NS5B inhibitor, as well as ledipasvir monotherapy or elbasvir monotherapy.³⁷ However, they showed sensitivity to the combination treatment simeprevir + GS-558093 or simeprevir + PegIFN.³⁷ These data suggest that NS5A P32del HCV are difficult to eliminate with a combination treatment of two DAAs including an NS5A inhibitor such as sofosbuvir/ledipasvir or glecaprevir/pibrentasvir. Combination treatments containing more than one drug, except NS5A inhibitors, should be required for NS5A P32del HCV.

Clinical Data on Retreatment with Sofosbuvir/Ledipasvir or Glecaprevir/Pibrentasvir in Patients with NS5A P32del HCV

To date, some patients with NS5A P32del HCV have received retreatment with IFN-free DAA therapy (→ Fig. 2). Akuta et al reported that a total of three patients were retreated by sofosbuvir/ledipasvir, and all three developed virologic failure.⁴⁹ Conversely, one patient with NS5A P32del HCV who

achieved SVR with sofosbuvir/ledipasvir was reported.⁵⁰ In addition, before retreatment, the patient had HCV that carried L31M, Q54H, A92K, and Y93H. In a phase 3 clinical trial of glecaprevir/pibrentasvir for HCV-infected Japanese patients with prior DAA experience (CERTAIN-1), a total of 32 patients were enrolled.⁵¹ Among them, two genotype 1b HCV-infected patients who had experienced virologic failure to daclatasvir + asunaprevir had NS5A P32del HCV before retreatment with glecaprevir/pibrentasvir. Both patients developed virologic failure again by retreatment, while the other 30 patients achieved SVR.^{13,51,52} In a phase 3 clinical trial of glecaprevir/pibrentasvir in Europe (MAGELLAN-1), a patient infected with genotype 1b HCV experienced virologic failure to daclatasvir + asunaprevir had NS5A P32del HCV before retreatment with glecaprevir/pibrentasvir and again developed virologic failure.⁵³ For real-world data, Osawa et al reported cases in which 30 patients with a previous history of DAA therapy, including 1 patient with NS5A P32del HCV, were retreated with glecaprevir/pibrentasvir.⁵⁴ A total of two patients, including the patient with NS5A P32del HCV, developed virologic failure. Uemura et al reported cases in which 42 patients with a previous DAA therapy history, including 3 patients with NS5A P32del HCV, were retreated with glecaprevir/pibrentasvir.⁵⁵ All three patients with NS5A P32del HCV experienced virologic failure, while the other patients achieved SVR. Collectively, NS5A P32del HCV is highly resistant to glecaprevir/pibrentasvir as well as sofosbuvir/ledipasvir, which is consistent with the experimental results from the murine model.

Clinical Data on Retreatments with Three DAAs and Sofosbuvir/NS5A Inhibitor Plus Ribavirin in Patients with NS5A P32del HCV

Among treatments containing more than one drug but not NS5A inhibitors, the efficiencies of 4 types of treatments for patients with NS5A P32del HCV have been reported:

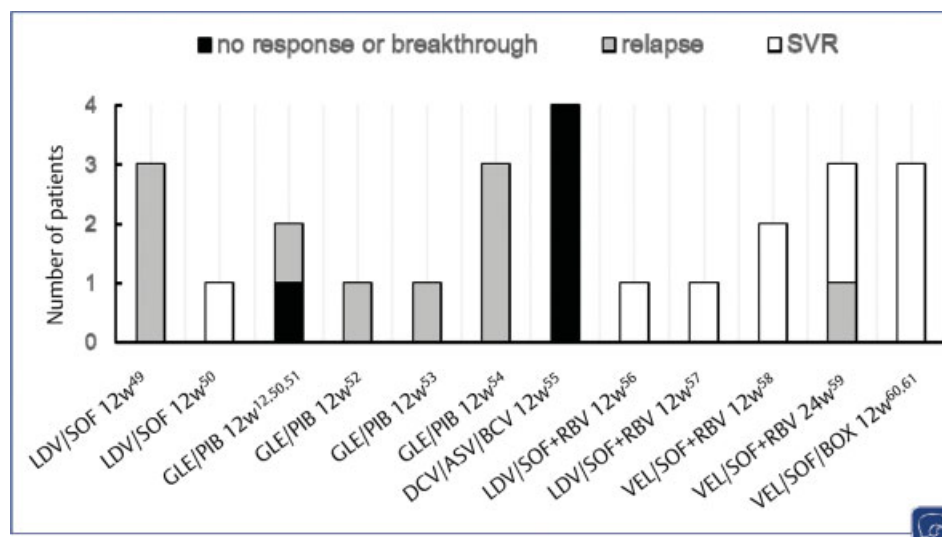


Fig. 2 Retreatment effect of patients infected with NS5A P32del hepatitis C virus (HCV). ASV, asunaprevir; BCV, beclabuvir; BOX, voxilaprevir; DCV, daclatasvir; GLE, glecaprevir; LDV, ledipasvir; PIB, pibrentasvir; RBV, ribavirin; SOF, sofosbuvir; VEL, velpatasvir.

daclatasvir/asunaprevir/beclabuvir for 12 weeks; sofosbuvir/ledipasvir + ribavirin for 12 weeks; sofosbuvir/velpatasvir + ribavirin for 24 weeks; and sofosbuvir/velpatasvir/voxilaprevir for 12 weeks. Takaguchi et al reported that a total of four patients with NS5A P32del HCV who had failed prior IFN-free DAA therapy were treated with daclatasvir/asunaprevir/beclabuvir and that none of them achieved SVR despite having wild-type NS3 D168.⁵⁶ Regarding daclatasvir/asunaprevir/beclabuvir treatment, its efficiency against patients without NS5A P32del HCV who had failed prior IFN-free DAA therapy was also low, which was reported to be 33% (17/51) in real-world settings.⁵⁶ Currently, daclatasvir/asunaprevir/beclabuvir are not recommended for any hepatitis C patients, including patients with NS5A P32del HCV. Suda et al⁵⁷ and Ikeda et al⁵⁸ both reported one patient infected with NS5A P32del HCV who was retreated with sofosbuvir/ledipasvir + ribavirin for 12 weeks in two separate clinical trials. The two patients achieved SVR. In a Japanese phase 3 clinical trial for HCV-infected patients with prior DAA experience, five patients with NS5A P32del HCV were retreated with sofosbuvir/velpatasvir + ribavirin.⁵⁹ Among them, four patients achieved SVR, and only one patient developed virologic failure. In regard to sofosbuvir/velpatasvir/voxilaprevir, a total of three patients with NS5A P32del HCV were treated for 12 weeks in clinical trials (POLARIS-1, POLARIS-4), and all of them achieved SVR.^{60,61} The efficiency of sofosbuvir/velpatasvir/voxilaprevir, which includes both an NS3/4A inhibitor and an NS5B inhibitor but not an NS5A inhibitor, is expected to be high in patients with NS5A P32del HCV.

Current Treatment Options for NS5A P32del HCV

According to the current American Association for the Study of Liver Diseases guidelines,⁶² for NS5A Inhibitor DAA-experienced genotype 1b patients with or without compensated cirrhosis, sofosbuvir/velpatasvir/voxilaprevir for 12 weeks is recommended. In the current European Association for the Study of the Liver guidelines,⁶³ for DAA-experienced patients with or without compensated cirrhosis protease inhibitors and/or NS5A inhibitor are recommended along with sofosbuvir/velpatasvir/voxilaprevir for 12 weeks. For these patients, especially patients with predictors of a poor response (advanced liver disease, multiple courses of DAA-based treatment, and complex NS5A resistance-associated amino acid change profile), sofosbuvir + glecaprevir/pibrentasvir is recommended. To date, three patients with NS5A P32del HCV have been reported to be treated with sofosbuvir/velpatasvir/voxilaprevir, and all of them achieved SVR. The efficacy of sofosbuvir + glecaprevir/pibrentasvir has not yet been reported in patients with NS5A P32del HCV. Although clinical evidence is weak, the EC₅₀ of each drug against NS5A P32del HCV and experimental data using HCV-infected mice suggest that both treatments are expected to be effective against NS5A P32del HCV.

However, sofosbuvir/velpatasvir/voxilaprevir will not be developed in some Asian countries, including Japan, due to the race-specific toxicity of voxilaprevir. It is difficult to use sofosbuvir in combination with glecaprevir/pibrentasvir in

some countries, including Japan, due to regulations related to the health insurance system. In these areas, other treatments containing more than one drug, except NS5A inhibitors such as sofosbuvir/velpatasvir + ribavirin, would be a suitable alternative choice. In regard to the sofosbuvir/velpatasvir + ribavirin treatment, 98% (46/47) of NS5A DAA-experienced patients with HCV genotype 1b achieved SVR24 with sofosbuvir/velpatasvir + ribavirin retreatment for 24 weeks in a Japanese clinical trial⁵⁹; thus, this regimen is the recommended treatment for DAA-experienced patients in the current guidelines of the Japan Society of Hepatology. Considering the high-level resistance of NS5A P32del HCV to velpatasvir,^{12,13,37,38} the effect of sofosbuvir/velpatasvir + ribavirin treatment on patients with the NS5A P32del HCV is highly dependent on their sensitivity to sofosbuvir and ribavirin. The EC₅₀'s of sofosbuvir and ribavirin against NS5A P32del HCV are similar to those of wild-type HCV.^{37,38} NS5A P32del HCV is detected only in genotype 1b. The efficacy of sofosbuvir + ribavirin, which may not be as high as that of sofosbuvir/ledipasvir or sofosbuvir/velpatasvir, requires a longer treatment duration but is still relatively beneficial for naive patients when the HCV genotype is limited to genotype 1b. For example, a phase 3b study in India demonstrated that sofosbuvir + ribavirin treatment for 24 weeks achieved 100% (23/23) SVR in treatment-naive genotype 1b patients.⁶⁴ A phase 3b study in China demonstrated that sofosbuvir + ribavirin treatment for 24 weeks achieved 94% SVR in NS5A inhibitor-unexperienced genotype 1b patients.⁶⁵ Considering the efficacy of 24-week sofosbuvir + ribavirin treatment in genotype 1b patients, velpatasvir/sofosbuvir + ribavirin treatment for 24 weeks for patients with NS5A P32del HCV is expected to be effective and a suitable alternative choice when sofosbuvir/velpatasvir/voxilaprevir or sofosbuvir + glecaprevir/pibrentasvir cannot be used.

Conclusion

NS5A P32del was detected in 5 to 10% of genotype 1b patients who experienced virologic failure with daclatasvir-containing therapies and sofosbuvir/ledipasvir treatment. Although most cases of NS5A P32del HCV are reported in Japan, some have been reported in clinical trials conducted in Europe⁵³ and Western countries.^{60,61} These reports suggest that NS5A P32del HCV is relevant in countries other than Japan. NS5A P32del HCV clones are generated by the complete deletion of three bases, which encode NS5A P32 from the original clones. In regard to deep sequencing analysis, deletion of three consecutive bases is sometimes unmapped on the reference sequence and consequently cannot be detected. In the examination of HCV resistance-associated amino acid changes, confirming whether NS5A P32del HCV clones can be mapped appropriately on the used reference sequence is important. To date, NS5A P32del was not detected in naive patients. However, the possibility that a patient with NS5A P32del HCV infects someone cannot be excluded, especially among high-risk populations, such as persons who inject drugs.^{66,67} From this aspect, analysis of HCV resistance-associated amino acid changes in naive patients is also informative. A recommendation therapy

against patients infected with NS5A P32del HCV remains unestablished. We should pay attention to the existence of NS5A P32del HCV, which is artificially generated by an NS5A inhibitor.

Conflicts of Interest

T.T. reports grants from the Japan Agency for Medical Research and Development, AMED, during the conduct of the study; grants and personal fees from Bristol-Myers Squibb, grants and personal fees from MSD K.K., grants and personal fees from AbbVie Inc, grants and personal fees from Gilead Sciences, grants from Chugai Pharmaceutical Co. Ltd, grants from Janssen Pharmaceutical K.K., outside the submitted work. H.H. reports grants from the Japan Agency for Medical Research and Development, AMED, during the conduct of the study; grants from MSD K.K., grants and personal fees from Bristol-Myers Squibb, outside the submitted work.

References

- Foster GR, Afdhal N, Roberts SK, et al; ASTRAL-2 Investigators; ASTRAL-3 Investigators. Sofosbuvir and velpatasvir for HCV genotype 2 and 3 infection. *N Engl J Med* 2015;373(27):2608–2617
- Mizokami M, Yokosuka O, Takehara T, et al. Ledipasvir and sofosbuvir fixed-dose combination with and without ribavirin for 12 weeks in treatment-naïve and previously treated Japanese patients with genotype 1 hepatitis C: an open-label, randomised, phase 3 trial. *Lancet Infect Dis* 2015;15(06):645–653
- Alqahtani SA, Afdhal N, Zeuzem S, et al. Safety and tolerability of ledipasvir/sofosbuvir with and without ribavirin in patients with chronic hepatitis C virus genotype 1 infection: analysis of phase III ION trials. *Hepatology* 2015;62(01):25–30
- Reau N, Robertson MN, Feng HP, et al. Concomitant proton pump inhibitor use does not reduce the efficacy of elbasvir/grazoprevir: a pooled analysis of 1,322 patients with hepatitis C infection. *Hepatol Commun* 2017;1(08):757–764
- Wei L, Jia JD, Wang FS, et al; C-CORAL Investigators. Efficacy and safety of elbasvir/grazoprevir in participants with hepatitis C virus genotype 1, 4, or 6 infection from the Asia-Pacific region and Russia: final results from the randomized C-CORAL study. *J Gastroenterol Hepatol* 2019;34(01):12–21
- Puoti M, Foster GR, Wang S, et al. High SVR12 with 8-week and 12-week glecaprevir/pibrentasvir therapy: an integrated analysis of HCV genotype 1-6 patients without cirrhosis. *J Hepatol* 2018;69(02):293–300
- Kumada H, Suzuki Y, Karino Y, et al. The combination of elbasvir and grazoprevir for the treatment of chronic HCV infection in Japanese patients: a randomized phase II/III study. *J Gastroenterol* 2017;52(04):520–533
- Naganuma A, Chayama K, Notsumata K, et al. Integrated analysis of 8-week glecaprevir/pibrentasvir in Japanese and overseas patients without cirrhosis and with hepatitis C virus genotype 1 or 2 infection. *J Gastroenterol* 2019;54(08):752–761
- Carrasco I, Arias A, Benítez-Gutiérrez L, et al. Baseline NS5A resistance associated substitutions may impair DAA response in real-world hepatitis C patients. *J Med Virol* 2018;90(03):532–536
- Li DK, Chung RT. Overview of direct-acting antiviral drugs and drug resistance of hepatitis C virus. *Methods Mol Biol* 2019;1911:3–32
- Dietz J, Susser S, Vermehren J, et al; European HCV Resistance Study Group. Patterns of resistance-associated substitutions in patients with chronic HCV infection following treatment with direct-acting antivirals. *Gastroenterology* 2018;154(04):976–988.e4
- Gottwein JM, Pham LV, Mikkelsen LS, et al. Efficacy of NS5A inhibitors against hepatitis C virus genotypes 1-7 and escape variants. *Gastroenterology* 2018;154(05):1435–1448
- Krishnan P, Schnell G, Tripathi R, et al. Integrated resistance analysis of CERTAIN-1 and CERTAIN-2 studies in hepatitis C virus-infected patients receiving glecaprevir and pibrentasvir in Japan. *Antimicrob Agents Chemother* 2018;62(02):e02217-17
- Gao M, Nettles RE, Belema M, et al. Chemical genetics strategy identifies an HCV NS5A inhibitor with a potent clinical effect. *Nature* 2010;465(7294):96–100
- Fridell RA, Qiu D, Wang C, Valera L, Gao M. Resistance analysis of the hepatitis C virus NS5A inhibitor BMS-790052 in an in vitro replicon system. *Antimicrob Agents Chemother* 2010;54(09):3641–3650
- Fridell RA, Wang C, Sun JH, et al. Genotypic and phenotypic analysis of variants resistant to hepatitis C virus nonstructural protein 5A replication complex inhibitor BMS-790052 in humans: in vitro and in vivo correlations. *Hepatology* 2011;54(06):1924–1935
- Wang C, Huang H, Valera L, et al. Hepatitis C virus RNA elimination and development of resistance in replicon cells treated with BMS-790052. *Antimicrob Agents Chemother* 2012;56(03):1350–1358
- Nettles RE, Gao M, Bifano M, et al. Multiple ascending dose study of BMS-790052, a nonstructural protein 5A replication complex inhibitor, in patients infected with hepatitis C virus genotype 1. *Hepatology* 2011;54(06):1956–1965
- Wang C, Sun JH, O'Boyle DR II, et al. Persistence of resistant variants in hepatitis C virus-infected patients treated with the NS5A replication complex inhibitor daclatasvir. *Antimicrob Agents Chemother* 2013;57(05):2054–2065
- McPhee F, Hernandez D, Zhou N, et al. Virological escape in HCV genotype-1-infected patients receiving daclatasvir plus ribavirin and peginterferon alfa-2a or alfa-2b. *Antivir Ther* 2014;19(05):479–490
- Uchida Y, Kouyama JI, Naiki K, et al. Development of rare resistance-associated variants that are extremely tolerant against NS5A inhibitors during daclatasvir/asunaprevir therapy by a two-hit mechanism. *Hepatol Res* 2016;46(12):1234–1246
- Kai Y, Hikita H, Morishita N, et al. Baseline quasispecies selection and novel mutations contribute to emerging resistance-associated substitutions in hepatitis C virus after direct-acting antiviral treatment. *Sci Rep* 2017;7:41660
- Itakura J, Kurosaki M, Hasebe C, et al. Complex pattern of resistance-associated substitutions of hepatitis C virus after daclatasvir/asunaprevir treatment failure. *PLoS One* 2016;11(10):e0165339
- Kobayashi M, Akuta N, Suzuki F, et al. Treatment efficacy of all-oral combinations of direct-acting antivirals in chronic hepatitis C patients with NS5A-P32 deletion. *Kanzo* 2017;58(12):671–673
- Iio E, Shintaro O, Shimada N, et al. Serial changes of NS5A P32deletion mutant in HCV genotype 1b patients after Daclatasvir/Asunaprevir failure. *Kanzo* 2018;59(04):230–233
- Teraoka Y, Uchida T, Imamura M, et al; Hiroshima Liver Study Group. Prevalence of NS5A resistance associated variants in NS5A inhibitor treatment failures and an effective treatment for NS5A-P32 deleted hepatitis C virus in humanized mice. *Biochem Biophys Res Commun* 2018;500(02):152–157
- Doi A, Hikita H, Sakamori R, et al. Nonstructural protein 5A/P32 deletion after failure of ledipasvir/sofosbuvir in hepatitis C virus genotype 1b infection. *Hepatology* 2018;68(01):380–383
- Tellinghuisen TL, Marcotrigiano J, Gorbalenya AE, Rice CM. The NS5A protein of hepatitis C virus is a zinc metalloprotein. *J Biol Chem* 2004;279(47):48576–48587
- Tellinghuisen TL, Marcotrigiano J, Rice CM. Structure of the zinc-binding domain of an essential component of the hepatitis C virus replicase. *Nature* 2005;435(7040):374–379
- Penin F, Brass V, Appel N, et al. Structure and function of the membrane anchor domain of hepatitis C virus nonstructural protein 5A. *J Biol Chem* 2004;279(39):40835–40843

- 31 Ascher DB, Wielens J, Nero TL, Doughty L, Morton CJ, Parker MW. Potent hepatitis C inhibitors bind directly to NS5A and reduce its affinity for RNA. *Sci Rep* 2014;4:4765
- 32 Knops E, Sierra S, Kalaghatgi P, Heger E, Kaiser R, Kalinina OV. Epistatic interactions in NS5A of hepatitis C virus suggest drug resistance mechanisms. *Genes (Basel)* 2018;9(07):E343
- 33 Nettles JH, Stanton RA, Brody J, et al. Asymmetric binding to NS5A by daclatasvir (BMS-790052) and analogs suggests two novel modes of HCV inhibition. *J Med Chem* 2014;57(23):10031–10043
- 34 Yin C, Goonawardane N, Stewart H, Harris M. A role for domain I of the hepatitis C virus NS5A protein in virus assembly. *PLoS Pathog* 2018;14(01):e1006834
- 35 Friborg J, Zhou N, Han Z, et al. In vitro assessment of re-treatment options for patients with hepatitis C Virus genotype 1b infection resistant to daclatasvir plus asunaprevir. *Infect Dis Ther* 2014
- 36 Dvory-Sobol H, Han B, Lu J, et al. In vitro resistance profile of hepatitis C virus NS5A inhibitor velpatasvir in genotypes 1 to 6. *J Viral Hepat* 2019;26(08):991–1001
- 37 Doi A, Hikita H, Kai Y, et al. Combinations of two drugs among NS3/4A inhibitors, NS5B inhibitors and non-selective antiviral agents are effective for hepatitis C virus with NS5A-P32 deletion in humanized-liver mice. *J Gastroenterol* 2019;54(05):449–458
- 38 Nitta S, Asahina Y, Kato T, et al. Impact of novel NS5A resistance-associated substitutions of hepatitis C virus detected in treatment-experienced patients. *Sci Rep* 2019;9(01):5722
- 39 Nitta S, Asahina Y, Matsuda M, et al. Effects of resistance-associated NS5A mutations in hepatitis C virus on viral production and susceptibility to antiviral reagents. *Sci Rep* 2016;6:34652
- 40 Rhim JA, Sandgren EP, Degen JL, Palmeter RD, Brinster RL. Replacement of diseased mouse liver by hepatic cell transplantation. *Science* 1994;263(5150):1149–1152
- 41 Azuma H, Paulk N, Ranade A, et al. Robust expansion of human hepatocytes in Fah^{-/-}/Rag2^{-/-}/Il2rg^{-/-} mice. *Nat Biotechnol* 2007; 25(08):903–910
- 42 Hasegawa M, Kawai K, Mitsui T, et al. The reconstituted 'humanized liver' in TK-NOG mice is mature and functional. *Biochem Biophys Res Commun* 2011;405(03):405–410
- 43 Washburn ML, Bility MT, Zhang L, et al. A humanized mouse model to study hepatitis C virus infection, immune response, and liver disease. *Gastroenterology* 2011;140(04):1334–1344
- 44 Tsuge M, Fujimoto Y, Hiraga N, et al. Hepatitis C virus infection suppresses the interferon response in the liver of the human hepatocyte chimeric mouse. *PLoS One* 2011;6(08):e23856
- 45 Kai Y, Hikita H, Tatsumi T, et al. Emergence of hepatitis C virus NS5A L31V plus Y93H variant upon treatment failure of daclatasvir and asunaprevir is relatively resistant to ledipasvir and NS5B polymerase nucleotide inhibitor GS-558093 in human hepatocyte chimeric mice. *J Gastroenterol* 2015;50(11):1145–1151
- 46 Kosaka K, Hiraga N, Imamura M, et al. A novel TK-NOG based humanized mouse model for the study of HBV and HCV infections. *Biochem Biophys Res Commun* 2013;441(01):230–235
- 47 Meuleman P, Libbrecht L, De Vos R, et al. Morphological and biochemical characterization of a human liver in a uPA-SCID mouse chimera. *Hepatology* 2005;41(04):847–856
- 48 Osawa M, Uchida T, Imamura M, et al. Efficacy of glecaprevir and pibrentasvir treatment for genotype 1b hepatitis C virus drug resistance-associated variants in humanized mice. *J Gen Virol* 2019;100(07):1123–1131
- 49 Akuta N, Sezaki H, Suzuki F, et al. Ledipasvir plus sofosbuvir as salvage therapy for HCV genotype 1 failures to prior NS5A inhibitors regimens. *J Med Virol* 2017;89(07):1248–1254
- 50 Iio E, Shimada N, Takaguchi K, et al. Clinical evaluation of sofosbuvir/ledipasvir in patients with chronic hepatitis C genotype 1 with and without prior daclatasvir/asunaprevir therapy. *Hepatol Res* 2017;47(12):1308–1316
- 51 Kumada H, Watanabe T, Suzuki F, et al. Efficacy and safety of glecaprevir/pibrentasvir in HCV-infected Japanese patients with prior DAA experience, severe renal impairment, or genotype 3 infection. *J Gastroenterol* 2018;53(04):566–575
- 52 Sano T, Akuta N, Suzuki F, et al. Role of NS5A-L31/Y93 double wild-type in failure of glecaprevir/pibrentasvir double therapy in two patients with a history of direct-acting antiviral agent failure: an ultra-deep sequencing analysis. *Intern Med* 2019;58(18):2657–2662
- 53 Poordad F, Pol S, Asatryan A, et al. Glecaprevir/Pibrentasvir in patients with hepatitis C virus genotype 1 or 4 and past direct-acting antiviral treatment failure. *Hepatology* 2018;67(04):1253–1260
- 54 Osawa M, Imamura M, Teraoka Y, et al; Hiroshima Liver Study Group. Real-world efficacy of glecaprevir plus pibrentasvir for chronic hepatitis C patient with previous direct-acting antiviral therapy failures. *J Gastroenterol* 2019;54(03):291–296
- 55 Uemura H, Uchida Y, Kouyama JI, et al. NS5A-P32 deletion as a factor involved in virologic failure in patients receiving glecaprevir and pibrentasvir. *J Gastroenterol* 2019;54(05):459–470
- 56 Takaguchi K, Toyoda H, Tsutsui A, et al. Real-world virological efficacy and safety of daclatasvir/asunaprevir/beclabuvir in patients with chronic hepatitis C virus genotype 1 infection in Japan. *J Gastroenterol* 2019;54(08):742–751
- 57 Suda G, Ogawa K, Yamamoto Y, et al; NORTE Study Group. Retreatment with sofosbuvir, ledipasvir, and add-on ribavirin for patients who failed daclatasvir and asunaprevir combination therapy. *J Gastroenterol* 2017;52(10):1122–1129
- 58 Ikeda H, Watanabe T, Shimizu H, et al. Efficacy of ledipasvir/sofosbuvir with or without ribavirin for 12 weeks in genotype 1b HCV patients previously treated with a nonstructural protein 5A inhibitor-containing regimen. *Hepatol Res* 2018;48(10):802–809
- 59 Izumi N, Takehara T, Chayama K, et al. Sofosbuvir-velpatasvir plus ribavirin in Japanese patients with genotype 1 or 2 hepatitis C who failed direct-acting antivirals. *Hepatol Int* 2018;12(04):356–367
- 60 Bourlière M, Gordon SC, Flamm SL, et al; POLARIS-1 and POLARIS-4 Investigators. Sofosbuvir, velpatasvir, and voxilaprevir for previously treated HCV infection. *N Engl J Med* 2017;376(22):2134–2146
- 61 Sarrazin C, Cooper CL, Manns MP, et al. No impact of resistance-associated substitutions on the efficacy of sofosbuvir, velpatasvir, and voxilaprevir for 12 weeks in HCV DAA-experienced patients. *J Hepatol* 2018;69(06):1221–1230
- 62 AASLD-IDSA HCV Guidance Panel. Hepatitis C guidance 2018 update: AASLD-IDSA recommendations for testing, managing, and treating hepatitis C virus infection. *Clin Infect Dis* 2018;67(10):1477–1492
- 63 European Association for the Study of the Liver. Electronic address: easloffice@easloffice.eu; European Association for the Study of the Liver. EASL Recommendations on Treatment of Hepatitis C 2018. *J Hepatol* 2018;69(02):461–511
- 64 Shah SR, Chowdhury A, Mehta R, et al. Sofosbuvir plus ribavirin in treatment-naïve patients with chronic hepatitis C virus genotype 1 or 3 infection in India. *J Viral Hepat* 2017;24(05):371–379
- 65 Wei L, Xie Q, Hou JL, et al. Sofosbuvir plus ribavirin with or without peginterferon for the treatment of hepatitis C virus: results from a phase 3b study in China. *J Gastroenterol Hepatol* 2018;33(06):1168–1176
- 66 Martinello M, Grebely J, Petoumenos K, et al. HCV reinfection incidence among individuals treated for recent infection. *J Viral Hepat* 2017;24(05):359–370
- 67 Rossi C, Butt ZA, Wong S, et al; BC Hepatitis Testers Cohort Team. Hepatitis C virus reinfection after successful treatment with direct-acting antiviral therapy in a large population-based cohort. *J Hepatol* 2018;69(05):1007–1014