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REVIEW ARTICLE

New antiviral therapies for chronic hepatitis C

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Abstract Chronic hepatitis C is an important health issue worldwide. The current standard therapy is based on a combination of pegylated-interferon (pegIFN) and ribavirin (RBV), but this treatment leads to only ~50% sustained virological response (SVR) in patients with HCV genotype 1 and high viral loads, who were mostly null-responders or relapsers. Among HCV genotypes other than HCV genotype 1, especially HCV genotype 4 patients show only 40–70% SVR by this treatment. Although new drugs also depend on the combination of pegIFN and RBV, it appears that these drugs improve not only rapid virological response (RVR) but also early virological response, leading to SVR in these patients. In the near future, we predict higher SVR rates in chronic hepatitis C patients treated with these new drugs.

Keywords EVR · Protease inhibitor · Polymerase inhibitor · Ribavirin · Vitamin D

Introduction

Hepatitis C virus (HCV) infection is a serious cause of chronic liver disease worldwide with more than 170 million infected individuals at risk of developing significant morbidity and mortality [1]. The current approved therapies for chronic hepatitis C are standard interferon (IFN) and the combination of pegylated-interferon (pegIFN) alpha 2a or 2b with or without ribavirin (RBV) therapy. This therapy leads to ~50% sustained virological response (SVR), but non-SVRs persist especially in patients infected with HCV genotype 1 and high viral load [2, 3]. A rapid virological response (RVR), defined as undetectable HCV RNA at week 4 of treatment, predicts a high likelihood of achieving SVR [4]. Among HCV genotype 1 patients, only 19–24% treated with pegIFN-alpha and RBV therapy can achieve RVR [5, 6]. Among these patients, only 60–70% treated with pegIFN-alpha and RBV therapy can achieve early virological response (EVR), in which HCV RNA disappears or shows 2-log-reduction at 12 weeks [7–9]. EVR is the most accurate predictor of not achieving an SVR [4]. Although to determine whether the patient's treatment duration could be shortened, RVR is more important than EVR for predicting SVR; patients with RVR have a high chance to achieve SVR and therefore they may not need newer antiviral therapy. The probability of eradicating HCV varies according to genotype (Table 1); HCV genotype 4 is also difficult to cure, as well as HCV genotype 1, and the chosen duration of therapy is 48 weeks [10]. The response of HCV genotype 4 is intermediate between HCV genotype 1 and HCV genotypes 2 and 3 [11]. Serious adverse effects and the limited SVR with this therapy emphasize the need for new, novel HCV therapies [12]. To obtain higher SVR, RVR and EVR must be improved on the strength of new therapeutic options.

Recently, various studies have focused on the outcome predictors for chronic hepatitis C patients receiving pegIFN plus RBV, especially in terms of racial factors. Treatment responses were better in Asians than in Caucasians, Hispanics or African-Americans [13–17] (Table 2). Recently, it was reported from several groups that genetic variations in interleukin (IL) 28B-SNP predict hepatitis C treatment-induced viral clearance [18–21]. The prevalence of responder phenotype of IL28B-SNP was higher in Asians

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Table 1 Sustained virological response (SVR) in hepatitis C virus according to genotypes

References	G	Number of patients	Naïve	Formula of therapy	Duration of treatment (weeks)	SVR (%)
McHutchison et al. [99]	1	3,070	Yes	PegIFN-α-2b 1.5 μg/kg per week + RBV 800–1,400 mg/day	48	39.8
				PegIFN-α-2b 1.0 μg/kg per week + RBV 800–1,400 mg/day	48	38.0
				PegIFN-α-2a 180 μg/week + RBV 1,000–1,200 mg/day	48	40.9
Yamada et al. [100]	1	192	Yes	PegIFN-α-2a 180 μg/week + RBV 600–1,000 mg/day	48	59.4
				PegIFN-α-2a 180 μg/week + placebo	48	24.0
Liu et al. [101]	1	110		PegIFN-α-2a 180 μg/week + RBV 1,000–1,200 mg/day	48	77.3
	2	50		PegIFN-α-2a 180 μg/week + RBV 800 mg/day	24	84.0
Liu et al. [17]	1	308		PegIFN-α-2a 180 μg/week + RBV 1,000–1,200 mg/day	48	76
				PegIFN-α-2a 180 μg/week + RBV 1,000–1,200 mg/day	24	56
Shiffman et al. [102]	2 or 3	1,469	Yes	PegIFN-α-2a 180 μg/week + RBV 800 mg/day	16	62
					24	70
Mangia et al. [103]	2	213		PegIFN-α-2b 1.0 μg/kg per week + RBV 1,000 or 1,200 mg/day	12 or 24	80
	3	70				60
El-Zayadi et al. [104]	4	110	Yes	PegIFN-α-2b 1.0 μg/kg per week + RBV 1,000–1,200 mg/day	48	55.0
				PegIFN-α-2b 1.0 μg/kg per week + RBV 1,000–1,200 mg/day	24	48.6
Roulot et al. [105]	4	242	Yes	PegIFN-α-2b 1.5 μg/kg per week + RBV 1,000–1,200 mg/day	48	32.4–54.9
Bonny et al. [106]	5	27	Yes	IFN-α-2b 3MU × 3/week + RBV 1,000–1,200 mg/day	48	60
Nguyen et al. [107]	6	34	Yes	PegIFN + RBV	48	74
	6	27	Yes	PegIFN + RBV	24	63
	1	70	Yes	PegIFN + RBV	48	49
	2 or 3	36	Yes	PegIFN + RBV	24	75
Fung et al. [108]	1	21	Yes	PegIFN + RBV	48	52
	6	21	Yes	PegIFN + RBV	48	86

G genotype, *Naïve* treatment-naïve

and in European-Americans than in African-Americans and Hispanics, which may reflect the racial factors.

Current data showed that HCV genotype 1 patients who failed the prior standard of care (pegIFN plus RBV for 48 weeks) had a lower chance to achieve SVR when they were treated with the same regimen with or without intensified or prolonged therapy [22–24]. It also seems that there was no additional benefit for treatment-naïve HCV genotype 1 patients with intensified therapy, although it was reported that higher rates of SVR were observed among patients ≥95 kg and those with a NAS (non-alcoholic fatty liver disease activity score) score >3 with induction dosing of pegIFN alpha-2a and/or higher RBV doses [25]. These studies show that new antiviral therapy has a more important role for these patients. In contrast, patients without RVR but with cEVR may benefit from prolonged therapy if they can tolerate the adverse events [10].

There are 'Specifically Targeted Antiviral Therapies for HCV (STAT-C)' and non-specific therapies for HCV [26, 27]. STAT-C includes HCV-specific inhibitors against internal ribosomal entry-site (IRES), p7, NS3 helicase, NS3/4A protease, NS5A, and NS5B polymerase. On the other hand, HCV-non-specific drugs include interferon, therapeutic vaccines and other drugs. In this review, we will discuss the availability of new drugs for the treatment of HCV infection.

Replication of HCV

The mechanism of entry of HCV through interactions between the envelope glycoproteins and specific cell surface receptors remains unclear at this time [28]. After entering into hepatocytes, HCV genomes are translated into single open reading frame of about 3,011 amino acids (Fig. 1). Viral and cellular proteases make this protein into structural (core, E1, E2 and p7) and nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B) [29]. HCV has two proteases: NS2 cysteine protease and NS3 serine protease [30]. Nonstructural proteins make HCV genomes into HCV RNA replication complexes. HCV RNA replicates through RNA-dependent RNA polymerase (NS5B).

Table 2 Sustained virological response (SVR) in hepatitis C virus-G1 according to racial factors

References	Race	Number of patients	Naïve	Formula of therapy	Duration of treatment (weeks)	SVR (%)
Liu et al. [17]	Asian (Taiwan)	308	Yes	PegIFN-α-2a 180 μg/week + RBV 1,000–1,200 mg/day	48	76
				PegIFN-α-2a 180 μg/week + RBV 1,000–1,200 mg/day	24	56
Yamada et al. [100]	Asians (Japan)	192	Yes	PegIFN-α-2a 180 μg/week + RBV 600–1,000 mg/day	48	59.4
				PegIFN-α-2a 180 μg/week + placebo	48	24.0
Muir et al. [16]	Blacks	100		PegIFN-α-2b 1.5 μg/kg per week + RBV 800–1,000 mg/day 48	19	
	Non-Hispanic Whites	100		PegIFN-α-2b 1.5 μg/kg per week + RBV 800–1,000 mg/day 48	52	
Rodriguez-Torres et al. [15]	Latino	269	Yes	PegIFN-α-2a 180 μg/week + RBV 1,000–1,200 mg/day	48	34
	Non-Latino	300	Yes	PegIFN-α-2a 180 μg/week + RBV 1,000–1,200 mg/day	48	49
Hepburn et al. [14]	Asians	36		IFN + RBV	48	61
	Whites	496				39
	Hispanics	79				23
	African-Americans	50				14
Conjeevaram et al. [13]	African-Americans	19	Yes	PegIFN-α-2a 180 μg/week + RBV 1,000–1,200 mg/day	48	28
	Caucasian Americans	20				52

Naïve treatment-naïve

Negative-stranded RNA is made, and positive-stranded RNA is made from negative-stranded RNA as a template. Then new virions are produced and egress from hepatocytes.

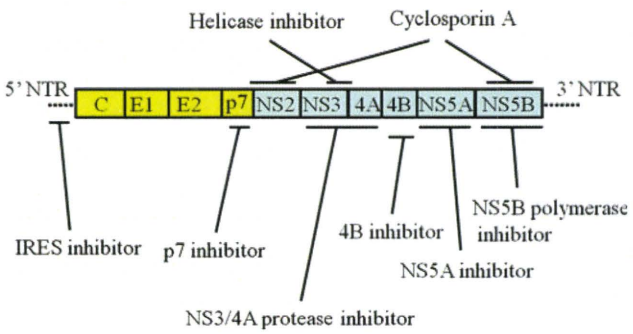

Fig. 1 Structure and targets of drugs of hepatitis C virus. NTR, non-translated region; IRES, internal ribosomal entry-site [27, 32–48, 93–98]

Drugs of STAT-C

NS3/4A protease inhibitors

As described above, HCV replication needs HCV NS3 serine protease and HCV NS4A enhances the enzyme activities of HCV NS3 protease. Specific inhibitors against HCV NS3 serine protease remarkably inhibit HCV RNA. First reports of a small molecule NS3 protease inhibitor, BILN 2061, for oral ingestion in human, appeared in 2003 [31]. Unfortunately, the development of BILN 2061 was hampered by cardiotoxicity in human liver-urokinase-type plasminogen activator $(uPA)^{+/+}$ severe combined immune deficient (SCID) mouse [32], but HCV-specific drugs have been shown to be useful for the treatment of HCV-infected patients. As shown in Table 3, NS3/4A protease inhibitors are being developed mainly in the USA. Various STAT-C compounds including HCV NS3 protease inhibitors, such as boceprevir (SCH 503034) and telaprevir (VX-950), have already entered phase-3 clinical development. It is important to understand that most protease inhibitors and polymerase inhibitors that we will be able to use are HCV genotype 1 specific.

Boceprevir

Boceprevir is currently being evaluated in combination with pegIFN alpha-2b and RBV. HCV SPRINT-1 study

Table 3 Drugs that directly target HCV (STAT-C) and phase of clinical trials (phases 2 and 3)

Drug	Company	Phase
Entry inhibitor		
ITX5061	iTherX (formerly Immusol)	2
Protease inhibitor		
Telaprevir	Vertex	3
Boceprevir (SCH503034)	Schering	3
ITMN-191/R7227	Intermune/Roche	2
TMC435	Medivir/Tibotec	2
BI 201335	Boehringer Ingelheim	2
MK-7009	Merck	2
NS5A inhibitor		
BMS-790052	Bristol-Myers Squibb	2
Polymerase inhibitor/Nucleoside polymerase inhibitor		
R7128	Roche/Pharmasset	2
Polymerase inhibitor/Non-nucleoside polymerase inhibitor		
PF-868554	Pfizer	2
ANA598	Anadys	2
VCH-759	ViroChem Pharma (acquired by Vertex)	2
IDX184	Idenix	2
Therapeutic Vaccines		
IC41	Intercell Novartis	2
CSL123	Chiron/CSL	2
GI 5005	Globeimmune	2

Hepatitis C new drug pipeline (http://www.hcvdrugs.com, accessed on 2/9/2010)

assessed the safety and efficacy of boceprevir, an oral inhibitor of HCV-NS3 protease, plus pegIFN alpha-2b and ribavirin (Table 4). SVR was significantly increased in the 28- and 48-week boceprevir arms compared to pegIFN alpha-2b and RBV control. RVR and EVR were highly predictive of SVR with boceprevir combinations. Rash-related adverse events were similar for boceprevir regimens and pegIFN alpha-2b and RBV control [33]. Null-responders to PegIFN alpha-2b plus RBV (4-week lead-in) therapy had high SVR after 44 additional weeks of boceprevir plus PegIFN alpha-2b plus RBV therapy, an important therapeutic advantage [34].

Telaprevir (VX-950)

Telaprevir (VX-950, Vertex Pharmaceuticals) is also a protease inhibitor specific to the HCV NS3/4A serine protease and rapidly reduce HCV RNA levels [35]. Telaprevir monotherapy for 14 days induced a median decline of more than $4.4\log_{10}$ units in the plasma HCV RNA level in patients with chronic HCV genotype 1 infection. Treatment with a telaprevir-based regimen significantly improved SVR rates in patients with genotype 1 HCV, albeit with higher rates of discontinuation because of adverse events [36].

The protease inhibitor for viral evaluation 1 study (PROVE1) was a phase-2b, randomized, double-blind, multicenter study of telaprevir in combination with pegIFN alpha-2a and RBV in treatment-naïve patients infected with HCV genotype 1 in the United States (Table 4). The rate of SVR was 41% (31 of 75 patients) in the pegIFN alpha-2a/RBV for 48 weeks (PR48) control group, as compared with 61% (48 of 79 patients) in the Telaprevir/pegIFN alpha-2a/RBV for 12 weeks, followed by P/R for 12 more weeks (T12PR24) group ($P = 0.02$), 67% (53 of 79 patients) in the T12PR48 group ($P = 0.002$), and 35% (6 of 17 patients) in the T12PR12 group. Viral breakthrough occurred in 7% of patients receiving telaprevir. Rash is the common reason for discontinuation.

The PROVE2 study was a multicenter, randomized, partially double-blind, placebo-controlled phase-2b trial in Europe to assess the efficacy and adverse event profile of various regimens combining telaprevir with pegIFN alpha-2a, with or without RBV, as compared with pegIFN alpha-2a and RBV alone in treatment-naïve patients infected with HCV genotype 1 [37] (Table 4). In this study, the rate of SVR for the T12PR12 (telaprevir/pegIFN alpha-2a/RBV for 12 weeks) and T12P12 (telaprevir/pegIFN alpha-2a for 12 weeks) groups combined was 48% (77 of 160 patients), as compared with 46% (38 of 82) in the PR48 (control) group ($P = 0.89$). The rate was 60% (49 of 82 patients) in the T12PR12 group ($P = 0.12$ for comparison with PR48 group), as compared with 36% (28 of 78 patients) in the T12P12 group ($P = 0.003$; $P = 0.20$ for comparison with PR48 group). The rate was significantly higher in the T12PR24 group [69% (56 of 81 patients)] than in the PR48 group ($P = 0.004$) (Table 4). The adverse events with increased frequency in the telaprevir-based groups were pruritus, rash, and anemia. These two studies suggest that treatment with a telaprevir-based regimen significantly improved SVR rates in patients with HCV genotype 1 [35, 36].

PROVE3 was a randomized phase-2 study assessing the safety and efficacy of telaprevir plus pegIFN alpha-2a with or without RBV in HCV genotype 1 patients who failed prior pegIFN alpha-2a plus RBV treatment [38]. Viral breakthrough rates were 11, 10, 21, and 3% in T12/PR24, T24/PR48, T24/P24, and PR48, respectively. Relapse rates were 28, 4, 53 and 52% in T12/PR24, T24/PR48, T24/P24, and PR48, respectively, 24 weeks after treatment. It was revealed that patients who failed prior pegIFN alpha-2a plus RBV therapy could successfully be treated with a telaprevir-based regimen and maintained SVR 1 year after the end of treatment. The general safety profile of these

Table 4 Results of clinical trials of new treatment options for HCV

References	Formula of therapy	Number of patients (genotype)	Duration of treatment (weeks)	SVR (%)
Kwo et al. (SPRINT-1) [33, 34]	PegIFN-α-2b 1.5 μg/kg per week (P) + RBV 800-1,400 mg/day (R)	104 (G1)	48	38
	4 weeks of P/R lead-in followed by P/R + Boc 800 mg TID for 24 weeks	103 (G1)	28	56
	4 weeks of P/R lead-in followed by P/R + Boc 800 mg TID for 44 weeks	103 (G1)	48	75
	P/R/Boc for 28 weeks	107 (G1)	28	55
	P/R/Boc for 48 weeks	103 (G1)	48	67
	P/low-dose R (400–1,000 mg/day)/Boc	59 (G1)	48	36
McHutchison et al. [36] (PROVE1)	PR48 control: PegIFN-α-2a(P)/RBV (R)	75 (G1)	48	41
	T12PR12: T/P/R for 12 weeks	17 (G1)	12	35
	T12PR24	79 (G1)	24	61
	T12PR48	79 (G1)	48	67
Hezode et al. [37] (PROVE2)	T12PR24: T/PegIFN-α-2a(P)/RBV (R) for 12 weeks, followed by P/R for 12 more weeks	81 (G1)	24	69
	T12PR12: T/P/R for 12 weeks	82 (G1)	12	60
	T12P12: T/P for 12 weeks	72 (G1)	12	36
	PR48 control	82 (G1)	48	46
Lalezari et al. [46]	R7128 1,500 mg bid/180 μg PegIFN-α-2a/ 1,000–1,200 mg RBV	11 (G1)	4	45% RVR
Sulkowski et al. [54]	albIFN 900 μg q2wk + RBV	442 (G1)	48	48.2
	albIFN 1,200 μg q2wk + RBV	440 (G1)	48	47.3
	PegIFN alpha-2a 180 μg + RBV	441 (G1)	48	51.0
Nelson et al. [55]	albIFN 900 μg q2wk + 800 mg RBV	(G2/3)	24	79.8
	albIFN 1,200 μg q2wk + 800 mg RBV	(G2/3)	24	80.0
	PegIFN alpha-2a 180 μg + 800 mg RBV	(G2/3)	24	84.8
Shiffman et al. [70]	Nitazoxanide (NTZ)/pegIFN/RBV	42 (G1)NR	24	7% cEVR
	Placebo/pegIFN/RBV	22 (G1)NR	24	0% EVR
Bacon et al. [71]	NTZ/pegIFN/RBV	75 (G1)	48	60% cEVR
	Placebo/pegIFN/RBV	37 (G1)	48	49% cEVR
Rossignol et al. [68]	NTZ 500 mg twice daily	23 (G4)	24	30.4
	Placebo	24 (G4)	24	0
Rossignol et al. [69]	PegIFN/RBV	40 (G4)	48	45
	NTZ 12 weeks followed by NTZ/pegIFN 36 weeks	40 (G4)	48	47.5
	NTZ 12 weeks followed by NTZ/pegIFN/RBV 36 weeks	40 (G4)	48	62.5
Inoue et al. [75]	IFN-α-2b 10 MU × 3/week	44 (G1)	24	31.8
	IFN-α-2b 10 MU × 3/week + cyclosporin A 100–200 mg/day	76 (G1)	24	55.2

Boc boceprevir, *T* telaprevir

regimens was similar to that observed in treatment-naïve patients. Grade 3 rash was observed in 5, 4, 3 and 0% of patients in T12/PR24, T24/PR48, T24/P24, and PR48, respectively. Grade 3 anemia was observed in 0, 6, 1 and 1% of patients in T12/PR24, T24/PR48, T24/P24, and PR48, respectively (Table 4).

Telaprevir demonstrated substantial antiviral activity against HCV genotype 2, while its activity against HCV genotype 3 was limited. Additional investigations of telaprevir for the treatment of HCV genotype 2 infection are needed [39].

TMC435

TMC435 is NS3/NS4A protease inhibitor under development for treatment of HCV infection. OPERA-1 is an

ongoing double-blind, placebo-controlled phase 2a trial to assess the antiviral activity, safety and pharmacokinetics of once-daily (QD) regimens of TMC435 in HCV genotype 1 treatment-naïve and treatment-experienced patients [40]. Interim analysis in treatment-naïve HCV genotype 1 patients at 28 days revealed that there were no TMC435-related treatment discontinuations, grade 3 or 4 adverse events or serious adverse events. Hepatic AST and ALT values improved during therapy. All three TMC435 doses (25, 75 and 200 mg QD) in combination with pegIFN/RBV showed antiviral activity superior to pegIFN/RBV alone.

Interim analysis of evaluation of the antiviral activity of TMC435 combined with pegIFN-2a/RBV for 28 days in non-responders or relapsers to previous IFN-based therapy also revealed that 4/9 (44%), 7/9 (78%) and 7/10 (70%) patients receiving 75, 150 and 200 mg QD TMC435 in combination with pegIFN-2a/RBV achieved HCV RNA below the lower limit of quantification (<25 IU/mL), compared to 0/9 patients in the pegIFN-2a/RBV group [41]. No serious adverse events or discontinuations due to adverse events were reported. Most common adverse events were headache, influenza-like illness, dyspnoea and nausea.

MK-7009

MK-7009 is a non-covalent competitive inhibitor of HCV NS3/4A protease, with demonstrated safety and efficacy when administered as monotherapy for 8 days. A phase-2a study of MK-7009 in combination with pegIFN/RBV was carried out in a randomized, placebo-controlled, double-blind study for treatment-naïve chronic hepatitis C patients [42]. MK-7009 was administered for 28 days with pegIFN/RBV in 1 of 5 regimens: placebo, 300 mg BID, 600 mg BID, 600 mg QD, or 800 mg QD; all patients continued pegIFN/RBV for an additional 44 weeks. The primary endpoint was the percent of subjects with undetectable HCVRNA (<10 IU/mL by Roche Cobas Taqman) at day 28 (RVR). There were no serious adverse events and no discontinuations due to an adverse event. The most common adverse events reported were nausea, headache, and flu-like symptoms. In the 300 mg BID, 600 mg BID, 600 mg QD, 800 mg QD, and placebo groups, 12/16 (75%), 15/19 (78.9%), 11/16 (68.8%), 14/17 (82.4%) and 1/18 (5.6%) subjects achieved RVR. MK-7009 in combination with pegIFN/RBV is a well-tolerated and potent inhibitor of HCV.

BI201335

BI201335, a potent and specific HCV NS3/4A protease inhibitor, has been studied with chronic genotype 1 HCV infection and 14-day monotherapy in treatment-naïve patients followed by combination with pegIFN/RBV for an additional 14 days, and in treatment-experienced patients for 28 days as combination therapy with pegIFN/RBV [43]. All dose groups achieved median viral load reductions of $\geq$3log10. On-treatment viral rebound was observed in most patients receiving monotherapy, but in only 3/19 treatment-experienced patients receiving triple combination therapy with lower dosages of BI201335. Recent study revealed that HCV NS3 variants that confer resistance to BI201335 were selected during treatment; these variants do not alter the sensitivity to pegIFN/RBV, as the majority of treatment-naïve patients with resistant virus subsequently displayed anti-viral responses during combination therapy.

BMS-650032

The HCV NS3 protease inhibitor BMS-650032 is a potent and selective inhibitor with in vitro picomolar potency that has demonstrated antiviral activity in both single and multiple ascending dose studies in subjects chronically infected with HCV genotype 1. BMS-650032 was safe and well tolerated at single doses up to 1,200 mg and up to 600 mg Q 12 h for 14 days in healthy subjects. Actively treated subjects experienced a mean decline in HCV RNA of $\sim$2.5 log10 at 24 h after a single 600 mg dose of BMS-650032 [44]. There were no deaths or discontinuations due to adverse events and all adverse events were mild to moderate.

HCV NS5B polymerase inhibitors

HCV NS5B RNA-dependent RNA polymerase (RdRp) is another attractive target for drug discovery. The same as hepatitis B virus, human immunodeficiency virus, and herpes virus, HCV is thought to be a target of polymerase inhibitor. There are two classes: nucleoside inhibitors and non-nucleoside inhibitors (NNIs) [45]. These drugs reduce the efficiency of further RNA elongation.

R7128

R7128 is the prodrug of 2′-deoxy-2′-C-methylcytidine (PSI-6130), a potent and selective inhibitor of HCV NS5B polymerase. R7128 is in clinical phase 2 trials for the treatment of HCV infection [45] (Table 3). Eighty-five percent of patients receiving R7128 1,500 mg twice daily (BID) with pegIFN alpha 2a plus RBV for 4 weeks achieved undetectable HCV RNA levels with safety and tolerability comparable to placebo with pegIFN alpha 2a plus RBV. R7128 is equally potent in vitro against HCV genotypes 1, 2, 3 and 4, and it may be clinically important to test R7128 in patients with these HCV genotypes because 20% of genotypes 2 and 3 HCV-infected patients

fail to achieve SVR by the currently favored treatment of 24 weeks of pegIFN plus RBV [46]. PSI-6130 presents a high barrier to resistance selection in vitro, and selects for variants exhibiting only low-level resistance with R1479 [47]. The development of HCV polymerase NNIs has been successfully validated in phase 2 clinical trials [48].

HCV-796

HCV-796 is a non-nucleoside allosteric inhibitor of HCV RNA-dependent RNA polymerase (NS5B) that is essential for viral replication. There was a phase 2, randomized, open-label study of the safety, antiviral activity, and pharmacokinetics of HCV-796 administered in combination with pegIFN alpha-2b/RBV versus pegIFN alpha-2b/RBV in HCV genotype-1-infected subjects who were either naïve to treatment or who had failed treatment. In the treatment-naïve pegIFN alpha-2b/RBV group, treatment-naïve HCV-796 plus pegIFN alpha-2b/RBV group, and non-responder plus pegIFN alpha-2b/RBV group, 6.9, 52.1, and 10.7% subjects achieved RVR and 58.1, 75.8, or 32.3% subjects achieved cEVR, respectively [49]. Grade 3/4 elevations in liver function tests occurred in 3 subjects receiving HCV-796. Other adverse events resulting in study discontinuation were infrequent in all three arms. There was no increase in anemia or neutropenia in HCV-796 arms.

Antiviral resistance to inhibitors

In the use of HCV NS3/4A protease inhibitors or HCV NS5B polymerase inhibitors, the emergence of several resistance mutants was reported [50–52] (Fig. 2a, b). In HCV NS5B polymerase inhibitors, NNIs produce more resistant mutants than nucleoside inhibitors and the effects of NNIs are more sensitive to these mutants. Moreover, it has been reported that these resistant mutations already existed before treatments [50]. Further treatment against these mutants should be considered in the future.

Non-specific therapies for HCV

New interferon

Albumin interferon

Phase 3 clinical trials with albuferon (albIFN alpha-2b), one of the longer-acting IFN alphas, are currently ongoing. This interferon is the fusion protein of human serum albumin and interferon alpha. Human serum albumin (MW 66,000) is made in liver and its half-life is about 20 days. Albuferon exhibits a prolonged half-life and duration of antiviral activity that offers reduced dosing regimen and indicates potential suitability for dosing intervals of 2–4 weeks [53]. In a phase 3 trial [54], albIFN alpha 2b 900 μg administered q2wk demonstrated comparable efficiency to pegIFN alpha 2a in patients with chronic HCV genotype 1. The overall incidence of serious or severe adverse events was similar between these two treatments [54]. SVR rates were 51.0% (225/441), 48.2% (213/442), and 47.3% (208/440) in the pegIFN alpha-2a, albIFN 900 and albIFN 1200 groups, respectively (Tables 4, 5).

A phase-3, randomized, active-controlled, multicenter study evaluating the efficacy/safety of albIFN alpha-2b in treatment-naïve patients with genotype 2/3 chronic hepatitis C was reported. Randomized 933 patients 1:1:1 to 1 of 3 treatment groups combined with oral RBV 800 mg/day: pegIFN alpha-2a 180 μg qwk, or albIFN 900 or 1,200 μg q2wk for 24 weeks were studied. In the intention-to-treat population, SVR rates were 84.8, 79.8, and 80.0% for pegIFN alpha-2a, albIFN 900 and 1,200 μg groups, respectively (Table 4). AlbIFN 900 μg q2wk demonstrated comparable efficacy to pegIFN alpha-2a, and comparable rates of serious or severe adverse events and discontinuations due to adverse events in patients with HCV genotypes 2/3 [53, 55]. Severe pulmonary infections and interstitial lung disease were rare. Rates were similar across treatment groups.

IFN lambda

IFN lambdas (IL28A/B and IL29), a novel Type III IFN, binds to a unique cell surface receptor, induces an intracellular antiviral response and also efficiently inhibit HCV replication in vitro with potentially less hematopoietic side effects than IFN-alpha because of limited receptor expression in hematopoietic cells [56]. Although the combined effects of IL29 and IFN alpha were primarily additive, the IL29/IFN gamma combination synergistically induced multiple genes and had the greatest antiviral activity [57]. A mathematical model estimated that well-tolerated doses of pegIFN lambda-1a are similar to reported pegIFN alpha therapy estimates in the ongoing clinical trial [58]. Recently, it was reported from several groups that genetic variations in IL28B predict hepatitis C treatment-induced viral clearance [18–21]. Treatment with IFN lambda in HCV pathogenesis may be important.

Therapeutic vaccine

An effective vaccine would represent significant progress in the management of chronic HCV infections [59–62]. IC41 is a synthetic peptide vaccine containing 7 relevant HCV T cell epitopes and the T helper cell (Th)1/Tc1 adjuvant poly-L-arginine [63]. IC41 has been shown to be

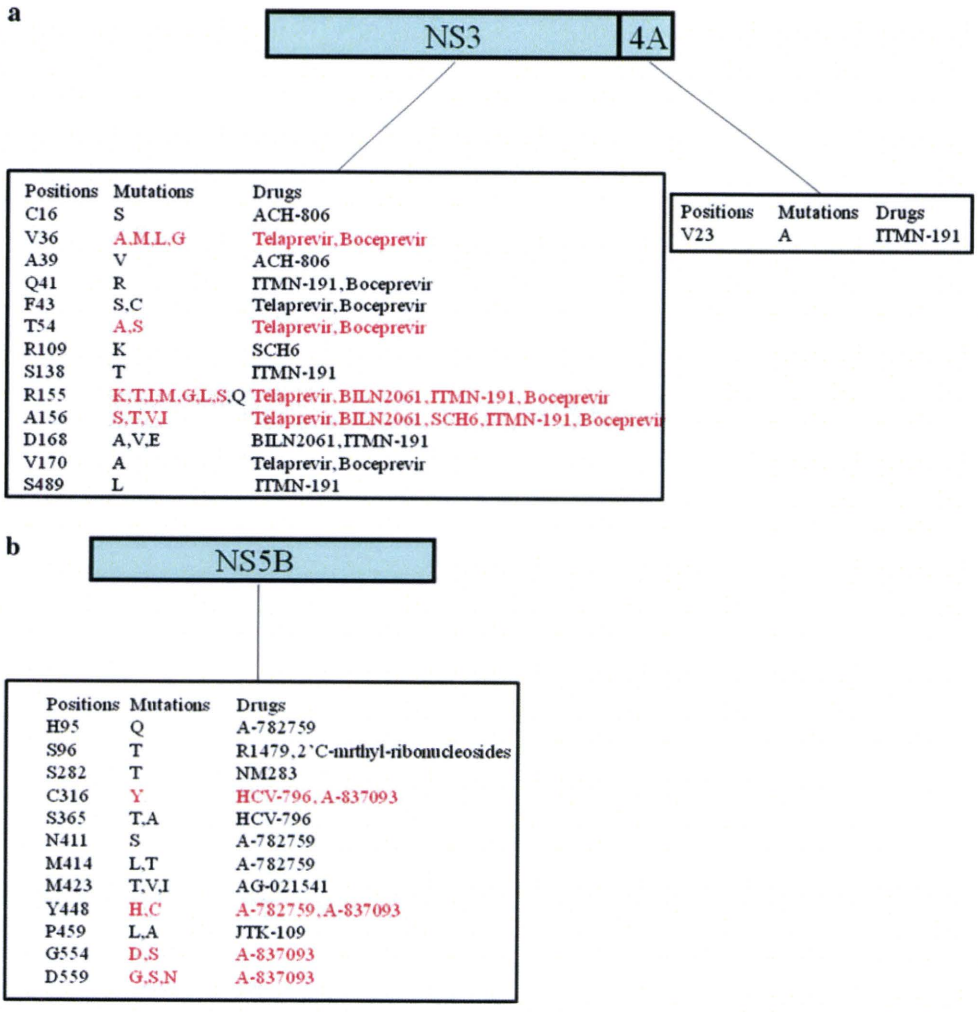

Fig. 2 Viral mutants for STAT-C genotype 1. **a** HCV NS3 and NS4 protein. **b** NS5B protein. *Positions* amino acids position and residues, *Mutations* resistance mutations. *Red colors* indicate in vivo studies [50–52]

safe and to induce HCV-specific IFN-gamma-secreting CD4+ and CD8+ T cells in healthy volunteers [64]. Of the 35 patients, each received six vaccinations with IC41 from 28 to 48 weeks of standard antiviral treatment and were followed up for another 6 months. IC41 vaccination did not prevent HCV relapse in patients with ongoing IFN standard treatment but HCV-specific T cell responses were inducible and were associated with lower relapse rates [59]. Optimized vaccine responses may enhance SVR rates obtained with standard treatment of chronic hepatitis C.

A key feature of most vaccines is the induction of neutralizing antibodies [61]. In many cases, infusion of neutralizing antibodies is also used for passive post-exposure prophylaxis. Recombinant E1E2 glycoproteins adjuvanted with MF59 containing a CpG oligonucleotide elicited strong CD4+ T helper responses but no CD8+ T cell responses. A recombinant NSs 3, 4, and 5 polyprotein also stimulated strong CD4+ T helper responses when adjuvanted with Iscomatrix containing CpG. Thus, a single immunization regimen was shown to be capable of eliciting these broad T cell responses, as well as neutralizing antibodies, meaning that these vaccines may have potential therapeutic use, as well as prophylactic efficacy, especially when combined with antiviral drugs [61].

GI-5005 is a whole heat-killed *S. cerevisiae* therapeutic vaccine expressing HCV NS3 and core antigens. GI-5005 elicits antigen-specific responses with the goal of improving the rate of immune-mediated elimination of HCV-infected hepatic cells [65]. Triple therapy of GI-5005 in combination with pegIFN and RBV regimen resulted in improved EVR, compared with the pegIFN and RBV regimen alone. Triple therapy was well tolerated with no significant new toxicities observed. Improvement at the end-of-treatment response was observed in naïve HCV genotype 1 patients in a triple therapy group [37/53 (70%)] compared to the control group [27/49 (55%)]. Although a phase-2 trial that evaluated triple therapy is now continuing to evaluate SVR, GI-5005 triple therapy as well as novel combination strategies for GI-5005 with other HCV inhibitory agents may be useful [62].

Table 5 Immune modifiers and other non-specific drugs for HCV and phase of clinical trials (phases 2 and 3)

Drug	Company	Phase
Interferon		
Albuferon (interferon/albumin fusion)	Human Genome Sciences	3
Omega interferon	Intarcia	2
Lactoferon	Biolex/OctoPlus	2
IET	Transition Therapeutics	2
Others		
Zadaxin: thymalphasin	SciClone Pharma/ Sigmatau	3
SCV-07	SciClone Pharma	2
MX3235	Migenix	2
Civacir	NABI	2
Suvus	Bioenvision	2
Alinia (nitazoxanide: NTZ)	Romark Laboratories	2
KPE02003002	Kemin Pharma	2
Lenocta (sodium stibogluconate SSG)	VioQuest Pharmaceuticals	2
CTS-1027 MMP inhibitor	Conatus	2
JKB-122	Jerkin	2
Fluvastatin	Oklahoma University HSC	2
Mito-Q	Antipodean	2
PYN17	Phynova	2
CB5300	Canopus	2
CF102	Can-Fite	2
Debio 025	Debiopharm	2
CYT107	Cytheris	2

Reference: Hepatitis C new drug pipeline (http://www.hcvdrugs.com, accessed on 2/9/2010)

Other antiviral drugs against HCV

Nitazoxanide

Alinia (nitazoxanide: NTZ) is a thiazolide that FDA approved for diarrhea caused by cryptosporidiosis infection. In patients co-infected with HCV and HIV, serum ALT levels were improved with the long-term use of this drug. Nitazoxanide was shown to enhance the antiviral activity of interferon in HCV replicon system [66]. It was recently reported to induce phosphorylation of eukaryotic initiation factor 2 alpha (eIF2α) via protein kinase activated by double-stranded RNA (PKR) activation [67].

Initially, the antiviral activity of NTZ was shown in HCV genotype 4 [68] (Table 4). The addition of NTZ to pegIFN or pegIFN-RBV improved virological response rates compared with pegIFN-RBV therapy without increase in adverse events [69] (Table 4). Compared with placebo, NTZ showed modest incremental early virological responses (cEVR and undetectable HCV RNA after 24 weeks of combination therapy) in patients with HCV genotype 1 who are NR to pegIFN plus RBV [70]. One hundred and twelve treatment-naïve patients with CHC genotype 1 underwent 2:1 randomization in 13 US centers in a double-blind, placebo-controlled study to receive either NTZ ($n = 75$) or placebo ($n = 37$) twice daily over a 4-week lead-in followed by continued NTZ or placebo plus pegIFN 180 μg weekly and weight-based RBV (1,000–1,200 mg/day) for 48 weeks [71].

In patients with HCV RNA levels >600,000 IU/mL, cEVR and EVR rates were higher in the NTZ ($n = 67$) versus placebo ($n = 31$) groups (57 vs. 39%, and 79 vs. 61%, respectively). There were 21 severe adverse events and no significant differences in adverse events between the two treatment groups. Further studies are ongoing.

MitQ (mitoquinone)

Increased oxidative stress and consequent mitochondrial damage are important pathways for apoptosis in HCV infection [72]. MitQ is a new, potent antioxidant that covalently bonds the antioxidant moiety of coenzyme Q10 to a triphenylphosphonium cation. The cation causes the attached antioxidant to accumulate several-hundred fold within mitochondria in vivo following oral administration, protecting them from oxidative damage and cell death. A phase-2 study of 28 days of MitQ revealed reduced serum aminotransferase in HCV infected patients [73]. MitQ may be useful for oxidative stress-related diseases such as chronic hepatitis C and reduce necroinflammation in HCV infection.

Cyclophilin inhibitors

Debio-025

Alisporivir (Debio-025), a non-immunosuppressive cyclosporine A (CsA) derivate that selectively inhibits cyclophilins (Cyps), is being developed by Debiopharm SA for the potential oral treatment of HCV infection [74]. Inoue et al. [75] reported that combination therapy of IFN alpha-2b and CsA for 24 weeks produced SVR in 42% of patients with both HCV genotype 1b and high viral loads (Table 5). Host cell Cyps are essential for efficient HCV replication in hepatocytes, and thus Cyps are regarded as a new therapeutic target of HCV [76, 77].

NIM811

Single dose of NIM811 (50–1,600 mg) was administered to 40 healthy volunteers. There were no severe adverse events, and no drug-related adverse events in the healthy

subjects. The non-immunosuppressive CsA analogues NIM811, DEBIO-025, and SCY635 have been observed to exert strong inhibitory effects on HCV replication and these compounds are now being evaluated in clinical trials [78, 79].

MX-3253

MX-3253 (celgosivir), an alpha-glucosidase I inhibitor, is an oral prodrug of castanospermine, a natural product derived from *Castanospermum austral*. This agent is not efficient as monotherapy for the treatment of HCV, but has demonstrated a synergistic effect in combination with pegIFN plus RBV [80].

Vitamin D

Recently, it was reported that low vitamin D serum level is related to severe fibrosis and low responsiveness to IFN-based therapy in genotype 1 chronic hepatitis C patients [81]. Abu-Mouch et al. also reported the beneficial effect of vitamin D combined with pegIFN and RBV in chronic hepatitis C [82]. Further studies will be needed.

Recently, HCV cell culture systems have been under development [83–87]. These systems allow us to test candidate antiviral agents in the context of a persistent HCV infection in cells whose metabolism is likely to approximate that of primary hepatocytes in vivo more closely. JFH1 systems [83, 86, 87] are specific to HCV genotype 2, but some are specific to HCV genotype 1 [84, 85]. Another cell-based model system that has proven to be extremely useful to study the early steps of virus binding and cell entry is the pseudoparticle system. HCV pseudoparticles are assembled by incorporating HCV glycoproteins E1 and E2 onto retroviral or lentiviral cores that are highly infectious and that can mimic the viral entry of HCV [88]. HCV replicons have proven to be extremely valuable for studies on the process of HCV replication, as well as for testing of novel antiviral compounds that specifically target the protease activity of NS3 or the polymerase activity of NS5 [89]. However, an apparent shortcoming of these models was that stable cell clones containing self-replicating replicons and expressing all viral proteins remained unable to release infectious HCV particles. The inability to secrete viral particles may be the consequence of adaptive mutations, which are needed to enhance viral replication rates but at the same time may block viral assembly. Importantly, the replication of cell-cultured HCV in this system was inhibited by IFN-alpha as well as by several HCV-specific antiviral compounds [83, 84].

The current data showed that HCV genotype 1 patients who failed a prior standard of care had a lower chance of achieving SVR [90–92], giving new antiviral therapies more important role for patients. In conclusion, most of the new drugs described above need the combination of peg-IFN and RBV at the same time to allow them to work in an anti-HCV manner. These drugs may be useful for the improvement of EVR and then SVR. Further studies will be needed to develop new drugs with less side-effects.

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Research Article

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Long-term use of entecavir in nucleoside-naïve Japanese patients with chronic hepatitis B infection☆

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Background & Aims: To evaluate the long-term efficacy of entecavir in nucleoside-naïve chronic hepatitis B patients.

Methods: One hundred and sixty-seven patients treated with entecavir 0.01 mg, 0.1 mg or 0.5 mg for 24–52 weeks in Phase II studies entered rollover study ETV-060 and received entecavir 0.5 mg daily. Responses were evaluated among patients with available samples.

Results: After 96 weeks in ETV-060 (120–148 weeks total entecavir treatment time), 88% (127/144) of patients had HBV-DNA <400 copies/ml; 90.1% (128/142) had alanine aminotransferase (ALT) ⩽1× the upper limit of normal (ULN) among those with abnormal baseline ALT; and 26% (32/121) achieved HBe seroconversion among those HBeAg(+) at baseline. A subset of 66 patients received entecavir 0.5 mg (approved dose) from Phase II baseline: at week 96 in ETV-060, 83% (48/58) had HBV-DNA <400 copies/ml, 88% (52/59) had ALT ⩽1× ULN, and 20% (10/49) achieved HBe seroconversion. Twenty-one out of 66 patients had paired baseline and on-treatment biopsies: 100% (21/21) and 57% (12/21) demonstrated histologic improvement, and improvement in fibrosis, respectively, over 3 years. The 3-year cumulative probability of resistance was 3.3% for all patients and 1.7% for the 0.5 mg subset.

Conclusions: Long-term entecavir for nucleoside-naïve patients resulted in high rates of virological, biochemical, and histological response, with minimal resistance.

Keywords: Entecavir; Nucleoside-naïve; Long-term treatment; Japanese; Chronic hepatitis B.

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Abbreviations: CHB, chronic hepatitis B; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; HAI, histologic activity index; ULN, upper limit of normal; PCR, polymerase chain reaction; ITT, intention-to-treat.

Research Article

Introduction

Chronic hepatitis B (CHB) affects 350–400 million people worldwide [1]. The prevalence is highest in the Asia–Pacific region, where 75% of all chronically infected individuals live, and up to 25% of CHB patients die of liver cirrhosis, hepatic decompensation or hepatocellular carcinoma (HCC) [2]. In Japan, the prevalence of CHB ranges from 0.8% to 4%, with geographic variation within the country [2–5]. The vast majority of CHB patients in Japan are infected with hepatitis B virus (HBV) of genotype C [6,7]. Infection with genotype C virus has been associated with delayed HBe seroconversion, more advanced liver disease, and increased probability of HCC development [8–11].

Recent studies have shown that CHB patients with moderate or elevated serum HBV-DNA are at the highest risk of developing long-term complications, including cirrhosis and HCC [11,12–14]. Yuen et al. showed that among Asian patients with CHB, disease progression was also seen in patients with persistently detectable viraemia and normal or minimally elevated levels of alanine aminotransferase (ALT), including patients who had achieved HBe seroconversion [12]. Consistent with these findings, current CHB treatment recommendations emphasize the importance of prolonged maximal HBV-DNA suppression and the avoidance of resistance [15–17].

Medications currently used for CHB include interferons (conventional and pegylated), lamivudine, adefovir, telbivudine, and entecavir. The interferons are efficacious in a subgroup of patients with genotype A infection, low baseline viral load and elevated baseline ALT but are often associated with treatment-limiting adverse events [18–20]. Lamivudine is well tolerated and initially efficacious, but the emergence of resistance in approximately 70% of patients after 4–5 years limits its benefit during long-term therapy [21,22]. Adefovir treatment is frequently associated with suboptimal HBV-DNA suppression and a cumulative probability of resistance of 29% at 5 years among HBeAg(−) patients, and resistance appears to be higher in the HBeAg(+) population [23–25]. Treatment with telbivudine leads to virological breakthrough, with resistance in 21.6% of HBeAg(+) and 8.6% of HBeAg(−) patients after only 2 years [26].

Entecavir has been shown to be highly effective at suppressing HBV-DNA replication to undetectable levels and normalizing ALT in Phase II studies of nucleoside-naïve CHB patients in Japan and in multinational studies [27–30]. Treatment for 24 weeks in the Japanese study ETV-047 showed that entecavir 0.5 mg daily resulted in superior viral load reduction compared with lamivudine 100 mg daily [28]. In the Japanese study ETV-053, treatment with entecavir 0.5 mg daily for 52 weeks resulted in significant histological improvement as well as viral load reduction and ALT normalization [27]. Immediately after completion of treatment in study ETV-047 or ETV-053, patients were eligible to enrol in rollover study ETV-060 and receive entecavir 0.5 mg daily. We present the long-term efficacy, safety, and resistance results for patients treated with entecavir in Phase II studies who rolled over into study ETV-060, for a total entecavir treatment time of up to 3 years (120–148 weeks). A subset of patients received the approved dose of entecavir (0.5 mg daily) continuously from Phase II baseline, and results for that cohort are also presented.

Patients and methods

Study design

Study ETV-060 was a rollover study designed to provide open-label entecavir to patients who completed previous entecavir therapy in Phase II studies ETV-047 or ETV-053 in Japan. In study ETV-047, 137 nucleoside-naïve patients were randomized to a range of daily doses of entecavir (0.01 mg [$n = 35$], 0.1 mg [$n = 34$], 0.5 mg [$n = 34$]) or lamivudine 100 mg [$n = 34$] for 24 weeks [34]). In study ETV-053, 66 nucleoside-naïve patients were randomized to entecavir 0.1 mg ($n = 32$) or entecavir 0.5 mg ($n = 34$) daily for 52 weeks [27]. Patients who completed 24 weeks of entecavir treatment in study ETV-047 ($n = 101$) or 52 weeks of entecavir treatment in study ETV-053 ($n = 66$) were enrolled in ETV-060 and received entecavir 0.5 mg daily in an open-label fashion. After 96 weeks of treatment in study ETV-060, patients could discontinue the study and were eligible to receive commercially available entecavir, which was approved by Japanese health authorities while study ETV-060 was ongoing. The current analysis describes results for patients who completed 96 weeks in study ETV-060 for a total entecavir treatment time of 120 weeks (patients from −047) or 148 weeks (patients from −053) (Fig. 1). Patients began dosing in ETV-060 immediately after completion of the previous study with no treatment gap or interruption.

During study ETV-060, clinical and laboratory assessments (serum chemistries, haematology, prothrombin time/INR, urinalysis) were made at baseline, at weeks 2 and 4, and every 4 weeks thereafter during dosing. Assessments of HBV-DNA by PCR assay and HBV serologies were performed at baseline, weeks 12 and 24, and subsequently every 24 weeks during dosing. Baseline liver biopsies in study ETV-053 were performed within 6 weeks of initiation of study therapy; or if a liver biopsy had been previously obtained within 52 weeks before initiation of protocol therapy, it was used as the baseline specimen for histological evaluation. Liver biopsies were evaluated using the Knodell Histologic Activity Index (HAI) and Knodell fibrosis scores and the New Inuyama classifications [31].

The study was conducted in compliance with the ethical principles of the Declaration of Helsinki, Good Clinical Practice guidelines, and Articles/Notifications of the Ministry of Health, Labour and Welfare in Japan. Written informed consent was obtained from all patients.

Study population

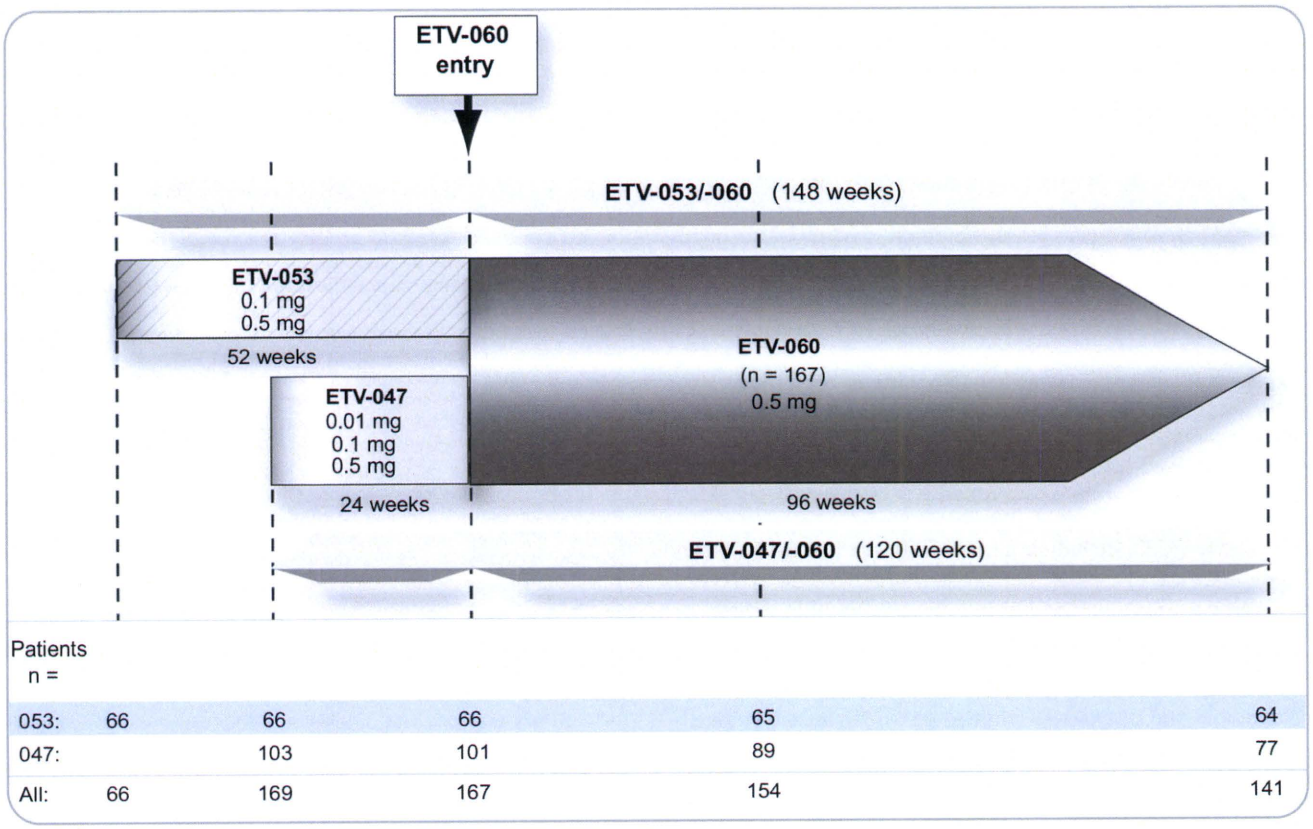
Inclusion criteria for studies ETV-047 and ETV-053 have been described previously [27,28]. Eligible patients were adults with CHB infection, compensated liver disease, and no more than 12 weeks prior treatment with anti-HBV nucleoside analogues. Patients could be HBeAg(+) or (−), and were required to have elevated ALT (1.25–10× the upper limit of normal [ULN] in ETV-047 and 1.3–10× ULN in ETV-053 at screening) and active viral replication (HBV-DNA $\geqslant 10^5$ copies/ml by PCR assay at screening in ETV-053 and $\geqslant 10^{7.6}$ copies/ml for patients in ETV-047). Patients were excluded from studies −047 and −053 if they had cirrhosis or evidence of liver decompensation, other forms of liver disease or suspected hepatic tumours, HIV infection or treatment with immunosuppressive therapy or interferon within 24 weeks prior to initiation of study medication. Pregnant and nursing women were also excluded.

Efficacy analyses

Efficacy end points included proportions of patients achieving the following: HBV-DNA <400 copies/ml, ALT normalization (ALT $\leqslant$1.0× ULN) among patients with abnormal ALT at baseline, and HBeAg loss and HBe seroconversion among patients HBeAg(+) at baseline. Histological end points are presented for the cohort that received entecavir 0.5 mg daily from Phase II baseline and include improvement in Knodell HAI and Knodell fibrosis scores among patients with evaluable biopsy pairs. Histological improvement was defined as a $\geqslant$2-point decrease in the Knodell necroinflammatory score and no worsening of fibrosis (worsening: $\geqslant$1-point increase in the Knodell fibrosis score). Improvement in fibrosis was defined as a $\geqslant$1-point decrease in the Knodell fibrosis score. Histological results were also assessed by the New Inuyama classification [31].

Safety analyses

Safety analyses included the incidence of adverse events, serious adverse events, laboratory abnormalities and discontinuations due to adverse events on treatment during ETV-060, including data for patients treated beyond 96 weeks. On-treatment ALT flares were defined as ALT >2× baseline and >10× ULN.



Patients n =					
053:	66	66	66	65	64
047:		103	101	89	77
All:	66	169	167	154	141

Fig. 1. Entecavir nucleoside-naïve long-term treatment cohort. One hundred and one patients completed 24 weeks of entecavir 0.01 mg, 0.1 mg or 0.5 mg treatment daily in study ETV-047, and 66 patients completed 52 weeks of entecavir 0.1 mg or 0.5 mg treatment daily in study ETV-053. Of these, 167 were enrolled in study ETV-060 with no interruption or gap in treatment. One hundred and forty-four patients remained on entecavir 0.5 mg daily through 96 weeks in study ETV-060 (for a total entecavir treatment time of 120–148 weeks).

Resistance monitoring

During treatment, HBV polymerase/reverse transcriptase substitutions were analyzed for all patients who had HBV-DNA ⩾400 copies/ml at weeks 100 and 120 (from Phase II [pre-treatment] baseline) for patients originating in study ETV-047, and at weeks 100 and 148 for patients originating in study ETV-053. Samples from all patients, who experienced virological breakthrough during ETV-060 (increase in HBV-DNA of ⩾1 $\log_{10}$ copies/ml from nadir in two consecutive measurements), were also analyzed for HBV polymerase/reverse transcriptase substitutions.

Assay methods

Serum HBV-DNA was determined by Roche Amplicor™ PCR assay (LOQ = 400 copies/ml; Roche Diagnostics K.K., Tokyo, Japan) in a central laboratory. Clinical laboratory tests, PCR assays for HBV-DNA, and serological tests were performed at SRL, Inc. (Tokyo, Japan), the central clinical laboratory designated by the trial sponsor. Genotypic analysis of HBV strains was performed using a PCR-based restriction fragment length polymorphism assay (SRL, Inc., Tokyo, Japan). On-treatment testing for resistance was carried out using a direct-sequencing PCR method.

Statistical analysis

Analyses of efficacy and safety end points were based on patients who received at least one dose of study medication in study ETV-060. Only descriptive summaries were performed. Parameters represented by continuous variables were summarized by the mean, median, standard deviation, minimum, and maximum. Analyses of HBV-DNA as a continuous parameter were applied after $\log_{10}$ transformation. In the analysis of binary end points, patients with missing on-treatment measurements were treated as missing (non-completer = missing). An additional sensitivity analysis using the last observation carried forward method was conducted for the end point of HBV-DNA <400 copies/ml at week 96. In this analysis, the last observed HBV-DNA levels were carried forward for patients without week 96 measurements, i.e., patients who either discontinued prior to week 96 or who were still on study but had a missing HBV-DNA measurement at week 96.

Results

One hundred and sixty-seven patients were treated with entecavir in Phase II studies ETV-047 or -053 and entered ETV-060 (Fig. 1). Twenty-three patients discontinued treatment during ETV-060 for the following reasons: adverse event (6), protocol violation (2), withdrawal of consent (4), pregnancy (1), loss to follow-up (4), insufficient effect (1), and complete response (4) or stability of disease condition (1) in the judgement of the investigator. Table 1 shows the baseline (pre-treatment) demographics and disease characteristics for all treated patients ($n = 167$); the cohort of patients who received the approved dose of entecavir (0.5 mg daily) from Phase II baseline through the end of treatment ($n = 66$); and the subset of patients who received 0.5 mg entecavir and had biopsies at baseline, week 48, and weeks 144–148. Among all treated patients, 72% were male, and the mean age was 43 years. Mean HBV-DNA was 7.88 $\log_{10}$ copies/

Table 1. Baseline demographics and disease characteristics of the entecavir nucleoside-naïve long-term treatment cohort ($n = 167$), the entecavir 0.5 mg cohort ($n = 66$), and the subset of patients from the entecavir 0.5 mg cohort with evaluable liver biopsies at baseline, week 48, and week 144–148 ($n = 19$). Patients were treated with different doses of entecavir in Phase II studies ETV-047 and ETV-053, and subsequently received 0.5 mg daily in rollover study ETV-060. This table describes characteristics at pre-treatment (Phase II) baseline.

Characteristic	Long-term treatment cohort $n = 167$	Entecavir 0.5 mg cohort $n = 66$	Entecavir 0.5 mg cohort with long-term liver biopsy $n = 19$
Male, *n* (%)	120 (71.9)	48 (72.7)	15 (78.9)
Age (years), mean ± SD	42.5 ± 11.0	43.2 ± 10.5	43.8 ± 10.3
Weight (kg), mean ± SD	65.9 ± 3.5	65.5 ± 12.2	66.8 ± 13.2
Ethnicity Japanese, *n* (%)	167 (100)	66 (100)	19 (100)
HBV-DNA, mean ± SD			
Log_{10} copies/ml by PCR	7.88 ± 1.01	8.03 ± 0.93	7.61 ± 0.95
HBeAg – positive, *n* (%)	141 (84.4)	55 (83.3)	13 (68.4)
ALT (IU/L), mean ± SD	151.2 ± 130.8	142.2 ± 87.7	140.5 ± 68.5
Abnormal ALT (ALT >1.0× ULN), *n* (%)	163 (97.6)	64 (97.0)	19 (100)
HBV genotype, *n* (%)			
A	4 (2.4)	1 (1.5)	0
B	5 (3.0)	1 (1.5)	0
C	154 (92.2)	63 (95.5)	19 (100)
Others	4 (2.4)	1 (1.5)	0

ml, mean ALT was 151 IU/l, and 84% (141/167) of patients were HBeAg(+). Ninety-two per cent (154/167) of patients were infected with HBV genotype C. Baseline demographics and disease characteristics were similar for all patient cohorts.

Virological response

Mean HBV-DNA levels fell rapidly during studies ETV-047 and ETV-053 [27,28]. For the cohort that entered ETV-060 from the two Phase II studies ($n = 167$), HBV-DNA fell from a mean of 7.88 log copies/ml at pre-treatment baseline to a mean of 3.41 log_{10} copies/ml at ETV-060 baseline. Viral load was further suppressed during treatment in ETV-060 and was maintained at low levels through 96 weeks (120–148 weeks total entecavir treatment time). Forty-nine per cent (82/167) of patients in the cohort had HBV-DNA <400 copies/ml at ETV-060 entry (Fig. 2A). By week 96 of the study, this proportion had increased to 88% (127/144). Of the 82 patients with HBV-DNA <400 copies/ml at ETV-060 entry, 81 patients (99%) maintained this response to the end of treatment. Eighty-five patients had HBV-DNA >400 copies/ml at ETV-060 entry; 62 (73%) achieved HBV-DNA <400 copies/ml during treatment in ETV-060, and 23 (27%) maintained >400 copies/ml at end of treatment. Among the 23 patients who discontinued treatment during ETV-060, 14 had HBV-DNA <400 copies/ml at the last on-treatment measurement. A sensitivity analysis using the last observation carried forward method was conducted based on the intention-to-treat (ITT) population. The last observed HBV-DNA levels for all subjects who either were still on study but had a missing PCR test at week 96 or discontinued prior to week 96 were carried forward; this maintained the total number of subjects in this cohort intact ($n = 167$). When the HBV-DNA end point was re-calculated using this method, 85% (142/167) of patients had HBV-DNA <300 copies/ml at week 96.

Biochemical response

Almost all patients (97.6%; 163/167) in the Phase II studies had abnormal ALT (ALT >1.0× ULN) at pre-treatment baseline (Table 1 and Fig. 3A). At the time of entry into study ETV-060, 81.0% (132/163) of those patients demonstrated normalized ALT levels (Fig. 3A). By ETV-060 week 48, that proportion had risen to 86.7%, and by week 96 (120–148 weeks total entecavir treatment time), the rate of ALT normalization was 90.1%.

Serological response

One hundred and forty-one patients (84%) were HBeAg(+) at pre-treatment baseline (Table 1 and Fig. 4A). At the time of entry into study ETV-060, 16.3% (23/141) of those patients had lost HBeAg and undergone HBe seroconversion (Fig. 4A). By week 96 of ETV-060 (120–148 weeks total entecavir treatment time), 38.8% (47/121) of patients had lost HBeAg, and 26.4% (32/121) had undergone HBe seroconversion. Among patients who underwent HBe seroconversion in ETV-060, the majority had achieved HBV-DNA suppression (<400 copies/ml) during treatment in study ETV-047 or ETV-053. One patient lost HBsAg and one patient underwent HBs seroconversion during treatment in study ETV-060.

Resistance

One hundred and sixty-four out of 167 patients were monitored for resistance through the end of treatment in ETV-060 (three patients refused consent for resistance testing). Five patients developed genotypic resistance to entecavir, which emerged during the third year of treatment, for a 3-year cumulative probability of resistance of 3.3%. Four of these five patients had received the lower (non-approved) doses of entecavir (0.01 mg or 0.1 mg) during the Phase II studies prior to ETV-060. Of the five patients with resistance, one patient had achieved HBV-DNA levels <400 copies/ml prior to developing resistance, and four patients experienced virological breakthrough. Fig. 5 provides HBV-DNA and ALT profiles for the patient who received continuous treatment with the approved 0.5 mg dose. This patient had detectable levels of HBV-DNA after 48 weeks of entecavir treatment in ETV-060. Genotypic resistance testing did not reveal any mutations associated with resistance to entecavir. The patient experienced virological breakthrough at week 96, which was associated with development of entecavir resistance (rt L180M, rt S202G, rt M204V).

Safety

Mean exposure to entecavir during study ETV-060 was 103.9 weeks (range: 5.1–140.6 weeks). Adverse events were reported for 99% (166/167) of patients, and most were mild to moderate in severity (Table 2). The most common clinical adverse event was nasopharyngitis (16.1%). Increased serum lactic acid

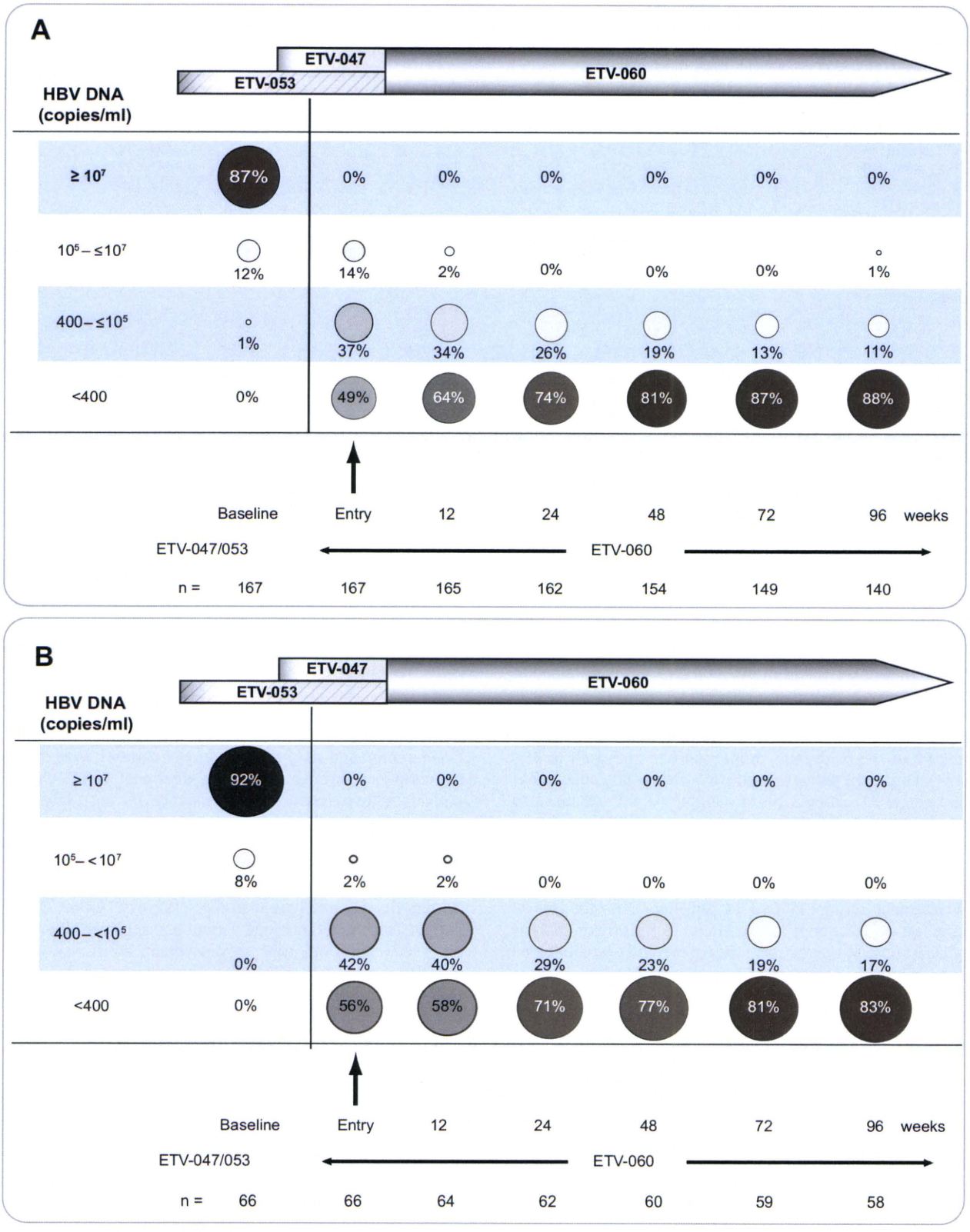


Fig. 2. Distribution of HBV-DNA levels over 96 weeks of treatment in rollover study ETV-060 (total entecavir treatment time, 120–148 weeks) for (A) the entecavir nucleoside-naïve long-term treatment cohort and (B) the entecavir 0.5 mg cohort.