Emerging Antivirals in the Future

8

C. Nelson Hayes, Michio Imamura, and Kazuaki Chayama

Abstract

The safety and effectiveness of antiviral therapy for chronic hepatitis C has improved markedly with the introduction of direct-acting antiviral drugs and a concomitant decrease in interferon use. Although DAAs are potent antivirals, the emergence of resistance against DAAs has spurred the development of new drugs. Second-generation NS5A inhibitors have a higher genetic barrier compared to first-generation NS5A inhibitors and are highly effective against strains that are resistant to first-generation NS5A inhibitors. While new drug development has primarily focused on DAAs, another way to counter DAA resistance is to develop combination therapies that target host factors in addition to viral factors because it is more difficult for the virus to overcome changes in the host environment. For example, miravirsen targets host microRNA-122, which is highly expressed in hepatocytes and essential for viral replication. Emergence of resistance mutations in such therapies is very low. Therefore, combined use of DAAs with other drugs is expected in the future to achieve high SVR rates while minimizing the risk of resistance.

C.N. Hayes • M. Imamura
Department of Gastroenterology and Metabolism, Applied Life Sciences, Institute of Biomedical and Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan

Liver Research Project Center, Hiroshima University, Hiroshima 734-8551, Japan

K. Chayama, MD, PhD (✉)
Department of Gastroenterology and Metabolism, Applied Life Sciences, Institute of Biomedical and Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan

Liver Research Project Center, Hiroshima University, Hiroshima 734-8551, Japan

Laboratory for Digestive Diseases, Center for Genomic Medicine, RIKEN, Hiroshima 734-8551, Japan
e-mail: chayama@hiroshima-u.ac.jp

K. Chayama (ed.), *Hepatitis C Virus Treatment*,
DOI 10.1007/978-981-10-2416-0_8

Keywords
Chronic hepatitis C • Second-generation NS5A inhibitors • Non-nucleoside polymerase inhibitors • Miravirsen • Resistance-associated variants

8.1 Abbreviations

DAA	Direct-acting antiviral
HCV	Hepatitis C virus
NI	Nucleoside inhibitor
NNI	Non-nucleoside inhibitor
PEG-IFN	Pegylated interferon
PI	Protease inhibitor
SVR	Sustained viral response

8.2 Introduction

Direct-acting antiviral (DAA) drugs strongly inhibit replication of hepatitis C virus (HCV) by directly targeting essential viral proteins. Triple combination therapy with pegylated interferon (PEG-IFN) plus ribavirin (RBV) and a protease inhibitor, e.g., telaprevir [1], simeprevir [2], or vaniprevir [3], is currently used in Japan for treatment of genotype 1 chronic HCV infection. However, several interferon-free all-oral DAA combination therapies have recently been approved, including daclatasvir plus asunaprevir therapy [4] and sofosbuvir plus ledipasvir [5] for treatment of genotype 1 infections and sofosbuvir plus RBV for treatment of genotype 2 infections [6]. DAAs have potent antiviral effects, and each of these therapies has been shown to have high safety and efficacy. However, drug resistance has nonetheless occurred in some patients, leading to treatment failure and raising questions about the best approach to re-treating these patients. Fortunately, a variety of new drugs are currently under development. Given the large number of DAAs in various stages of clinical development, the number of treatment options is only expected to increase, and effective treatment for all patients is a major goal. This chapter outlines current drug resistance challenges and discusses trends in ongoing and future drug development.

8.2.1 NS3/NS4A Protease Inhibitors

The first DAAs to be approved were the protease inhibitors telaprevir and boceprevir. The 9.6 kb HCV RNA genome contains a single open reading frame coding for a single 3000-amino-acid polypeptide, which must then be cleaved into three structural and six nonstructural proteins. Cellular proteases cleave the three structural

proteins, which include the core protein and two envelope proteins, and the nonstructural proteins are cleaved at four sites by the virally encoded proteases NS2 and NS3 along with the NS4A cofactor. Telaprevir mimics the carboxy-terminal region of the HCV NS3/NS4 serine protease [7] and interferes with viral replication by preventing cleavage of the polyprotein. While telaprevir triple therapy improves SVR rates to around 70 %, aside from its inconvenient thrice-daily dosing regimen, the therapy is associated with a high frequency of adverse events, including pruritus, rash, and nausea [8], and has been reported to lead to treatment discontinuation in 18 % of patients [9]. Clinical trials in Japan reported comparable SVR rates but a higher incidence of adverse events, due in part to the relatively high fixed dose relative to body weight in Japanese patients [10].

The defining characteristic of DAAs is their high target specificity for viral proteins, but a consequence of this specificity is the relatively low barrier to resistance it presents to this highly adaptable RNA virus. Inter-genotypic variation in the NS3 domain restricts the use of first-generation protease inhibitors to HCV genotype 1 [11], but even within genotype 1, resistance occurs more frequently in genotype 1a than 1b due to a synonymous codon at R155 that reduces the number of nucleotide changes needed to achieve a favorable amino acid substitution [12]. Compensatory mutations such as V36M that restore viral fitness may also occur, allowing the virus to compete with wild-type virus in the absence of the drug [13], and cross-resistance prevents the use of related protease inhibitors.

8.2.2 Second-Wave Protease Inhibitors

Second-wave PIs, such as simeprevir, asunaprevir, faldaprevir, and paritaprevir, attempt to overcome these problems by increasing the barrier to resistance and broadening the antiviral activity to other genotypes. Although the historically difficult-to-treat genotype 1 is the most prevalent genotype worldwide with 46.2 % of cases, it has also received the most focus in drug development efforts, whereas genotype 4, which is overrepresented in low-income countries, remains difficult to treat with current therapies [14]. Another goal of second-wave PIs is to improve patient compliance by reducing the dosing schedule and reducing side effects [15]. In light of these improvements, telaprevir and boceprevir should be avoided as a first-line treatment.

Simeprevir has been approved in the USA (150 mg dose) and Japan (100 mg dose) for use in triple therapy with PEG-IFN and ribavirin. Simeprevir is a once-daily macrocyclic PI active against genotypes 1, 2, 4, 5, and 6 [16]. Simeprevir triple therapy achieves SVR rates of up to approximately 80 % [17–19], with similar incidence and severity of adverse events to PEG-IFN and ribavirin alone. With response-guided therapy, up to 96 % of prior relapsers were reported to achieve SVR, but viral breakthrough or relapse was common [20, 21]. Despite these promising results, the simeprevir-resistant Q80K mutation occurs frequently (9–48 %) in genotype 1a patients [22], potentially requiring screening of genotype 1a patients for the Q80 mutation and an alternative therapy if necessary.

8.2.3 Second-Generation Protease Inhibitors

Second-generation PIs attempt to go a step further and provide pan-genotypic activity against all HCV genotypes as well as resistance mutations affecting first-generation PIs. Grazoprevir (MK-5172) is a much anticipated once-daily second-generation PI currently undergoing advanced clinical testing. Grazoprevir is not sensitive to most variants affecting first-generation inhibitors and has a higher barrier to resistance, with SVR rates ranging from 89 % to 100 % [23]. In the randomized, open-label phase II C-WORTHY clinical trial, treatment-naive HCV genotype 1 patients were treated with grazoprevir plus the NS5A inhibitor elbasvir (MK-8742) with or without ribavirin for 12 weeks [24]. The SVR12 rate was 93 % for patients without ribavirin and 93 % with ribavirin. While no patients discontinued due to adverse events, virological failure occurred in 4 % of patients due to emergence of resistance-associated variants against grazoprevir or elbasvir. In another C-WORTHY study examining treatment duration in treatment-naive cirrhotic patients and prior null responders, SVR12 rates up to 100 % were achieved among prior null responders treated for 18 weeks with grazoprevir, elbasvir, and ribavirin [25]. The regimen is now undergoing phase III clinical testing in the C-Edge series of studies examining safety and efficacy of the therapy in a variety of difficult-to-treat patient populations, including those with HIV coinfection, chronic kidney disease, severe liver damage, or blood disorders. Results of a phase III C-Edge HIV coinfection study involving 12 weeks of grazoprevir plus elbasvir therapy indicate that 96 % of patients achieved SVR12, including all 35 patients with cirrhosis [26], while several patients relapsed, in two cases due to reinfection. Adverse events were mild and included fatigue, headache, and nausea. Improvements in drug development will continue to extend the reach of DAA therapy to patients with previously unmet treatment needs, although achieving 100 % SVR may prove challenging.

8.3 Second-Generation NS5A Inhibitors

NS5A inhibitors are among the most effective antivirals available, with picomolar efficacy and pan-genotypic activity, and might serve as the backbone of future HCV therapies. However, first-generation NS5A inhibitors are highly vulnerable to resistance variants, such as L31M and Y93H. Compared to the first-generation NS5A inhibitors daclatasvir and ledipasvir, second-generation inhibitors under development, such as ACH-3120, GS-5816, and MK-8742, have a higher genetic barrier and are expected to be highly effective against resistance mutations that affect first-generation drugs. Tables 8.1 and 8.2 show sensitivity of first- and second-generation NS5A inhibitors to wild-type and first-generation resistance mutations for genotype 1a and 1b using an HCV replicon [27]. The second-generation NS5A inhibitor ACH-3120 has been shown to have overall higher efficacy against NS5A mutations compared to ledipasvir and daclatasvir. For example, ledipasvir and daclatasvir show EC50 fold changes of 119 and 18 against NS5A-L31V variants relative to

Table 8.1 NS5A inhibitor susceptibility to amino acid substitutions in genotype 1a (modified from reference 7 [27])

Genotype 1a	ACH-3120		Ledipasvir		Daclatasvir	
NS5A amino acid substitution	EC50 (nM)	EC50 Fold change	EC50 (nM)	EC50 Fold change	EC50 (nM)	EC50 Fold change
Wild type	0.012	1	0.0061	1	0.015	1
M28T	0.27	23	0.0093	15	2.8	187
M28V	0.013	1	0.016	3	0.0058	0.39
Q30E	0.85	71	20	3279	61	4067
Q30H	0.011	1	0.63	103	2.5	167
Q30K	0.75	63	66	10,819	83	5533
Q30R	0.041	3	2.8	459	3.0	200
L31M	0.019	2	17	2787	1.9	127
L31V	0.016	1	2.4	393	11	733
P32L	0.095	2	0.97	41	1.5	29
H58D	0.1	8	–	–	1.9	127
Y93H	61	5083	30	4918	17	1133
K24R-Q30R	2.4	200	23	3770	17	1133

Table 8.2 NS5A inhibitor susceptibility to amino acid substitutions in genotype 1b (modified from reference 7 [27])

Genotype 1b	ACH-3120		Ledipasvir		Daclatasvir	
NS5A amino acid substitution	EC50 (nM)	EC50 Fold change	EC50 (nM)	EC50 Fold change	EC50 (nM)	EC50 Fold change
Wild type	0.0040	1	0.00077	1	0.0030	1
L28M	0.0045	1	0.0034	4	0.0031	1
L31F	0.0090	1	0.0077	3	0.046	5
L31M	0.0030	1	0.011	14	0.0094	3
L31V	0.0026	1	0.092	119	0.055	18
P32L	0.0025	1	0.0074	10	0.019	6
Y93H	0.0061	2	2.1	2727	0.12	40
Y93N	0.016	4	2.7	3506	0.23	77
L28M-Y93H	2.4	600	199	258,441	7.8	2600
L31M-Y93H	0.047	12	210	272,727	163	54,333
L31V-Y93N	0.95	238	256	332,467	265	88,333
P58A-Y93H	0.047	12	22	28,571	5.9	1967
P58S-T64A-Y93H	0.39	98	2.8	3636	1.1	467

wild type, whereas ACH-3102 shows no change in sensitivity. Similarly, ledipasvir and daclatasvir show EC50 changes of 2727 and 40 against the NS5A-Y93H wild type, while ACH-3102 shows only a slight reduction in sensitivity with a fold change of 2. Even against the double variant NS5A-L31M-Y93H, ACH-3120 remains relatively sensitive, with an EC50 fold change of 12, whereas ledipasvir and daclatasvir become almost completely ineffective, with fold changes of 272,727 and 54,333, respectively. This result suggests that treatment with a second-generation NS5A inhibitor may be an effective re-treatment option for patients who experienced treatment failure with daclatasvir and asunaprevir therapy due to NS5A-L31 and NS5A-Y93 mutations. Clinical trials are currently underway to examine the combination of ACH-3120 and sofosbuvir. In a phase 2 clinical trial, 36 treatment-naive genotype 1 HCV patients were treated with 50 mg ACH-3120 and 40 mg sofosbuvir once daily for 6 or 8 weeks [28]. All patients achieved SVR regardless of treatment period. In the future, ACH-312 and sofosbuvir combination therapy should be examined as a potential re-treatment option for patients who failed to clear the virus during daclatasvir and asunaprevir therapy.

8.4 Non-nucleoside Polymerase Inhibitors

Although both targeting the HCV RNA-dependent RNA polymerase, the polymerase inhibitor DAAs are divided into two drug classes, nucleoside and non-nucleoside inhibitors, that target different steps in RNA synthesis and have different mechanisms and resistance profiles. Following the success of the nucleoside inhibitor sofosbuvir, clinical testing of non-nucleoside inhibitors such as beclabuvir has begun. Beclabuvir, which inhibits polymerase activity by targeting the thumb 1 domain of the NS5B polymerase, has exhibited pan-genotypic antiviral activity against genotypes 1, 3, 4, 5, and 6 in vitro [29]. In a phase II clinical trial, 66 patients were treated for 12 or 24 weeks with beclabuvir in combination with daclatasvir (NS5A inhibitor) and asunaprevir (protease inhibitor), resulting in an SVR rate of 92 % [30]. However, beclabuvir is susceptible to mutations at NS5B-A421 and NS5B-P495 [31, 32]. A breakthrough occurred in genotype 1a patients with treatment-emergent NS3-R155K + NS5A-Q30R-L31M + NS5B-P495L mutations, and NS3-V36M-R155K+ NS5A-M28A-Q30R-H58P + NS5B-P495L mutations emerged at the time of relapse in a patient with preexisting NS3-V36M + NS5A-H58P mutations. In a phase III clinical trial, an SVR rate of 98 % (81/83) was observed for treatment-naive genotype 1b patients, and an SVR rate of 100 % (28/28) was observed for previously treated patients after 12 weeks of treatment [33]. Even among genotype 1a patients, SVR rates of 90 % (206/229) and 85 % (64/75) were observed in treatment-naive patients and previously treated patients, respectively. Furthermore, even genotype 1b patients with preexisting NS5A-L31I/M or NS5A-Y93H mutations were able to achieve SVR (Table 8.3). In an HCV replicon study involving genotype 1b NS5A-L31M-Y93H double mutants, no inhibitory effect was observed for daclatasvir and asunaprevir alone, but the three-way combination of daclatasvir, asunaprevir, and beclabuvir effectively suppressed

Table 8.3 SVR rates for combination therapy with daclatasvir, asunaprevir, and beclabuvir in patients with NS5A amino acid mutations (Modified from [33])

Genotype	NS5A amino acid substitution	Treatment naive ($n=312$)	Previously treated ($n=103$)
Genotype 1a	M28L/I/T/V	12/17 (71 %)	8/9 (89 %)
	Q30H/R	0/5 (0 %)	1/1 (100 %)
	L31M	2/2 (100 %)	2/2 (100 %)
	Y93H/C	1/2 (50 %)	0
Genotype 1b	L28M/V	1/1 (100 %)	1/1 (100 %)
	R30Q	3/3 (100 %)	1/1 (100 %)
	L31I/M	3/3 (100 %)	1/1 (100 %)
	Y93H	6/6 (100 %)	3/3 (100 %)

HCV replication [34]. This result suggests that addition of beclabuvir might be effective in re-treating patients who fail to respond to daclatasvir and asunaprevir therapy. On the other hand, the frequency of resistance mutations affecting non-nucleoside polymerase inhibitors and their effects on combination therapy are not well understood and must be further examined to determine the safest course of treatment for these patients.

8.5 Paritaprevir/Ritonavir, Ombitasvir, and Dasabuvir

Although many sofosbuvir-based therapies are being evaluated, several clinical trials have examined AbbVie's alternative DAA combination therapy consisting of paritaprevir with ritonavir (ABT-450/r, a protease inhibitor), ombitasvir (ABT-267, an NS5A inhibitor), and dasabuvir (ABT-333, an NNI polymerase inhibitor), with or without ribavirin. In a phase III clinical trial, treatment-naive genotype 1 patients who were treated with paritaprevir/r, ombitasvir, dasabuvir, and ribavirin for 12 weeks achieved an SVR12 rate of 96 % (genotype 1a, 95 %; genotype 1b, 98 %) [35]. In another phase III trial, 99.5 % of genotype 1b patients and 97 % of genotype 1a patients achieved SVR12 (36).

The combination therapy also showed promise for patients with cirrhosis or non-response to prior interferon therapy. In a phase III trial, genotype 1 patients who failed to achieve SVR during prior interferon therapy were treated with paritaprevir/r, ombitasvir, dasabuvir, and ribavirin for 12 weeks and achieved an SVR rate of 96 % [37]. In a phase III clinical study of patients with Child-Pugh class A compensated cirrhosis who were treated with paritaprevir/r, ombitasvir, dasabuvir, and ribavirin for 12 or 24 weeks, 91 % of patients in the 12-week arm achieved SVR, and 96 % of patients in the 24-week arm achieved SVR [38].

In December 2014, the FDA approved the Viekira Pak (co-formulated ombitasvir, paritaprevir, and ritonavir co-packaged with dasabuvir tablets) for treatment of genotype 1 infection, including patients with compensated cirrhosis. In September 2015, Japan approved AbbVie's VIEKIRAX (paritaprevir/ritonavir co-formulated

with ombitasvir) for treatment of genotype 1 HCV infection, partly on the basis of results of the GIFT-1 clinical phase III clinical trial in which 94 % of non-cirrhotic patients and 91 % of cirrhotic patients were able to achieve SVR12 [39]. These approvals, along with the FDA's approval of Gilead's Harvoni (ledipasvir co-formulated with sofosbuvir) for genotype 1 in October 2014 and its expanded approval for genotypes 4, 5, and 6 in November 2015, signal an important trend toward fixed, once-daily dosing. While this change should simplify therapy implementation and improve patient compliance, it also limits the discretion of physicians to adjust the dosage or substitute an alternative DAA in response to patient needs, especially among cirrhotic patients. Shortly after approving the Viekira Pak, the FDA warned of serious liver injury or death in some patients with cirrhosis. While therapy was contraindicated or not recommended for many of these patients, treating cirrhotic patients remains a priority, and early discontinuation of DAA therapy could promote antiviral resistance. Interferon-free therapy for patients with advanced liver disease is a recent and unprecedented development, and the long-term outcomes and risks have yet to be determined.

8.6 Host Factor-Targeting Antivirals

8.6.1 Entry Inhibitors

While DAAs have improved treatment prospects for most patients, their safety and effectiveness is less clear in immunocompromised or coinfected patients, as well as patients with advanced liver disease. Prevention of graft reinfection in patients who receive liver transplantation is another concern that may require alternative or complementary approaches. These patients may benefit from HCV entry inhibitors, which interfere with early interactions between the HCV envelope proteins and host factors, by disrupting attachment to hepatocyte receptors or by interfering with post-binding events or viral fusion [40]. Saikosaponins, particularly SSb2, derived from *Bupleurum kaoi* root, have been shown to prevent HCV entry by targeting HCV E2 and inhibiting viral attachment and fusion events [41]. Synergistic interactions between DAAs and host-targeting agents such as antibodies against CD81, SR-B1, or CLDN1 might also help to improve efficacy in difficult-to-treat patients while reducing toxicity [42].

8.6.2 Cyclophilin A Inhibitors

While entry inhibitors prevent entry of HCV into uninfected cells, cyclophilin inhibitors act against already infected cells by disrupting the interaction between HCV NS5A and the HCV replication complex [43, 44]. Addition of the cyclophilin inhibitor SCY-635 was shown to restore interferon-stimulated gene activity in HCV-infected cells by reducing phosphorylation of two negative regulators of ISG activity, PKR and eIF2α [45]. Although the mechanism of action of cyclophilin inhibitors

is incompletely understood, Chatterji et al. showed that cyclophilin A and NS5A are essential for the creation of double-membrane vesicles required for HCV RNA replication [46].

8.6.3 Silymarin

The milk thistle extract silymarin is an antioxidant with hepatoprotective effects on the liver by promoting hepatocyte regeneration and reducing inflammation and fibrogenesis [47] and has long been used for treatment of *Amanita phalloides*-induced liver failure [48]. High-dose intravenous silymarin has been successfully used to treat HCV patients who fail to respond to PEG-IFN plus ribavirin therapy [49–51].

8.6.4 Miravirsen

Highly expressed in the liver, the microRNA miR-122 plays an essential role in HCV replication and presents a potential host antiviral drug target [52]. Miravirsen, a 15-nucleotide locked nucleic acid (LNA), is a modified phosphorothioate antisense oligonucleotide that binds to miR-122 and inhibits its function [53]. Miravirsen has shown pan-genotypic inhibitory effects on replication against genotypes 1 through 6. MiR-122 binds both to the 5′UTR (S1 and S2) [54] and to the 3′UTR (S3) [55] of the HCV RNA genome. Long-term HCV replicon studies of miravirsen activity have not revealed mutations in the miR-122-binding region [56]. Efficacy of miravirsen against chronic hepatitis C patients has been examined in a phase II clinical trial. In this study, 36 genotype 1 patients were assigned to receive placebo or 3, 5, or 7 mg/kg of miravirsen administered weekly by subcutaneous injection for 5 weeks, and HCV RNA levels were monitored for 18 weeks [57]. In the miravirsen-treated patients, blood HCV RNA titers were significantly reduced in a dose-dependent manner that persisted even after the end of therapy. The average HCV RNA reduction was 0.4 log IU/mL in the placebo group, 1.2 log IU/mL ($P=0.01$) in the 3 mg/kg group, 2.9 log IU/mL ($P=0.003$) in the 5 mg/kg group, and 3.0 log IU/mL ($P=0.002$) in the 7 mg/kg group. In addition, after 14 weeks of follow-up, HCV RNA became undetectable in four patients in the 7 mg/kg group and one patient in the 5 mg/kg group. Following the end of treatment, HCV RNA increased again in some patients, but no mutations in the S1, S2, and S3 HCV miR-122-binding sites were observed. However, mutations at A4C and C3U in the full-length 5′UTR were recognized in these patients [56]. In an HCV replicon study, miravirsen sensitivity did not differ between A4C mutant and wild-type strains, but resistance in C3U mutants was sevenfold higher than wild type, which may be problematic during treatment with miravirsen. However, in combination with DAAs, IFN, or RBV, C3U mutations show comparable sensitivity to wild type (Table 8.4). The combination of miravirsen with these agents may help to effectively suppress emergence of resistance mutations.

Table 8.4 Susceptibility of various drugs to 5′UTR substitutions (Modified from [56])

Drug	Fold change relative to wild type	
	A4C substitution	C3U substitution
Miravirsen	1	7
Telaprevir	2	1
VX-222	1	1
Daclatasvir	ND	1
IFN-α2b	ND	1
Daclatasvir	2	0.3
Ribavirin	ND	1

ND no data

8.7 Conclusions

Development of new HCV drugs unaffected by resistance-associated variants that limit current DAA drugs is anticipated. In principle, an SVR rate for treatment of chronic hepatitis C approaching 100 % is possible. However, a number of issues remain with respect to the emergence of resistance mutations, re-treatment following treatment failure, and treatment of patients with renal function decline or decompensated cirrhosis. Although many new drugs are under development, recent clinical trials have focused on shortening the duration of therapy, identifying effective DAA combinations for genotype 1 as well as other genotypes, improving resistance and safety profiles, and evaluating the need for ribavirin. The recent trend toward co-formulated once-daily fixed-dose tablets simplifies the treatment landscape and should improve patient compliance but at the cost of reduced flexibility and potentially greater risk for cirrhotic patients. With the development of new drugs and more effective treatments, it is hoped that all patients with chronic hepatitis C can be successfully treated.

References

1. Kumada H, Toyota J, Okanoue T, Chayama K, Tsubouchi H, Hayashi N. Telaprevir with peginterferon and ribavirin for treatment-naive patients chronically infected with HCV of genotype 1 in Japan. J Hepatol. 2012;56(1):78–84.
2. Hayashi N, Izumi N, Kumada H, Okanoue T, Tsubouchi H, Yatsuhashi H, et al. Simeprevir with peginterferon/ribavirin for treatment-naive hepatitis C genotype 1 patients in Japan: CONCERTO-1, a phase III trial. J Hepatol. 2014;61(2):219–27.
3. Hayashi N, Mobashery N, Izumi N. Vaniprevir plus peginterferon alfa-2a and ribavirin in treatment-experienced Japanese patients with hepatitis C virus genotype 1 infection: a randomized phase II study. J Gastroenterol. 2015;50(2):238–48.
4. Kumada H, Suzuki Y, Ikeda K, Toyota J, Karino Y, Chayama K, et al. Daclatasvir plus asunaprevir for chronic HCV genotype 1b infection. Hepatology. 2014;59(6):2083–9.
5. Mizokami M, Yokosuka O, Takehara T, Sakamoto N, Korenaga M, Mochizuki H, et al. Ledipasvir and sofosbuvir fixed-dose combination with and without ribavirin for 12 weeks in

treatment-naive and previously treated Japanese patients with genotype 1 hepatitis C: an open-label, randomised, phase 3 trial. Lancet Infect Dis. 2015;15(6):645–53.
6. Omata M, Nishiguchi S, Ueno Y, Mochizuki H, Izumi N, Ikeda F, et al. Sofosbuvir plus ribavirin in Japanese patients with chronic genotype 2 HCV infection: an open-label, phase 3 trial. J Viral Hepat. 2014;21(11):762–8.
7. Perni RB, Almquist SJ, Byrn RA, Chandorkar G, Chaturvedi PR, Courtney LF, et al. Preclinical profile of VX-950, a potent, selective, and orally bioavailable inhibitor of hepatitis C virus NS3-4A serine protease. Antimicrob Agents Chemother. 2006;50(3):899–909.
8. Jacobson IM, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, et al. Telaprevir for previously untreated chronic hepatitis C virus infection. N Engl J Med. 2011;364(25):2405–16.
9. Sherman KE, Flamm SL, Afdhal NH, Nelson DR, Sulkowski MS, Everson GT, et al. Response-guided telaprevir combination treatment for hepatitis C virus infection. N Engl J Med. 2011;365(11):1014–24.
10. Chayama K, Hayes CN, Yoshioka K, Moriwaki H, Okanoue T, Sakisaka S, et al. Accumulation of refractory factors for pegylated interferon plus ribavirin therapy in older female patients with chronic hepatitis C. Hepatol Res: Off J Jpn Soc Hepatol. 2010;40(12):1155–67.
11. Halfon P, Locarnini S. Hepatitis C virus resistance to protease inhibitors. J Hepatol. 2011;55(1):192–206.
12. Lok AS, Gardiner DF, Lawitz E, Martorell C, Everson GT, Ghalib R, et al. Preliminary study of two antiviral agents for hepatitis C genotype 1. N Engl J Med. 2012;366(3):216–24.
13. Wyles DL. Beyond telaprevir and boceprevir: resistance and new agents for hepatitis C virus infection. Top Antivir Med. 2012;20(4):139–45.
14. Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, et al. Global distribution and prevalence of hepatitis C virus genotypes. Hepatology. 2015;61(1):77–87.
15. Wendt A, Adhoute X, Castellani P, Oules V, Ansaldi C, Benali S, et al. Chronic hepatitis C: future treatment. Clin Pharmacol: Adv Appl. 2014;6:1–17.
16. Rosenquist A, Samuelsson B, Johansson PO, Cummings MD, Lenz O, Raboisson P, et al. Discovery and development of simeprevir (TMC435), a HCV NS3/4A protease inhibitor. J Med Chem. 2014;57(5):1673–93.
17. Jacobson IM, Dore GJ, Foster GR, Fried MW, Radu M, Rafalsky VV, et al. Simeprevir with pegylated interferon alfa 2a plus ribavirin in treatment-naive patients with chronic hepatitis C virus genotype 1 infection (QUEST-1): a phase 3, randomised, double-blind, placebo-controlled trial. Lancet. 2014;384(9941):403–13.
18. Manns M, Marcellin P, Poordad F, de Araujo ES, Buti M, Horsmans Y, et al. Simeprevir with pegylated interferon alfa 2a or 2b plus ribavirin in treatment-naive patients with chronic hepatitis C virus genotype 1 infection (QUEST-2): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet. 2014;384(9941):414–26.
19. Forns X, Lawitz E, Zeuzem S, Gane E, Bronowicki JP, Andreone P, et al. Simeprevir with peginterferon and ribavirin leads to high rates of SVR in patients with HCV genotype 1 who relapsed after previous therapy: a phase 3 trial. Gastroenterology. 2014;146:1669.
20. Izumi N, Hayashi N, Kumada H, Okanoue T, Tsubouchi H, Yatsuhashi H, et al. Once-daily simeprevir with peginterferon and ribavirin for treatment-experienced HCV genotype 1-infected patients in Japan: the CONCERTO-2 and CONCERTO-3 studies. J Gastroenterol. 2014;49(5):941–53.
21. Zeuzem S, Berg T, Gane E, Ferenci P, Foster GR, Fried MW, et al. Simeprevir increases rate of sustained virologic response among treatment-experienced patients with HCV genotype-1 infection: a phase IIb trial. Gastroenterology. 2014;146(2):430–41.e6.
22. Schneider MD, Sarrazin C. Antiviral therapy of hepatitis C in 2014: do we need resistance testing? Antiviral Res. 2014;105c:64–71.
23. Gentile I, Buonomo AR, Borgia F, Zappulo E, Castaldo G, Borgia G. MK-5172: a second-generation protease inhibitor for the treatment of hepatitis C virus infection. Expert Opin Investig Drugs. 2014;23(5):719–28.

24. Sulkowski M, Hezode C, Gerstoft J, Vierling JM, Mallolas J, Pol S, et al. Efficacy and safety of 8 weeks versus 12 weeks of treatment with grazoprevir (MK-5172) and elbasvir (MK-8742) with or without ribavirin in patients with hepatitis C virus genotype 1 mono-infection and HIV/hepatitis C virus co-infection (C-WORTHY): a randomised, open-label phase 2 trial. Lancet. 2015;385(9973):1087–97.
25. Lawitz E, Gane E, Pearlman B, Tam E, Ghesquiere W, Guyader D, et al. Efficacy and safety of 12 weeks versus 18 weeks of treatment with grazoprevir (MK-5172) and elbasvir (MK-8742) with or without ribavirin for hepatitis C virus genotype 1 infection in previously untreated patients with cirrhosis and patients with previous null response with or without cirrhosis (C-WORTHY): a randomised, open-label phase 2 trial. Lancet. 2015;385(9973):1075–86.
26. Sulkowski MS, Sherman KE, Dieterich DT, Bsharat M, Mahnke L, Rockstroh JK, et al. Combination therapy with telaprevir for chronic hepatitis C virus genotype 1 infection in patients with HIV: a randomized trial. Ann Intern Med. 2013;159(2):86–96.
27. Patel D, Zhao Y, Fabrycki J, Yang G, Podos S, Kocinsk H, et al. P0805: achievement of SVR12 despite the presence of HCV variants resistant to first generation NS5A inhibitors in genotype-1 hepatitis C patients after 8-week therapy of ACH-3102 in combination with sofosbuvir. J Hepatol. 62:S636.
28. Gane E, Schwabe C, Mader M, Suri V, Donohue M, Huang M, et al. LP06: sustained virologic response after ACH-3102 and sofosbuvir treatment for 8 or 6 weeks: a phase 2 "proxy" study. J Hepatol. 62:S266.
29. Liu M, Tuttle M, Gao M, Lemm JA. Potency and resistance analysis of hepatitis C virus NS5B polymerase inhibitor BMS-791325 on all major genotypes. Antimicrob Agents Chemother. 2014;58(12):7416–23.
30. Everson GT, Sims KD, Rodriguez-Torres M, Hezode C, Lawitz E, Bourliere M, et al. Efficacy of an interferon- and ribavirin-free regimen of daclatasvir, asunaprevir, and BMS-791325 in treatment-naive patients with HCV genotype 1 infection. Gastroenterology. 2014;146(2):420–9.
31. Shi N, Hiraga N, Imamura M, Hayes CN, Zhang Y, Kosaka K, et al. Combination therapies with NS5A, NS3 and NS5B inhibitors on different genotypes of hepatitis C virus in human hepatocyte chimeric mice. Gut. 2013;62(7):1055–61.
32. Abe H, Hayes CN, Hiraga N, Imamura M, Tsuge M, Miki D, et al. A translational study of resistance emergence using sequential direct-acting antiviral agents for hepatitis C using ultra-deep sequencing. Am J Gastroenterol. 2013;108(9):1464–72.
33. Reddy KR, Bourliere M, Sulkowski M, Omata M, Zeuzem S, Feld JJ, et al. Ledipasvir and sofosbuvir in patients with genotype 1 hepatitis C virus infection and compensated cirrhosis: an integrated safety and efficacy analysis. Hepatology. 2015;62:79.
34. Friborg J, Zhou N, Han Z, Yang X, Falk P, Mendez P, et al. In vitro assessment of re-treatment options for patients with hepatitis C virus genotype 1b infection resistant to daclatasvir plus asunaprevir. Inf Dis Ther. 2014.
35. Feld JJ, Kowdley KV, Coakley E, Sigal S, Nelson DR, Crawford D, et al. Treatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. N Engl J Med. 2014;370(17):1594–603.
36. Ferenci P, Bernstein D, Lalezari J, Cohen D, Luo Y, Cooper C, et al. ABT-450/r-ombitasvir and dasabuvir with or without ribavirin for HCV. N Engl J Med. 2014;370(21):1983–92.
37. Zeuzem S, Jacobson IM, Baykal T, Marinho RT, Poordad F, Bourliere M, et al. Retreatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. N Engl J Med. 2014;370(17):1604–14.
38. Poordad F, Hezode C, Trinh R, Kowdley KV, Zeuzem S, Agarwal K, et al. ABT-450/r--ombitasvir and dasabuvir with ribavirin for hepatitis C with cirrhosis. N Engl J Med. 2014;370(21):1973–82.
39. Kumada H, Chayama K, Rodrigues Jr L, Suzuki F, Ikeda K, Toyoda H, et al. Randomized phase 3 trial of ombitasvir/paritaprevir/ritonavir for hepatitis C virus genotype 1b-infected Japanese patients with or without cirrhosis. Hepatology. 2015;62(4):1037–46.

40. Fofana I, Jilg N, Chung RT, Baumert TF. Entry inhibitors and future treatment of hepatitis C. Antiviral Res. 2014;104:136–42.
41. Lin LT, Chung CY, Hsu WC, Chang SP, Hung TC, Shields J, et al. Saikosaponin b2 is a naturally occurring terpenoid that efficiently inhibits hepatitis C virus entry. J Hepatol. 2015;62(3):541–8.
42. Xiao F, Fofana I, Thumann C, Mailly L, Alles R, Robinet E, et al. Synergy of entry inhibitors with direct-acting antivirals uncovers novel combinations for prevention and treatment of hepatitis C. Gut. 2015;64(3):483–94.
43. Hopkins S, Bobardt M, Chatterji U, Garcia-Rivera JA, Lim P, Gallay PA. The cyclophilin inhibitor SCY-635 disrupts hepatitis C virus NS5A-cyclophilin A complexes. Antimicrob Agents Chemother. 2012;56(7):3888–97.
44. Hopkins S, Gallay P. Cyclophilin inhibitors: an emerging class of therapeutics for the treatment of chronic hepatitis C infection. Viruses. 2012;4(11):2558–77.
45. Daito T, Watashi K, Sluder A, Ohashi H, Nakajima S, Borroto-Esoda K, et al. Cyclophilin inhibitors reduce phosphorylation of RNA-dependent protein kinase to restore expression of IFN-stimulated genes in HCV-infected cells. Gastroenterology. 2014;147(2):463–72.
46. Chatterji U, Bobardt M, Tai A, Wood M, Gallay PA. Cyclophilin and NS5A inhibitors, but not other anti-hepatitis C virus (HCV) agents, preclude HCV-mediated formation of double-membrane-vesicle viral factories. Antimicrob Agents Chemother. 2015;59(5):2496–507.
47. Feher J, Lengyel G. Silymarin in the prevention and treatment of liver diseases and primary liver cancer. Curr Pharm Biotechnol. 2012;13(1):210–7.
48. Mengs U, Pohl RT, Mitchell T. Legalon(R) SIL: the antidote of choice in patients with acute hepatotoxicity from amatoxin poisoning. Curr Pharm Biotechnol. 2012;13(10):1964–70.
49. Ferenci P, Scherzer TM, Kerschner H, Rutter K, Beinhardt S, Hofer H, et al. Silibinin is a potent antiviral agent in patients with chronic hepatitis C not responding to pegylated interferon/ribavirin therapy. Gastroenterology. 2008;135(5):1561–7.
50. Polyak SJ, Ferenci P, Pawlotsky JM. Hepatoprotective and antiviral functions of silymarin components in hepatitis C virus infection. Hepatology. 2013;57(3):1262–71.
51. Dahari H, Shteingart S, Gafanovich I, Cotler SJ, D'Amato M, Pohl RT, et al. Sustained virological response with intravenous silibinin: individualized IFN-free therapy via real-time modelling of HCV kinetics. Liver Int: Off J Int Assoc Study Liver. 2015;35(2):289–94.
52. Jopling CL, Yi M, Lancaster AM, Lemon SM, Sarnow P. Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. Science. 2005;309(5740):1577–81.
53. Li YP, Gottwein JM, Scheel TK, Jensen TB, Bukh J. MicroRNA-122 antagonism against hepatitis C virus genotypes 1–6 and reduced efficacy by host RNA insertion or mutations in the HCV 5′UTR. Proc Natl Acad Sci U S A. 2011;108(12):4991–6.
54. Jopling CL, Schutz S, Sarnow P. Position-dependent function for a tandem microRNA miR-122-binding site located in the hepatitis C virus RNA genome. Cell Host Microbe. 2008;4(1):77–85.
55. Henke JI, Goergen D, Zheng J, Song Y, Schuttler CG, Fehr C, et al. microRNA-122 stimulates translation of hepatitis C virus RNA. EMBO J. 2008;27(24):3300–10.
56. Ottosen S, Parsley TB, Yang L, Zeh K, van Doorn LJ, van der Veer E, et al. In vitro antiviral activity and preclinical and clinical resistance profile of miravirsen, a novel anti-hepatitis C virus therapeutic targeting the human factor miR-122. Antimicrob Agents Chemother. 2015;59(1):599–608.
57. Janssen HL, Reesink HW, Lawitz EJ, Zeuzem S, Rodriguez-Torres M, Patel K, et al. Treatment of HCV infection by targeting microRNA. N Engl J Med. 2013;368(18):1685–94.