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Michael J. Sofia *Editor*

HCV: The Journey from Discovery to a Cure

Volume II

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32
Topics in Medicinal Chemistry

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Michael J. Sofia
Editor

HCV: The Journey from Discovery to a Cure

Volume II

With contributions by

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Preface

The story of how the battle against hepatitis C was won began in 1975 with the realization that a previously unknown virus, non-A non-B hepatitis (NANBH), was responsible for a liver disease that plagued millions of individuals worldwide and took the lives of hundreds of thousands. It wasn't until the efforts of Harvey Alter, Michael Houghton, and their collaborators that in 1989 the hepatitis C virus (HCV) was identified as the new virus. Through their efforts, the development of a way to screen the blood supply was achieved and the risk of contracting this disease was dramatically reduced. However, there were still tens of millions of individuals who remained infected and transmitting the disease either sexually, via IV drug use or by coming in contact with contaminated blood by other means. The need for a cure was critical. This two-volume book attempts to chronicle the scientific story of the discovery of the virus, the development of tools important to the search for a cure, and the many drug discovery and development efforts that eventually delivered curative therapies to the millions of chronically infected HCV patients. It also attempts to put context around the impact of this work for the patient and society.

In conceiving this book, *HCV: The Journey from Discovery to a Cure*, I wanted to not simply have a series of isolated accounts of drug discovery efforts that led to marketed products. I wanted to take the reader along the entire historical scientific journey from the beginning to the end. It is rare in the annals of science that within a lifetime the full story of the identification of the key causative agent for a disease is found, and a cure is identified and made available to patients. In fact, cures of diseases are extremely rare, and the cure for HCV is the only example of a cure for a chronic viral disease. Therefore, I felt that the entire story needed to be told in one place.

In this two-volume account of how an HCV cure was achieved, the journey is communicated by those scientists and clinicians, including five Lasker Award Laureates, who were making those critical contributions integral in making this achievement happen. It begins with accounts of the discovery of the virus, elucidation of the virus life cycle and the role of each viral protein, development of the replicon system, and the use of interferon as early therapy. It continues with sections

focused on each of the key viral drug discovery targets. Each of these drug discovery sections first provides a general overview of the evolution of medicinal chemistry efforts against the target followed by detailed accounts of the discovery of each drug that is now a marketed HCV therapy. Yet the account of how HCV was cured would not be complete without addressing the evolution of innovative clinical trials and how combination therapies evolved to deliver therapies that are now pan-genotypic, provide exceptionally high cure rates in 8–12 weeks, and exhibit high barriers to resistance. Finally, the true indicator of medical achievement is not the commercial launch of a drug but the benefits that medicines bring to the patient and society; therefore, Volume 2 of the book ends with several perspectives speaking to the benefits achieved by an HCV cure and the possibilities for eliminating HCV as a global health threat.

What this two-volume book does not attempt to do is capture the vast body of work that was published over the 24 years that spanned the time from identification of the virus in 1989 to the approval of the first interferon-free HCV cure, sofosbuvir, in 2013. It also doesn't attempt to capture in detail the stories of the many failed avenues of investigation or accounts of the many investigational drugs that never made it to regulatory approval. However, this book does capture what I feel are the seminal contributions to the field and the important drug discovery success stories that matter to patients.

Finally, I have to thank all the chapter authors who committed a great deal of time outside of their busy schedules to tell the stories contained in this book. Each of them made their contribution because they too saw the need to tell the full story and wanted to be part of it. I also must thank all those researchers and clinicians who have contributed to the HCV cure story over 24 years but whose names are not explicitly mentioned in this work. Your contributions are not lost on those who have authored these chapters.

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Part I
HCV NS5A Inhibitors

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NS5A as a Target for HCV Drug Discovery

Donald R. O'Boyle II and Min Gao

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Abstract Discovery and development of HCV inhibitors is one of the most successful stories in the history of antiviral research. After more than 30 years of effort by academic and pharmaceutical researchers, HCV infection is a curable disease. In fact, HCV is the first chronic infectious disease to be cured with combinations of direct antiviral agents. Among these antiviral agents, NS5A inhibitors are the most potent. The unprecedented low pM potency, pan-genotype coverage, and well-tolerated clinical profile have made NS5A inhibitors an essential component of all interferon-/ribavirin-free regimens in currently approved HCV therapies. Since NS5A has no known enzymatic activity and is not a traditional antiviral target, this review focuses on the challenges and concerns that arose during the discovery of this class of inhibitor, the mode of action/inhibition, and the value of NS5A inhibitors in the treatment of HCV infection.

Keywords Combination therapy, Daclatasvir, Genotype coverage, NS5A inhibitors, Resistance, Synergistic effect

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1 General Properties of NS5A

The nonstructural protein 5A (NS5A) is a phosphoprotein required for HCV RNA replication and virion assembly in vitro and in vivo [1, 2]. NS5A from genotype 1 (GT1) is comprised of approximately 447 amino acids (aa) that can be divided into several distinct domains (Fig. 1). The first 33 residues of NS5A are highly conserved in all genotypes and form an amphipathic alpha helix [3] that is essential to modulate the association between NS5A and the endoplasmic reticulum membrane (ER) for recruitment to lipid droplets [4, 5]. The remaining NS5A monomer consists of three structural domains (I, II, and III) separated from each other by two inter-domain regions called low-complexity sequences (LCS) [6]. NS5A has the ability to bind RNA, preferentially the polypyrimidine tracks of 3′ untranslated regions (UTR) [7]. Although each domain is able to bind independently to 3′-UTR [6], the distinct genetically defined functions of these domains suggest that differential binding to other targets may enable NS5A to play diverse roles at different stages of the HCV life cycle (RNA replication and virion assembly).

Domain I (aa 34-213) is the most conserved region among the different HCV genotypes, is essential for replication, and has been crystalized as a dimer in different conformations (Fig. 2) [8–10]. The dimer is oriented to form a groove between two monomers. The groove has been suggested to be an RNA-binding site [8]; however, NS5A dimerization appears to occur via direct contacts between NS5A monomers and not via RNA [5]. Mutation analysis showed that the first LCS (Fig. 1) is important for NS5A dimerization [5]. The various dimeric forms of NS5A were found using different expression/purification and crystallization conditions (Fig. 2a–c); however, the differing NS5A crystals indicate the protein monomers can interact in multiple ways to form dimeric complexes (Fig. 2a–d). Since NS5A protein performs multiple functions in vivo, different NS5A functions may require different conformations to accommodate each role: protein-protein interactions (dimer formation, host protein interactions), protein-membrane interactions (with NS5A amphipathic helix), protein-nuclei acid interactions (RNA binding), and regulatory posttranslational modifications (serine phosphorylation) [5, 11, 12]. Additional NS5A conformational changes may occur prior to the release of the

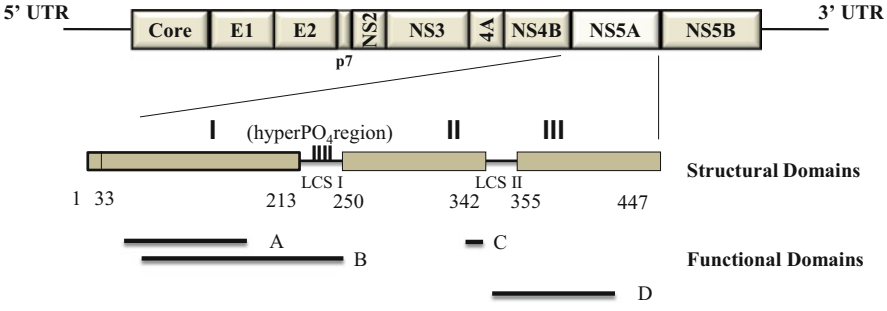

Fig. 1 Schematic drawing of HCV and NS5A. Structural and functional domains of NS5A

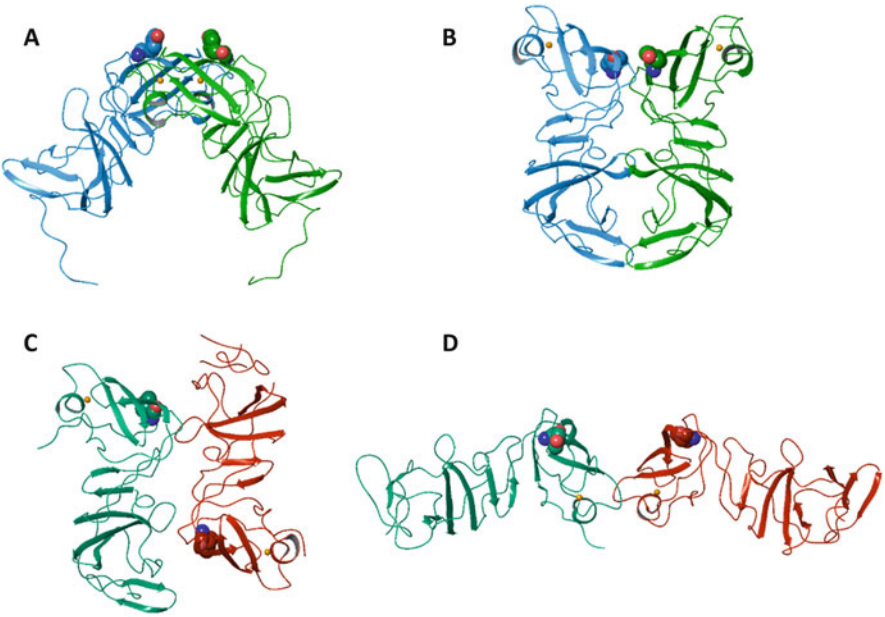

Fig. 2 The crystal structures for GT1b and 1a NS5A domain 1 dimers are displayed in ribbon representation. Tyrosine 93, a major resistant mutation, and Zinc (orange) are displayed as spheres. The GT1b monomers are blue and green, while the GT1a monomers are teal and red. (**a**) The first GT1b dimer structure [8] forms a potential RNA-binding pocket. (**b**) The 1b monomers in the second dimer [9] structure are in parallel to form an extensive interface. The first genotype GT1a NS5A domain 1 structure [10] contains two dimers. (**c**) The A and B monomers share the same interface as the dimer shown in (**b**); however, the monomers are antiparallel. (**d**) Monomers C and D form an extended N-terminal, head-to-head dimer

NS5A protein from the HCV poly-protein during replication complex formation. These conformational changes in NS5A are likely to be essential and represent multiple drug discovery targets that can be blocked via inhibitor binding.

Domain I also coordinates a single zinc (Zn++) atom per protein molecule. The coordination of the Zn++ by four NS5A cysteine residues (Cys^{39}, Cys^{57}, Cys^{59}, and Cys^{80}) [7, 8] suggests this is a structurally important metal ion required for NS5A folding and stability. Genetic data demonstrated that Zn++ binding is essential for multiple NS5A functions [7]. The coordination of Zn++ may also be important for the formation of higher-order structures such as oligomers/polymers. Domains II (aa 250–342) and III (aa 355–447) are less conserved among HCV genotypes than domain I [13] and natively unfolded [14, 15]. Domain II interacts with cyclophilin A (CypA), a cellular protein that stimulates RNA binding and is required for HCV replication [16]. This is consistent with the observation that CypA inhibitors such as cyclosporine (CsA) inhibit HCV replication [17]. Domain III is not required for HCV RNA replication but is essential for virion assembly [18, 19].

In addition to these structural domains, four functional domains (A, B, C, and D) of NS5A were mapped genetically using in vitro intragenic complementation experiments [20]. Domains A, B, and C have distinct roles in HCV RNA replication, while domain D is associated with virion assembly [18, 19].

NS5A has two phosphorylated forms, p56 and p58, that differ in electrophoretic mobility on SDS-PAGE. Basal phosphorylation of NS5A (p56) by host cellular protein kinases occurs at the center and near the C terminus (LCS II and domain III), whereas hyperphosphorylation of NS5A (p58) occurs in LCS I within a stretch of serine residues [21, 22] (Fig. 1). Ross-Thriepland and Harris [23] recently reviewed the cellular kinases involved in NS5A phosphorylation, the phosphorylated residues, and the functions of phosphorylation. The hyperphosphorylation of NS5A has a negative impact on HCV replicon replication in cell culture systems [21].

Adaptive mutations that greatly enhance RNA replication of GT1 HCV replicons [24] in vitro were identified by selection. Many of the mutations impact NS5A hyperphosphorylation: inhibition of p58 formation is associated with an increase in HCV RNA replication [11, 25–27]. The most effective adaptive mutation is S2204I, which impairs NS5A hyperphosphorylation. On the other hand, GT2a JFH-1 replicons replicate very well in cell culture without adaptive mutations [24]. To determine why adaptive mutations are needed for efficient replicon replication of genotypes other than GT2aJFH-1, a pooled lentivirus-based human cDNA library was screened. A single cellular protein, SEC14L2, was identified [25]. SEC14L2 promotes HCV infection by enhancing vitamin E-mediated protection against lipid peroxidation. It supports HCV replicon and infectious virus replication in cell culture without adaptive mutations. Observations that hyperphosphorylation is dependent on the presence of other nonstructural HCV proteins, such as NS4A [26, 28] and NS2 generated by NS2-3 auto-cleavage [29], or polyprotein consisting of N3-NS5A with active NS3 protease activity [30, 31] strongly suggest that a conformational change in NS5A may affect its hyperphosphorylation.

As a nonstructural protein without known enzymatic activity, NS5A relies on interactions with other viral and cellular proteins to exert multiple functions. These interactions do not occur simultaneously and must be temporally regulated to exert different essential roles during different stages of the HCV life cycle.

2 NS5A as a Target for HCV Drug Discovery

Based on clinical experience with HIV therapy, a combination regimen was predicted to be the most effective strategy for effective HCV treatments. Early on, it was noted that NS5A inhibitors possess several characteristics that make them attractive candidates as a component for combination therapy: (1) exceptional potency which drives a rapid initial viral RNA decline, (2) broad or pan-genotype coverage, and (3) mechanistically unique class with no cross resistance with other direct antiviral agents (DAA).

Traditional targets for antivirals are enzyme-based viral proteins, such as polymerase, protease, integrase, etc. In fact, more than half of the approved antiviral drugs are active site inhibitors represented by nucleos(t)ide analogs. Discovery of all new drugs requires the development of numerous in vitro assays (including binding, enzymatic, and cell-based) as well as co-crystallization with inhibitors and enzymes to insure the rational design of targeted inhibitors. Also, the data derived from in vitro assays are used routinely to predict the antiviral effects of inhibitors in the clinic. Since NS5A is not an enzyme target, development of assays enabling drug discovery was a challenge. The interactions of NS5A with many viral and cellular proteins could amplify the toxic effects as well as the antiviral effects. In addition, the host-cell environment affects the anti-HCV properties of NS5A inhibitors. Since the NS5A protein is a nontraditional antiviral target, sections that follow discuss the challenges and concerns that arose during the discovery of the first NS5A inhibitor daclatasvir (DCV, BMS-790052), the mode of action/inhibition, and the value of NS5A inhibitors in the treatment of HCV infection.

3 Discovery Challenges

The first NS5A inhibitor, BMS-858, was identified through an HCV replicon-based high-throughput screen of over 1 million compounds [32–34]. The path from BMS-858 to the discovery and development of DCV as a clinical candidate was littered with puzzles and questions. The astonishing in vitro potency of NS5A inhibitors was a puzzle that demanded focus on its mode of action (MOA). During the early stages of drug discovery, it was necessary to determine if the target of NS5A inhibitors was a cellular kinase or NS5A-kinase complex. Inhibition of p58 was associated with the activity of NS5A inhibitors (Fig. 3, left panel with compound BMS-529 [11, 34, 35]). The phenotype appeared to link with MOA since resistant NS5A lost sensitivity to p58 inhibition [34]. However, a similar phenotype was observed for NS3/NS4A protease inhibitors: the inhibition of NS5A p58 was lost with an NS3-resistant variant in the presence of a NS3 protease inhibitor [34]. These observations suggested that the inhibition of p58 is due to the NS3 protease inhibitor binding to its NS3 protease target and causing a conformational change of the kinase substrate, NS5A. Resistant variants selected with certain human kinase inhibitors also mapped to NS5A [36]. The caveat with these results is that under the selective pressure from a kinase inhibitor, it is easier to select resistance from a viral protein (the kinase substrate NS5A) than a cellular kinase itself. DCV-like molecules did not inhibit the activity of multiple kinases in vitro (Gao M, unpublished data).

The most convincing evidence that the target of DCV-like molecules is NS5A and not cellular kinase(s) was derived from the results of two experiments: (1) DCV inhibits p58 production of NS5A and replication of a JFH-1 replicon and virus without adaptive mutations [37] and (2) inhibition of p58 and HCV replicon activity can be separated (Fig. 3, right panel) [38]. Compound BMS-158 inhibited the HCV GT1b replicon with a median effective concentration (EC_{50}) of 0.5 nM; the resistant

	EC_{50} (nM)	
	WT	Resist (Y93H)
BMS-529	0.04	0.4
BMS-158	0.5	280

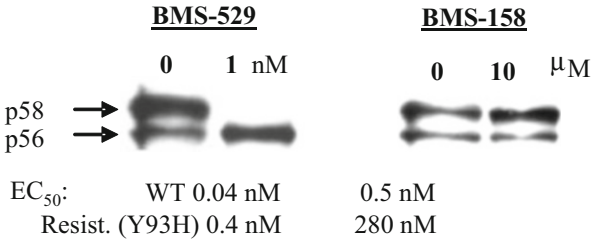

Fig. 3 Inhibition of HCV replicon replication and p58 production can be separated. EC_{50} values of compounds BMS-529 and BMS-158 were determined in HCV WT and Y93H replicons. Both compounds inhibited replicon through the same mechanism as shown by Y93H resistance. Western immunoblotting: GT1b plasmid was expressed in a vaccinia virus transient expression system treated with either DMSO (no compounds) or BMS-529 or BMS-158. Cell lysates were separated by SDS-PAGE, and NS5A proteins were identified by using an anti-NS5A antibody

variant NS5A Y93H is inhibited at EC_{50} of 280 nM. However, when NS5A without adaptive mutations was expressed from a vaccinia virus expression system compound, BMS-158 did not inhibit p58 (Fig. 3, right panel), in contrast to compound BMS-529 (left panel). This series of experiments convinced us that DCV did not inhibit kinase activity and enhanced our confidence that the target of DCV-like molecules is NS5A.

Drug discovery efforts also focused on whether inhibitors bind directly to NS5A. A biotinylated DCV-like molecule inhibited wild-type HCV replicon (EC_{50} of 33 nM) but was inactive toward the variant Y93H replicon ($EC_{50} > 10$ μM), whereas its diastereomer, used as a control, was inactive toward WT and Y93H ($EC_{50} > 10$ μM). NS5A was pulled down efficiently with the active inhibitor but not by the inactive diastereomer, suggesting selective binding to NS5A [35]. A different group reported a similar result [39]. Since the biotinylated DCV-like molecule binds WT NS5A and resistant NS5A with similar affinity, the correlation between specific inhibitor binding and antiviral activity was not firmly established using this approach. However, direct binding of NS5A inhibitors DCV and AZD7295 to bacterially expressed domain I with a Kd in the nM range has been reported [40]. Decreased binding affinity of these inhibitors to resistant variants L31V and Y93H confirmed specific binding and established a correlation between specific binding and anti-HCV effects. Interestingly, binding of these inhibitors does not affect NS5A dimerization, while RNA binding to NS5A inhibits inhibitor binding, suggesting that DCV-like molecules favor a dimeric structure of NS5A that does not

bind RNA [40]. Direct binding of ledipasvir (LDV) to a full-length NS5A containing a C-terminal His-tag produced from a baculovirus system was also reported [41]. Specificity was validated by (1) diminished binding of the resistant mutant NS5A (Y93H) to LDV and (2) competition of LDV binding to NS5A by DCV. Interestingly, LDV binding to NS5A was competed by DCV but not by the biotinylated compound BMS-671 [41], suggesting the binding mode of a monomer-like compound may be different from a dimer. These experiments establish the direct and specific binding of NS5A inhibitors to NS5A protein.

Phylogenetic analysis of nucleotide sequences identified at least six major genotypes [1 through 6] and many subtypes of HCV [42]. The highest priority for early HCV drug discovery was the development of an inhibitor with GT1a and 1b coverage, but the identification of an NS5A inhibitor with broad genotype coverage was the goal. The identification of NS5A inhibitors with broad genotype coverage required the development of many new research tools. When the first NS5A inhibitor was discovered, a GT1b replicon was the only genotype available. All the early structure-activity relationships (SAR) were established with the GT1b replicon [34, 43]. Although the major resistance residues of NS5A selected in GT1b replicon (L31 and Y93) are conserved in GT1a, none of the NS5A inhibitors discovered early (BMS-858, BMS-824, and BMS-346) were potent inhibitors of the GT1a replicon [34, 44]. The binding mode (dimeric vs. monomeric) and cap "structures" of the inhibitors were investigated to improve GT1a inhibition; however, the conservation of key resistance residues (L31 and Y93) provided the foundation for the design of potent inhibitors with broad or pan-genotype inhibition. Daclatasvir (DCV, BMS-790052) [35], a compound that preserves the symmetry present in BMS-346, inhibits most genotypes, and the second generation of NS5A inhibitors (velpatasvir (VEL), pibrentasvir (PIB), and elbasvir (ELB)) has significantly improved inhibition profiles for genotypes and resistance variants (Fig. 4 and Table 1).

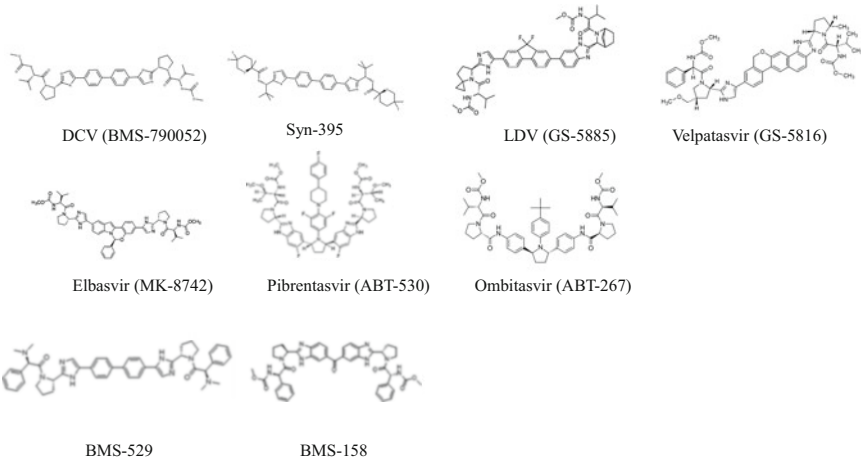

Fig. 4 Structures of different NS5A inhibitors used in this chapter

Table 1 In vitro replicon potency, genotype coverage, and resistance profiles of selected NS5A inhibitors[a]

Replicons (EC_{50}, nM)[a]	DCV (BMS-790052) [35, 45, 46]	LDV (GS-5885) [47, 48]	VEL (GS-5816) [49, 50]	EBR (MK-8742) [51, 52]	PIB (ABT-530) [53]	OMB(ABT-267) [54]
GT1a WT	0.05, *0.006*	0.031, *0.051*	0.031, *0.014*	0.004, *0.007*	0.002, *0.0007*	0.014, *0.003*
M28T	*4.1*	*1.8*	*0.11*	*0.11*	*0.0015*	*24.5*
M28V	*0.007*	*0.08*		*0.009*	*0.002*	*0.16*
Q30E	*150*	*44.1*	*0.25*		*0.002*	
Q30H	*8.7*	*5.3*	*0.032*	0.03	*0.0007*	*0.008*
Q30K	*146*		*0.15*			
Q30R	*7.3*	*12.4*	*0.031*	0.5	*0.001*	*2.2*
L31M	*2.1*	*14.6*	*0.22*	*0.07*	*0.0008*	*0.005*
L31V	*20*		*0.95*	0.5	*0.001*	
H58D	*3*	*32.7*	*0.10*	*0.04*	*0.0008*	*0.67*
Y93C	*11.1*	*48.7*	*0.052*	0.2	*0.001*	*4.6*
Y93H	*32*	*86.4*	*8.5*	2.4	*0.005*	*113*
Y93N	*282*	*>500*	*38.6*	*6.6*	*0.005*	*182*
Q30H-Y93H	553		*39.7*			
Q30R-Y93H	>1,000		*2.8*		*0.19*	
GT1b WT	0.009, *0.003*	0.004	0.015, *0.016*	0.003	0.004, *0.002*	0.005, *0.0008*
L31F	*0.013*			0.05		*0.008*
L31V	*0.061*		*0.037*	0.01		*0.007*
Y93H	*0.049*	*5.3*	*0.057*	0.05	0.001	0.06
L31F-Y93H	*14.9*					*8.1*
L31V-Y93H	*21.7*				0.002	*9.7*
GT2a (JFH)	0.071, *0.049*	21	*0.009*	0.003	0.005, 0.*001*	0.0008
GT2 (L31M)	6.9–13	*249*	*0.008–0.017*	3	*0.002*	0.012, *0.001*
T24A					*0.001*	*0.05*
F28S	>2,000				*0.001*	

GT3a	0.15, *0.25*	*168*	*0.004*	0.14	0.002, *0.0007*	0.019, *0.004*
M28T					*0.001*	*2.9*
A30K	*15.4*		*0.20*	7.0		
L31F	*80*			20		
Y93H	*688*		*2.9*	68	*0.002*	*30*
GT4a	0.012	0.39	*0.009*	0.003	0.002, *0.0008*	0.002, *0.0004*
L28V					*0.0009*	*0.008*
GT5a	0.033, 0.005	*0.15*	*0.059*		0.001, *0.0009*	0.003, *0.0009*
L28I	6.9				*0.001*	*0.072*
L31V	2.2				*0.0008*	*0.22*
L31F					*0.002*	*0.26*
GT6a	0.054, 0.05	1.1	0.007		0.003, *0.001*	0.37, *0.082*
L31M	43.9					
L31V					*0.001*	*0.56*

[a]EC_{50} values in italic were derived from transient replication assays

Cytotoxicity, an indicator of off-target activity, is generally easy to monitor and well separated from antiviral activity for enzyme targets such as polymerase and protease. For NS5A inhibitors, many different in vitro cell-based counterscreens (including a panel of DNA and RNA viruses and multiple cell lines derived from a variety of origins) as well as in vivo preclinical animal toxicity studies were used to evaluate off-target activity [34, 35]. The unprecedented potency and specificity of NS5A inhibitors yielded remarkable in vitro therapeutic indices (CC_{50}/EC_{50}) and in vivo safety margins. Indeed, clinical data have shown that NS5A inhibitors are well tolerated with a favorable adverse effect profile and low potential for drug-drug interactions [55, 56].

4 Anti-HCV Effects In Vitro and In Vivo

All NS5A inhibitors have been identified and evaluated with in vitro replicon or infectious virus assays. This class of inhibitors has produced the most potent antiviral agents reported to date, with EC_{50} values in the low pM range for all HCV genotypes (Table 1). DCV, the first NS5A inhibitor to enter the clinic, has broad genotype coverage with low pM potency in in vitro replicons, except for the GT2a variant containing the NS5A substitution L31M (EC_{50} 6.9–13 nM, Table 1). Analysis of baseline sequences of GT2a NS5A from >400 clinical specimens indicated that the most prevalent NS5A polymorphism associated with resistance is L31M (88%) [57]. Therefore, GT2a NS5A L31M can be considered "wild type (WT)" for NS5A inhibitor drug discovery. Indeed, the second-generation NS5A inhibitors, represented by VEL, PIB, and OMB, have true pan-genotype coverage with $EC_{50} < 0.5$ nM against all genotypes including GT2a expressing the NS5A L31M variant (Table 1). In addition to potency, the kinetics of antiviral suppression by NS5A inhibitors was found to vary based on the genotype- or strain-specific stability or half-life of the functional HCV replicase complex [58, 59]. This also modulates the effectiveness of NS5A inhibitors.

The in vitro replicon potency of NS5A inhibitors appears to correlate well with the initial HCV decline observed in infected patients treated with NS5A inhibitors. From a virology point of view, the antiviral effect of a specific inhibitor is determined mainly by two factors: intrinsic potency and resistance barrier. Because of the exceptional potency of NS5A inhibitors, patients generally experienced an initial sharp HCV RNA decline, indicative of the inhibition of wild-type virus. For example, the EC_{50} values of DCV for GT1a and GT1b replicons are 0.050 nM and 0.004 nM, respectively. The difference observed in GT1a and GT1b replicon potency was mirrored in a 14-day multiple ascending dose study, where the mean maximal decline in viral load was 3.6 $\log_{10}$ for GT1a-infected patients and 4.5 $\log_{10}$ for GT1b-infected patients who received 100 mg DCV once a day (QD) (Table 2). Rapid and sharp declines in HCV at early treatment time points in patients receiving NS5A inhibitors is another characteristic of this class of inhibitors. A 2 $\log_{10}$ viral RNA decline was reached 4 h after the first 60 mg dose of DCV [45]. This marked

Table 2 HCV RNA decline observed in monotherapies with selected NS5A inhibitors

Inhibitor	Dose	# of patients	Duration (days)	Max. viral decline				
				GT1a	GT1b	GT2	GT3	GT4
DCV (BMS-790052) [45]				Mean (log_{10})				
	60 mg QD	4 for GT1a	14	3.8				
	100 mg, QD	3/1 for GT1a/1b	14	3.6	4.5			
	30 mg, BID	2/2 for GT1a/1b	14	2.6	5.7			
LDV (GS-5885) [60]				Median (log_{10})				
	10 mg, QD	10/10 for GT1a/1b	3	3.2	3.3			
	90 mg, QD	10 for GT1a	3	3.1				
VEL (GS-5816) [61]								
	50 mg, QD	8/4 for GT1a/3	3	3.6			2.6	
	100 mg QD	8 for GT1a	3	3.6				
	150 mg, QD	7/8/8/6/2 for GT1a/1b/2/3/4	3	4.0	4.0	4.4	3.3	3.5
EBR (MK-8742) [62]				Mean (log_{10})				
	50 mg QD	5/5/5 for GT1a/1b/3	5	4.2	5.1		3.1	
	100 mg, QD	5 for GT3	5				3.4	
FIB (ABT-530) [63]				Mean (log_{10})				
	40 mg, QD	8 for GT1	3	4.1				
	120 mg QD	8 for GT1	3	4.5				
OMB (ABT-267) [64]				Mean (log_{10})				
	5 mg, QD	4 for GT1	3	2.9				
	50 mg, QD	4 for GT1	3	2.8				

Table 3 SVR12 for FDA approved combination therapies containing selected second-generation NS5A inhibitors

Combination	Non-NS5A inhibitor in the combination	Usage	Genotypes	Duration (weeks)
Harvoni (LDV/SOF) [65, 66]	SOF: nucleotide analog	90/400 mg, QD	1, 4–6	8–24
Epclusa (VEL/SOF) [67]		100/400 mg	1–6	12
Vosevi (VEL/SOF/VOX) [68]	VOX: NS3/4A protease inhibitor	100/400/ 100 mg	1–6	12
Zepatier (EIB/GRA) [69]	GRA: NS3/4A protease inhibitor	50/100 mg	1, 4	12–16
Mavyret (PIB/GLE) [70]	GLE: NS3/4A protease inhibitor	40/100 mg	1–6	12

and robust antiviral effect suggested the use of NS5A inhibitors could shorten treatment duration significantly, making therapy more tolerable. The first- and second-generation NS5A inhibitors (Table 2) have strong antiviral effects in monotherapy trials in different genotypes tested, confirming that the exceptional in vitro potency of NS5A inhibitors translated to in vivo efficacy.

NS5A inhibitors are the most potent antiviral agents developed to date and are components of all interferon/ribavirin-free regimens in currently approved HCV therapy (Table 3). FDA approved Harvoni (SOF-nucleotide/LDV-NS5A) as a once-daily single-tablet regimen to treat HCV in adults in 2014 and in children in 2017 [65, 66]. Subsequently, NS5A inhibitors were combined successfully with other DAAs, such as NS3 protease inhibitors. The treatments offer excellent efficacy and safety profiles, especially treatments containing the second generation of NS5A inhibitors (Table 3). The FDA approved Epclusa in 2016 and Vosevi and Mavyret in 2017 as fixed-dose combinations for treatment of HCV GT1–6 [67, 68, 70]. The "one-pill for all" regimen greatly simplified therapy by precluding the need to screen genotypes prior to treatment. The overall cure rates have reached $\geq$92% with 8–16 weeks of treatment for all genotypes (Table 3). With certain patient populations, the cure rate is almost 100% [71, 72].

5 Resistance

Infection with HCV results in a highly heterogeneous virus population, a consequence of its rapid replication turnover rate (~10^{12} virions/day) and the lack of a proofreading function in the NS5B polymerase. Therefore, mutations at every position of the HCV genome are possible, and variants resistant to individual DAAs are predicted to preexist at baseline (BL) in infected subjects.

In addition to intrinsic potency, the resistance barrier of an inhibitor determines its antiviral effect. A slow second phase of viral decline or a slight viral rebound was observed at later time points during the 14-day monotherapy study of DCV [45, 46]. This observation was consistent with an accumulation of resistant variants and suggested that the adaptation or selection of resistant variants enhanced their fitness. The emergence of resistance suggests that DCV, like NS3 protease inhibitors and NS5B polymerase allosteric inhibitors, may have a low genetic barrier to resistance. A single-nucleotide change (UAU or UAC to AAU or AAC) at residue 93 (Tyr to Asn) of GT1a NS5A is sufficient for HCV to acquire clinical resistance to DCV. Furthermore, the accumulation or acquisition of additional mutations generates linked substitutions such as Q30D/H/L/R-Y93C/H/N that confer higher levels of resistance [46, 47, 49, 52, 54].

All amino acid (aa) substitutions associated with resistance to this class of inhibitors have been mapped to the N-terminal 100 residues for all genotypes (Table 1). The most prevalent resistant substitutions for GT1–6 are shown in Fig. 5 [45–54, 60–64, 73–76]. Observation of the same substitutions in vitro and in vivo confirms the utility of the replicon system for assessing resistance in response to treatment with NS5A inhibitors. In general, GT1a variants conferred higher levels of resistance than GT1b variants, possibly explaining why viral breakthrough was more common among patients with GT1a. Some single amino acid substitutions confer low-to-moderate levels of resistance (Q30H: 1,450- and

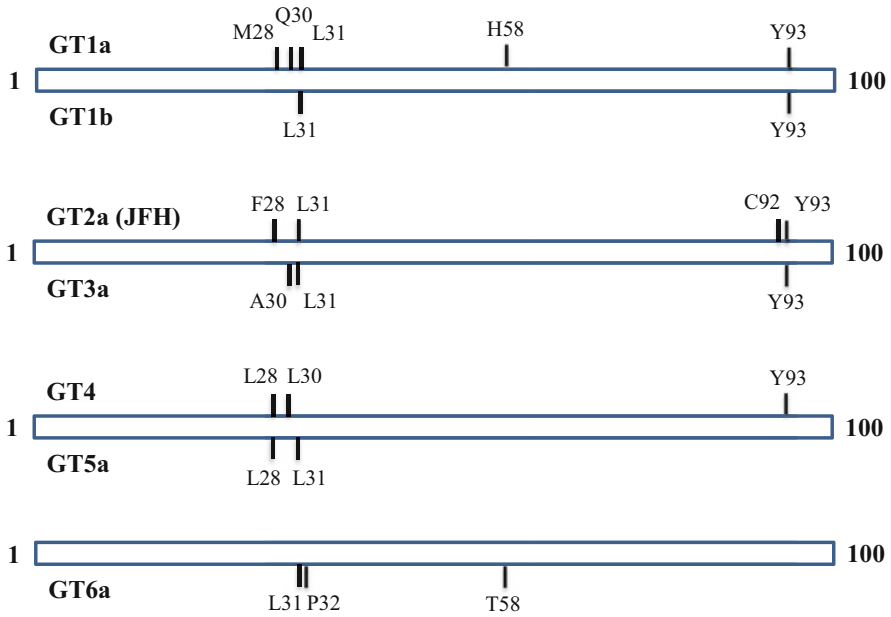

Fig. 5 Major NS5A resistance-associated substitutions observed in GT1-6. Most, if not all, substitutions are mapped to the first 100 aa of NS5A

2.3-fold; Y93H 5,333- and 607-fold resistance to DCV and VEL, respectively), but linked substitutions such as Q30H-Y93H confer higher levels of resistance (92,167- and 2,836-fold resistance to DCV and VEL, respectively; Table 1). However, the second-generation NS5A inhibitor, pibrentasvir (PIB, ABT-530), has not only a pan-genotype coverage profile but also a high barrier to resistance in vitro (Table 1). For example, the GT1a Y93N variant has high levels of resistance to DCV, LDV, OMB, and VEL (EC_{50} values of 282, >500, 182, and 38.6 nM, respectively) but is still very sensitive to PIB (EC_{50} of 0.005 nM). In fact, EC_{50} values of PIB for all tested variants from GT1–GT6 are less than 1 nM, including GT1a Q30R-Y93H variant with linked substitutions (Table 1).

Resistance of variants to different NS5A inhibitors varies significantly, mainly determined by inhibitory pressure, fitness of the variants, and genetic background of HCV before treatment. The inhibitory pressure of inhibitors and the fitness of variants are relatively easy to measure, while monitoring the genetic background of HCV replicons and patient specimens before treatment, especially minor variants, can be a challenge. A correlation between the influence of naturally occurring polymorphisms on DCV activity in vitro and in vivo has been observed [77]. A Q30R variant with a low level of in vitro resistance to DCV (EC_{50} ~7 nM) was observed at viral breakthrough in a GT1a-infected patient. Because the level of DCV observed in the plasma of the patient was high (Ctrough $\geq$117 nM), a rigorous investigation was initiated to determine the basis for resistance. A baseline polymorphism (E62D) found in this patient did not show resistance to DCV when it was introduced into a GT1a replicon; but the linked variant, Q30R-E62D, conferred high-level resistance in vitro (EC_{50} = 153 nM) and is likely to be responsible for viral breakthrough in vivo. These data showed that a BL polymorphism with minimal impact on the anti-HCV effect of DCV could enhance the emergence of resistance and significantly affect clinical outcome. Further support was obtained by evaluating hybrid replicons in which the entire NS5A coding region of GTla was replaced with the corresponding region of specimens collected from the infected patient. This work established a clear, systematic approach to monitor resistance to NS5A inhibitors in the clinic.

Although NS5A inhibitors have a relatively low resistance barrier compared to *sofosbuvir*, the resistance barrier becomes less important with combination treatment/therapy. Effective control of HIV infection/resistance using combination therapies provided a clear path for the development of HCV inhibitors. From the beginning of HCV drug discovery, development of combination therapies was the goal for an HCV cure. To be an effective combination therapy, individual inhibitors should (1) target different viral proteins or different stages of the viral life cycle, (2) have no detectable overlapping toxicity in preclinical animal studies, and (3) have minimal or no drug-drug interactions. To identify effective combination treatments that included DCV, in vitro combination studies were performed. As shown in Fig. 6, numerous resistant colonies were observed (Fig. 6a, b) when HCV replicon cultures were treated with a single agent, DCV, ASV (asunaprevir, NS3 protease inhibitor), or BCV (beclabuvir, NS5B non-nucleoside polymerase inhibitor), at a concentration 30-fold above the inhibitor EC_{50} [78]. Dual

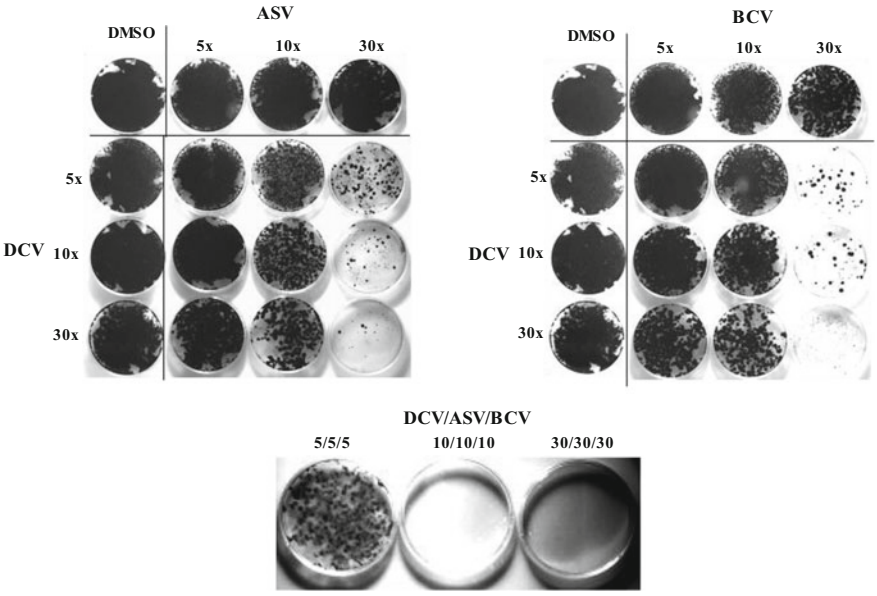

Fig. 6 Combination treatment reduces the emergence of resistant colonies. GT1b HCV replicon cells were incubated for 4 weeks with BMS-790052 (DCV), BMS-791325 (BCV), or BMS-650032 (ASV) as monotherapy and dual therapy (top left and right) and triple therapy (bottom) at 5×, 10×, and 30× EC_{50}. Colonies were visualized by crystal violet staining. Data shown are representative of the results of three independent experiments

combinations, DCV + ASV or DCV + BCV (concentrations 15-fold above each inhibitor EC_{50}), reduced the number of resistant colonies compared to the single agents. A triple combination of DCV + ASV + BCV at concentrations tenfold above each inhibitor EC_{50} eliminated HCV replicon (Fig. 6c). The power of combination therapy for curing HCV has been confirmed in clinical studies (Table 3).

6 Mode of Action

The mode of action (MOA) of NS5A inhibitors is not fully understood; however, several models have been proposed based on experimental results and mathematical modeling. The models provide insights into how these inhibitors affect the biologic functions of NS5A and HCV.

The pM potency of NS5A inhibitors in vitro translated to remarkable initial viral decline in vivo, suggesting the MOA of NS5A inhibitors could be related to the roles of NS5A during the HCV life cycle. Clinical data indicated that the initial viral decline observed with NS5A inhibitor treatment was faster than any other antiviral agents reported. This led to mathematical modeling that predicted DCV efficiently

blocks two distinct stages of the viral life cycle, RNA synthesis and virion assembly/secretion, with mean effectiveness of 99% and 98%, respectively. The model also yielded a more precise estimate of 45 min for the HCV serum half-life [79–81]. Experiments done in vitro with infected cells corroborated a prediction based on the model: intracellular HCV RNA had a similar pattern of decline when cells were treated with either DCV or an HCV polymerase inhibitor (NM107), but only DCV treatment yielded a rapid initial decline of extracellular viral titers compared to a delayed and slow decline with NM107 [80]. A second in vitro study confirmed these results [80]. In this study, the kinetics of antiviral suppression by NS5A inhibitors were compared to protease inhibitors and NS5B polymerase inhibitors. Despite their potency, NS5A inhibitors were slow to inhibit HCV RNA synthesis, compared to the protease and polymerase inhibitors. However, NS5A inhibitors rapidly depleted intracellular infectious virus and RNA-containing HCV particles, indicating the inhibition of intracellular virion assembly. Inhibition of virion assembly has not been confirmed by resistance analysis; no NS5A-resistant variants associated with virion assembly have been reported. In addition, the inhibition of different stages of the HCV life cycle (RNA replication and virion assembly) does not explain the potency of NS5A inhibitors since HCV virion assembly is not present in the replicon system in cell culture.

HCV replication in replicon cells is inhibited by a ratio of DCV to NS5A estimated to be approximately 1 to 47,000. This ratio suggests that a small number of inhibitor molecules impact the function of a large number of NS5A protein molecules, and it may be related to the potency of NS5A inhibitors [82–85]. Based on the crystal structures of NS5A protein dimers and the structural analysis of NS5A inhibitor and NS5A protein co-crystals, it has been proposed that NS5A forms polymers and/or oligomers requiring only small amounts of NS5A inhibitors to affect the higher-order forms [8, 9, 41].

Biophysical methods were used to observe the intrinsic self-association of NS5A domain 1 (GT1a and GT1b) which existed as a heterogeneous mixture in solution and exhibited dynamic equilibria between monomers and higher-order structures [85]. The formation of large and irreversible protein aggregates was induced by DCV [85]. NS5A inhibitor binding to a variety of NS5A species inside cells (monomers, dimers, and multiples of NS5A dimers) was observed using NS5A compounds containing cross-linking functionalities (azide, bis-azide) [86]. NS5A inhibitor binding to HCV processing intermediates was observed using elution studies performed with the biotin affinity compound BMS-671 [86]. Release of NS5A proteins from BMS-671 required detergent with heating. The harsh elution conditions suggest BMS-671 is "wrapped up" by the NS5A proteins during the folding process [10, 86]. Lending support to this hypothesis are (1) the targeted disruption of only new HCV membranous-web replication centers by NS5A inhibitors, with little to no effect on existing replication centers, and (2) the detection of NS5A inhibitors associated with monomeric NS5A during SDS-PAGE [39, 86].

A series of experiments exploring the possibility that a single inhibitor may inhibit the function(s) of NS5A oligomers revealed a novel synergy mechanism between specific pairs of NS5A compounds of similar structure (Fig. 7) [84, 87].

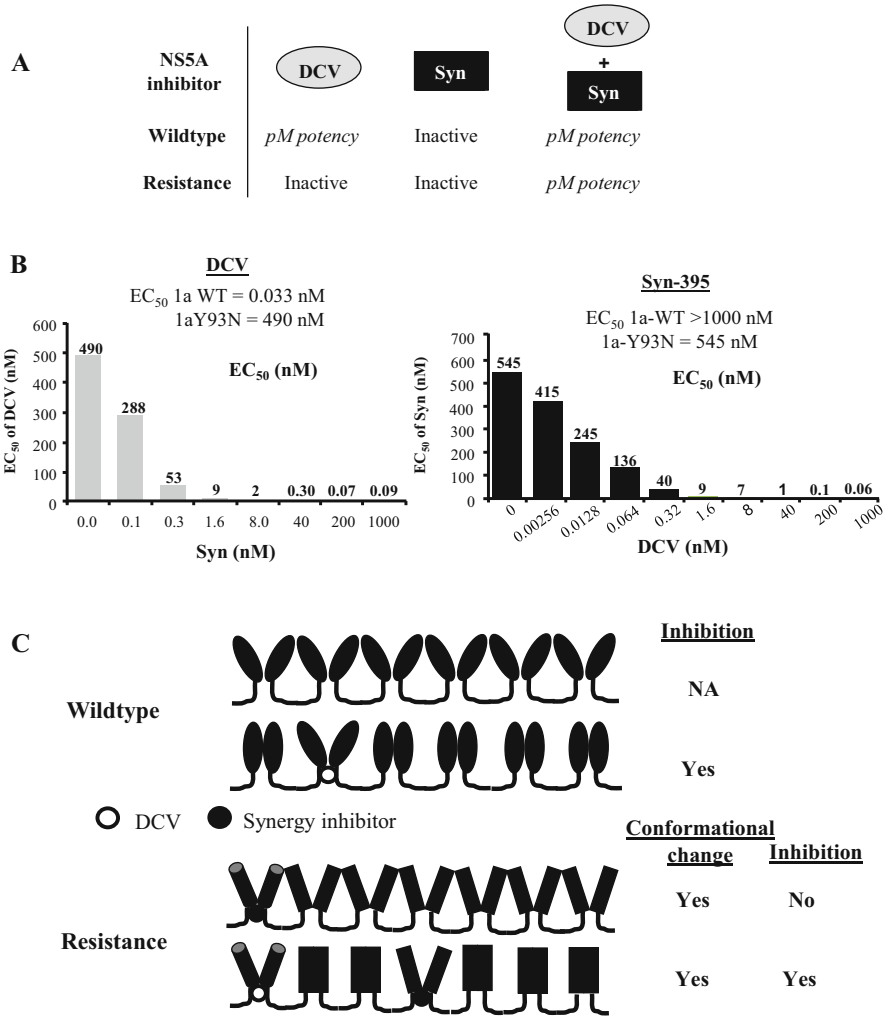

Fig. 7 Synergistic anti-HCV effect of NS5A compounds. (**a**) Cartoon representation of the synergistic effect of a pair of NS5A compounds, DCV and Syn-395. (**b**) Synergistic effect in a GT1a Y93N replicon. Left panel, EC_{50} values of DCV were determined in the absence or presence of different concentrations of Syn-395; right panel, EC_{50} values of Syn-395 were determined in the absence or presence of different concentrations of DCV. (**c**) Working model of the synergistic effect of a pair of NS5A compounds. NS5A molecules form oligomers in the replication complex and communicate with each other. For WT NS5A, DCV binds to NS5A, affecting multiple NS5As and disrupting the function(s) of the entire oligomer. Although DCV can bind resistant NS5A, it does not disrupt NS5A function(s); however, DCV binding causes a conformational change that accommodates the binding of the second inhibitor on adjacent NS5A molecules and disrupts function(s) of the oligomer

DCV exhibits an EC50 of 0.033 nM against a WT GT1a replicon, but it has no apparent activity against some NS5A-resistant variants such as Y93N ($EC_{50} > 100$ nM, Fig. 7b). A structurally related compound, referred to as a synergist (Syn-395, Fig. 7a), is inactive against both WT and resistant variants ($EC_{50} > 100$ nM). However, the combination of DCV and Syn-395 has remarkably potent inhibitory activity, with EC_{50} values in the pM range against DCV-resistant variants as well as the WT replicon (Fig. 7a). Specifically, the presence of Syn-395 enhances the potency of DCV approximately 2,600-fold against the Y93N variant, from 339 nM in the absence of Syn-395 to 0.13 nM in the presence of 40 nM Syn-395 (Fig. 7b, left panel). A similar synergistic effect was observed in a reciprocal experiment: Syn-395 is inactive against both WT and resistant variants ($EC_{50} > 500$ nM). The presence of DCV enhances the potency of Syn-395 against the Y93N variant from 545 nM in the absence of DCV to 1 nM in the presence of 40 nM DCV (Fig. 7b, right panel). This result illustrates a cooperative interaction between DCV and Syn-395 inhibiting HCV replication in the presence NS5A protein carrying a resistance substitution. This synergistic effect of a pair of NS5A inhibitors observed in the GT1a replicon is conserved among different HCV genotypes [81, 87]. The synergistic inhibitory effect of specific pairs of NS5A compounds, such as DCV and Syn-395, suggests that the conformational changes in NS5A protein induced by DCV only accommodate the binding of synergists of specific structure. The results indicate that Syn-395 binds to both WT- and DCV-resistant NS5A, but the binding of Syn-395 is not able to inhibit virus replication. However, Syn-395 binding, either adjacent to or a few NS5A dimers to either side of DCV, potentiates the effect of DCV by introducing a conformational change that resensitizes the resistant NS5A toward DCV inhibition (Fig. 7c). These experiments clearly demonstrated the functional communication between NS5A molecules during HCV replication.

7 Conclusion

A new class of anti-HCV inhibitor targeting the NS5A protein has become an essential component of combination therapies for eradicating this chronic viral disease. Lessons learned during the discovery of the class illustrate the general challenges of developing drugs for novel targets. Understanding the mode of inhibition of HCV NS5A inhibitors may illuminate specific opportunities to discover drugs with a similar mechanism for diseases other than HCV.

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Compliance with Ethical Standards

Conflict of Interest: Dr. Gao worked for Bristol Myers Squibb.

Ethical Approval: This chapter does not contain any studies with human participants or animals performed by any of the authors.

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The Discovery and Development of Daclatasvir: An Inhibitor of the Hepatitis C Virus NS5A Replication Complex

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Abstract The discovery of the hepatitis C virus (HCV) NS5A replication inhibitor daclatasvir (**1**) had its origins in a phenotypic screening lead. However, during the optimization campaign, it was observed that some members of the chemotype underwent a radical dimerization under the assay conditions. This redirected the effort to focus on palindromic molecules, a species subsequently shown to complement the dimeric nature of the NS5A protein. The most challenging aspect of the discovery program was extending antiviral activity to encompass GT-1a virus which was accomplished only after the development of extensive structure-activity relationships. In clinical trials, oral administration of daclatasvir (**1**) produced a profound effect on viral load with onset that was more rapid than had been seen previously with either NS3 protease or NS5B polymerase inhibitors. A groundbreaking clinical trial that combined daclatasvir (**1**) with the protease inhibitor asunaprevir (**52**) established that a chronic HCV infection could be cured with small molecule therapy in the absence of immune stimulation, setting the stage for approval of this regimen for the treatment of GT-1b-infected subjects by the Japanese health authorities on July 4, 2014.

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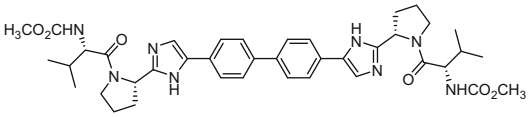
1 Introduction

The discovery of the hepatitis C virus (HCV) nonstructural 5A (NS5A) replication complex inhibitor daclatasvir (**1**) began with the development of a genotype 1b (GT-1b) replicon that was implemented as a phenotypic screen using a design that conferred a stringent triaging of hit molecules [1–11]. This screening campaign was part of a broader strategy that pursued the identification of mechanistically orthogonal inhibitors of HCV that could be used in combination therapy, an approach that anticipated clinical use of drug cocktails to minimize the selection of resistant viruses. More specifically, the replicon screen comprised of simultaneously assessing the antiviral activity of test molecules toward a sub-genomic HCV GT-1b construct and a bovine viral diarrhea virus (BVDV) replicon, both replicating in the same human liver Huh-7 cell line background and seeded in the same well of a 96-well plate [6]. HCV inhibition was determined indirectly using a fluorescence resonance energy transfer (FRET)-based assay that assessed NS3 protease activity toward a synthetic substrate incorporating both a fluorescence donor [(5-((2-aminoethyl)amino)naphthalene-1-sulfonic acid (EDANS)] in the amino terminus and a fluorescence acceptor [4-((4-(dimethylamino)phenyl)azo)benzoic acid, succinimidyl ester (DABCYL)] at the carboxyl terminus. The BVDV replicon incorporated a firefly luciferase reporter gene that provided an orthogonal readout of replication activity based on the emission of light, reflecting the amount of enzyme present after adding a substrate. The third piece of information harvested from the assay, which was also the first experimental data obtained, was AlamarBlue cell viability staining which assessed mitochondrial function, providing an indication of the cytotoxicity of test compounds. This assay configuration was used to interrogate a representative selection of the Bristol-Myers Squibb proprietary compound collection and identified the iminothiazolidinone **2** as hit that met the criteria of exhibiting activity toward the HCV replicon at concentrations at least tenfold lower than either inhibitory activity toward the BVDV replicon or cytotoxicity [12]. The specific details of the antiviral profile of **2** in the screening assays and toward a panel of viruses are summarized in Table 1. Compound **2** had an interesting background since it had its origin in a prospective library that had been prepared as part of a campaign to embellish the Bristol-Myers Squibb proprietary compound collection. A notable structural feature of **2** is that it had been designed to include a phenyl substituent at C-5, unusual since C-5 benzylidene derivatives are far more prevalent in the literature, a function of convenient access by way of a Knoevenagel condensation reaction between a C-5 unsubstituted iminothiazolidinone and an aldehyde [13, 14].

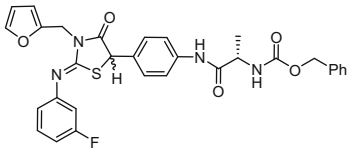
Table 1 Antiviral activity of **2** in the GT-1b HCV replicon and toward a panel of viruses

Assay	EC_{50} (μM)	CC_{50} (μM)
GT-1b HCV replicon	0.57	>50
BVDV replicon	24	>100
BVDV virus	17	>150
HIV-1	41	41
HRV	>100	>100
RSV	23	23

HIV-1 human immunodeficiency virus-1, *HRV* human rhinovirus, *RSV* respiratory syncytial virus



1 (BMS-790052, daclatasvir)



2 (BMS-858)

The data accumulated for **2** that are compiled in Table 1 revealed a profile of significant and selective inhibitory potency toward the GT-1b HCV replicon with an EC_{50} value of 570 nM, a finding that encouraged further study of the chemotype [15]. Adding to the intrigue with **2** as a lead inhibitor was the generation and validation of resistant mutants arising in the replicon in response to selective pressure that mapped to the amino terminus of the NS5A protein, specifically a Tyr98His and a Leu31Val/Gln54Leu combination [12]. At the time of this discovery, little was known about the specific functions of the NS5A protein in viral replication although, perhaps not surprisingly for such a small virus, it was known to be an essential protein [16–20].

The *Z*-configuration of the 2-arylimino moiety of **2** was assigned based on prior studies which indicated that this topology minimized steric effects. However, attempts to separate the individual stereoisomers at C-5 were thwarted by facile racemization of the benzylic center after chiral chromatographic separation [15, 21]. Seminal insights into structure-activity relationships (SARs) were gleaned from a survey that explored variation of the three major peripheral elements of the molecule – the furanylmethyl substituent, the arylimino moiety, and the amino acid amide. Changes to both the furanylmethyl and arylimino moieties in the context of the Cbz-alanine amide were found to influence potency, with compounds incorporating polar heterocycles being the more potent, while more lipophilic substituents

generally exhibited poorer replicon inhibitory activity. Overall, a good dynamic range of potency was observed with the structural variations sampled, with EC_{50} values ranging from 10 to 2.5 μM. However, more profound and precise effects on HCV GT-1b inhibitory potency were associated with changes to the Cbz-alanine amide element, with the key findings summarized in Fig. 1. The unnatural alanine analogue **3** was 170-fold less potent, while the glycine homologue **4** was eightfold weaker than **2**. The strong dependence of antiviral activity on the absolute configuration of the amino acid element was reproduced in the proline derivatives **5** and **6**, both of which were fivefold more potent than their alanine congeners [15]. Replacing the Cbz element with a dihydrocinnamate moiety resulted in an erosion of potency in both series, as exemplified by **7** and **9**. However, a significant increase in potency was observed with the phenylacetamide moiety found in **8** and **10**, with both compounds expressing single digit nanomolar EC_{50} values in the replicon and confirming the inherent advantage offered by proline. Only weak antiviral effects were observed with the many other amino acids and additional structural variations that were explored at this site of the pharmacophore, with some representative examples of those sampled compiled in Fig. 2 [15].

While these collective SARs appeared to be coherent and were readily interpretable, as highlighted by the sensitivity of potency to changes to the amino acid element, as exploration of **2** and its analogues continued, it became apparent that the iminothiazolidinone chemotype was unstable under some conditions [15, 22]. The first indication of a problem was the observation that a sample of **2** degraded upon standing as a solution in DMSO for several days, with the thiohydantoin **15** (R_3 = $NHCO_2CH_2Ph$) and thiourea **17** (R_3 = $NHCO_2CH_2Ph$) determined to be two of the degradation products after a preparative experiment (Scheme 1). In the replicon, **15** exhibited a modest inhibitory activity, EC_{50} = 13 μM,

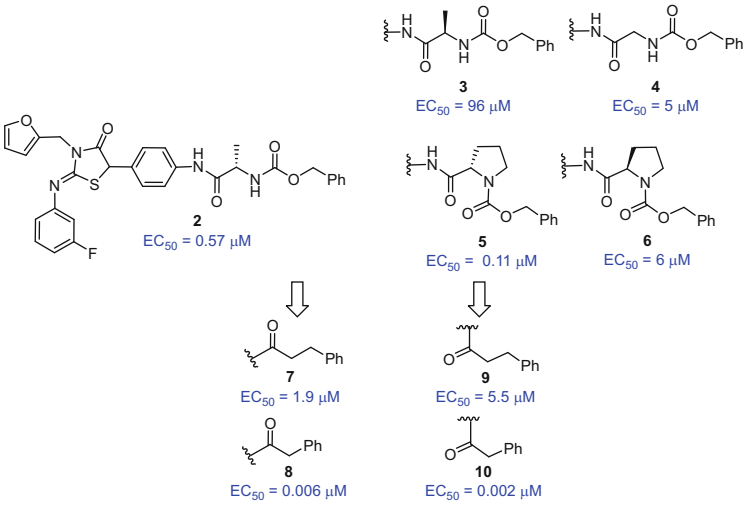


Fig. 1 Structure-activity relationships associated with variation of the amino acid moiety of **2**

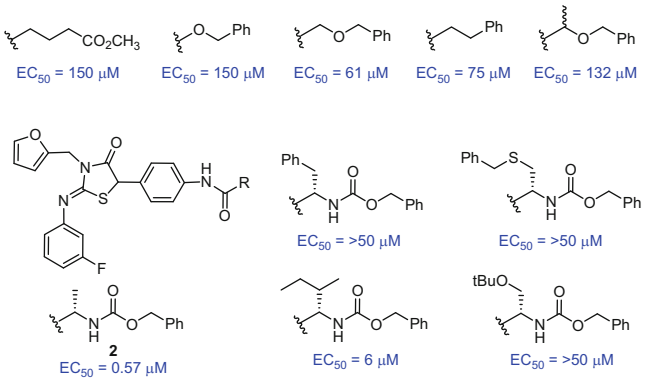
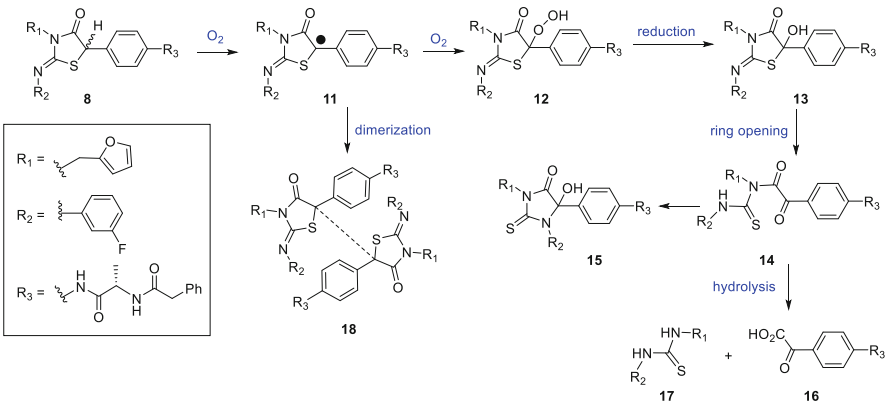


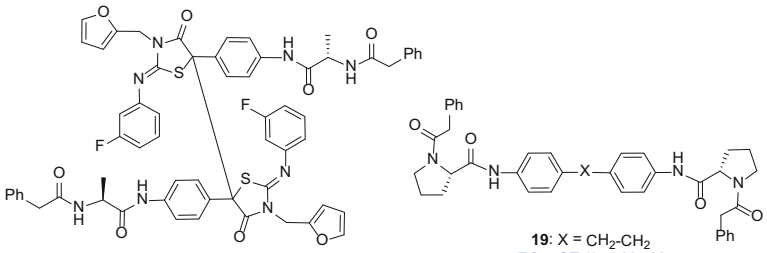
Fig. 2 Structure-activity relationships associated with the amino acid element of **2**



Scheme 1 Chemical degradation path elucidated for **2**

while **17** was essentially inactive. Compounding the intrigue, incubating the more potent **8** in replicon media until degradation was complete and then assessing the antiviral activity of the preparation in the GT-1b replicon demonstrated that the HCV inhibitory activity and potency were fully preserved [15, 22]. A more detailed analysis of the degradation products from **8** guided by high-performance liquid chromatography (HPLC) biogram analysis, in which fractions taken from the chromatography column are concentrated and assessed for replicon inhibition, led to the identification of two potent constituents present in just trace amounts in the cell media [22, 23]. A preparative experiment allowed isolation of a sufficient quantity of each component to allow for a more detailed characterization. ^{1}H-NMR and mass spectrum analyses indicated that the two compounds were dimers with an isomeric relationship and assigned as **18** based on the absence of the C-5 hydrogen atom of the iminothiazolidinone ring in the ^{1}H-NMR spectrum and connectivity that was

confirmed after the analysis of the ^{13}C-NMR spectrum. Although neither the exact stereo-composition of the benzylic centers of **18** nor the precise relationship between these two compounds was determined, it is of note that the isomer that was less mobile on a reversed phase column converted to the more mobile isomer upon heating in CD_3CN at 50°C, an observation made while conducting an NMR experiment as part of the structure determination process [22]. With the acquisition of these data, the mechanistically holistic picture of the degradation process that is presented in Scheme 1 was proposed. Abstraction of the C-5 hydrogen atom of **8** by oxygen, which is a diradical in the ground state, would lead to the C-5 radical **11** that is stabilized in a classic captodative fashion by the adjacent C=O moiety, the sulfur atom, and the phenyl substituent [24, 25]. Combination of **11** with O_2 and hydrogen atom acquisition would lead to the hydroperoxide species **12** which in DMSO would be reduced to alcohol **13**, an unstable ring system that would be expected to undergo ring opening to give **14**. Reclosure of **14** would then afford the thiohydantoin **15**, which had been isolated in the original DMSO degradation experiment, while hydrolytic decomposition of **14** would afford acid **16** and the thiourea **17**, the latter of which had also been isolated. However, in cell culture media, the stability of radical **11** is presumably such that dimerization can occur, a process that may well be facilitated by aggregative association of **8** and/or **11** in the aqueous medium.



The replicon inhibitory potency of the dimers **18a** and **18b** was striking, with the less mobile isomer eluting from the reversed phase chromatography column exhibiting an EC_{50} value of 600 pM in the GT-1b replicon, while the thermodynamically more stable and chromatographically more mobile isomer was 70-fold weaker, $EC_{50} = 43$ nM [15, 22]. With the elucidation of the structures of **18a** and **18b**, consideration was given to the concept that the NS5A-inhibiting pharmacophore represented by these dimers may be the embedded symmetrical bibenzyl element. This notion was based on an appreciation of the precise SARs surrounding the amino acid moiety in contrast to the relatively more nebulous effects associated with structural variation to the iminothiazolidinone substituents. Under this concept, the iminothiazolidinone ring system of **2** was suggested to act as a scaffolding element by which a bivalent NS5A inhibitor pharmacophore was convened through a radical-mediated dimerization process [15, 22]. This hypothesis was readily tested in the context of the proline-based chemotype, with **19** demonstrating an EC_{50} value of 30 nM and confirming the pharmacophore proposal. More interestingly, the unsaturated synthetic precursor **20**

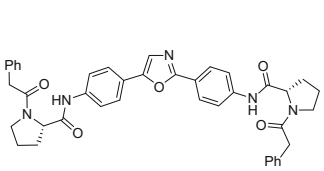
was almost 350-fold more potent than **19**, $EC_{50} = 0.086$ nM, providing further insight into the topography of the NS5A-inhibiting pharmacophore.

2 Optimization of Dimeric NS5A Replication Complex Inhibitors to Daclatasvir

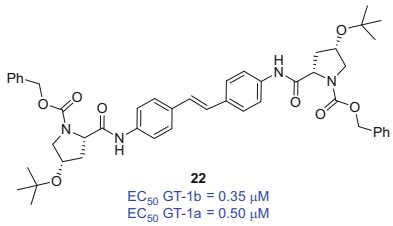
The discovery of the structurally simpler symmetrical pharmacophore represented by **19** and **20** turned out to be prescient since X-ray crystallographic structure data for NS5A constructs that were published several years after this discovery revealed a dimeric, C2-symmetric protein complex [26–28]. Of note, this structure was determined using a protein construct lacking the membrane-associating amino terminus but, nevertheless, containing some of the key elements of the anticipated binding site for NS5A inhibitors based on the location of resistance mutations. The availability of a structure of the amino terminus of NS5A, acquired by NMR methodology, facilitated the construction of models of NS5A that, when combined with the resistance mutation data, suggested that these compounds bound across the NS5A dimer interface at a site residing between the membrane and the core of the protein. Dimerization of the NS5A protein in cells was subsequently confirmed as was association of NS5A with RNA, a prediction from the X-ray data based on the preponderance of basic amino acids lining the inner surface of the U-shaped dimeric form of NS5A in the solid state [29–32].

While the antiviral activity of **20** was attractive, there were concerns around elements embedded within the structure, with the olefin observed to be configurationally unstable in some analogues, while the potential for release of one or both aniline moieties after the action of an amidase or protease in vivo raised the specter of toxicity. However, a considerably more challenging problem arose when **20** was evaluated in a newly developed GT-1a replicon where the compound was found to be poorly active, with an EC_{50} value that was in excess of 10 μM. The GT-1a strain of HCV is clinically relevant, with prevalence that varies across the globe; consequently, securing activity toward this genotype was considered to be a critical objective. Enhancing GT-1a inhibitory activity became the immediate focus of further study, and the approach adopted, while primarily directed toward the peripheral elements, was broad-based in prosecution. Modifications to all facets of the molecule were explored, some of which were also directed toward simultaneously addressing the structural liabilities highlighted above. However, as described below, introducing and retaining GT-1a coverage while optimizing ADME properties would end up posing a considerable challenge. While deeper insight into SARs for GT-1b inhibition emerged from the initial phases of this effort, only a small number of compounds were identified that exhibited modest but reproducible GT-1a inhibition. Among these were the oxazole **21** and the substituted proline derivative **22**, both of which were inhibitory in the GT-1a replicon with EC_{50} values of less than 1 μM [33]. However, attempts to optimize these molecules were unproductive, in each case leading to SAR cul-de-sacs. As the studies progressed, the 2-ethyl-substituted benzamide derivative **23** and its unsaturated homologue **24** were discovered to

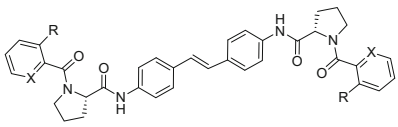
exhibit weak inhibition of GT-1a and GT-1b replicons, with modeling studies suggesting that the effect of substitution was not a function of modulating the conformation between the phenyl ring and proline carbonyl [34]. The pyridine derivative **25** further advanced the SAR associated with this cap element but, more importantly, formed the basis of the discovery of the isoquinoline derivative **26**, in which the ethyl substituent was incorporated into a fused ring, as the first compound to exhibit potent and balanced antiviral activity toward GT-1a and GT-1b replicons [34]. The promise of this compound was further underscored when it was screened in replicons or hybrid replicons representing genotypes 2a, 3a, and 5a where the EC_{50} values ranged from 2.2 to 14 nM.



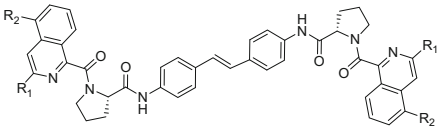
21
EC_{50} GT-1b = 0.002 μM
EC_{50} GT-1a = 0.345 μM



22
EC_{50} GT-1b = 0.35 μM
EC_{50} GT-1a = 0.50 μM

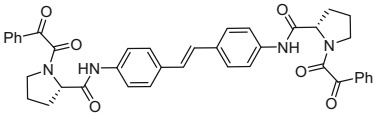


23
R = CH_2CH_3; X= CH
EC_{50} GT-1b = 1.13 μM
EC_{50} GT-1a = ~1.00 μM
24
R = $CH{=}CH_2$; X= CH
EC_{50} GT-1b = 1.2 μM
EC_{50} GT-1a = 1.1 μM
25
R = CH_2CH_3; X= N
EC_{50} GT-1b = 0.046 μM
EC_{50} GT-1a = 0.72 μM

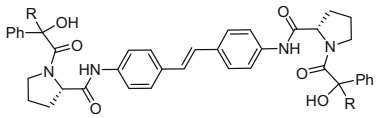


26
R_1 = Cl, R_2 = OMe,
EC_{50} GT-1b = 7 nM
EC_{50} GT-1a = 19 nM
27
R_1 = R_2 = H,
EC_{50} GT-1b = 22 nM
EC_{50} GT-1a = 390 nM
28
R_1 = OCH_3; R_2 = H,
EC_{50} GT-1b = 32 nM
EC_{50} GT-1a = >10,000 nM
29
R_1 = Cl; R_2 = H,
EC_{50} GT-1b = 240 nM
EC_{50} GT-1a = >10,000 nM

Further examination of the SARs revealed that GT-1a inhibitory activity was much more sensitive to the nature and the substitution pattern of the isoquinoline ring than GT-1b [34]. For example, **27**, the parental analogue of **26**, exhibited GT-1a and GT-1b EC_{50} values that were 20- and 3-fold weaker than that of **26**, respectively. In addition, the methoxy-substituted derivative **28** and its chloro-substituted analogue **29** retained potent GT-1b inhibition, but their GT-1a EC_{50} values were >10 μM. However, a more fruitful avenue of investigation was found when the effects of deannulating the isoquinoline ring were probed [35]. The α-keto amide **30** preserved the GT-1a inhibition exhibited by **26**, while the derived secondary alcohols **31** and **32** demonstrated that planarity at this site was not a specific requirement. The tertiary alcohol homologues **33** and **34** added further to the SARs, with the (*S*,*S*)-analogue **34** the more potent isomer, particularly toward the GT-1b replicon where the EC_{50} value was 6 pM. Another encouraging observation was made with **35** which, although poorly active in the GT-1a replicon, demonstrated 64% bioavailability following oral dosing to rats, indicating that securing systemic exposure after oral delivery of these symmetrical stilbene derivatives was feasible.

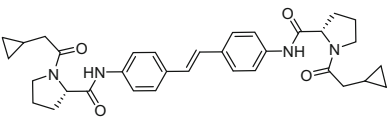


30
EC_{50} GT-1b = 8 nM
EC_{50} GT-1a = 447 nM

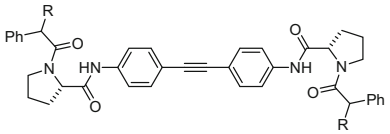


31: R = H, (*R*,*R*)
EC_{50} GT-1b = 0.022 nM
EC_{50} GT-1a = 640 nM
32: R = H, (*S*,*S*)
EC_{50} GT-1b = ~4.2 nM
EC_{50} GT-1a = 1020 nM

33: R = CH_3, (*R*,*R*)
EC_{50} GT-1b = <4.6 nM
EC_{50} GT-1a = 6850 nM
34: R = CH_3, (*S*,*S*)
EC_{50} GT-1b = 0.006 nM
EC_{50} GT-1a = 84 nM



35
EC_{50} GT-1b = 62 nM
F in rats = 64%



36: R = (*R*,*R*)-$N(CH_3)_2$
EC_{50} GT-1b = <4.6 nM
EC_{50} GT-1a = 8.8 nM
37: R = (*S*,*S*)-$N(CH_3)_2$
EC_{50} GT-1b = <4.6 nM
EC_{50} GT-1a = 628 nM

38: R = (*R*,*R*)-$NHCO_2CH_2$
EC_{50} GT-1b = 0.013 nM
EC_{50} GT-1a = 26 nM

Replacing the stilbene element with an alkyne, which resolved a *cis-trans* isomerization issue observed with some analogues, added further to the understanding of the topography of the pharmacophore. Additional probing of the amino acid terminal region using this alkyne-based scaffold identified potent arylglycine-based analogues for which the GT-1a EC_{50} values for some compounds, including **36**–**38**, were single digit nM [35]. Notably, the change in stereochemical preference in evolving the chemotype from the mandelamide analogues **33** and **34** to the phenylglycine analogues **36** and **37** further highlighted the relatively intricacy and sensitivity of the GT-1a inhibition SARs that were being uncovered during this phase of the project. Equally intriguing was the accumulating evidence indicating that the GT-1b virus was highly tolerant of many structural changes, an observation that could not readily be explained based on the differences in amino acid composition at the putative binding site of the compounds.

A concurrent effort examined scaffold modifications directed toward the identification of a less problematic replacement for the anilide moiety that would decrease or eliminate the potential for aniline release in vivo which led to the emergence of two noteworthy SAR findings. Firstly, the replacement of the anilide moieties of **36** and **38** with a benzimidazole, a design intended to preserve both the H-bond donor and acceptor properties of the anilide, resulted in a 4- to 30-fold reduction in potency toward the GT-1a replicon, as exemplified by **39** and **40** [36]. Secondly, a mix and match SAR exercise accentuated the sensitivity of GT-1a inhibitory potency to topological parameters, exemplified by the 70-fold difference in GT-1a inhibitory potency between regioisomers **41** and **42**.

These SAR findings were attributed to the altered topology of the peripheral pharmacophoric elements with respect to the core, a shortcoming that was ultimately addressed by the biphenyl derivatives **43** and **44** which recapitulate the linearity

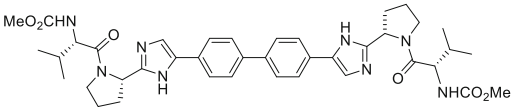
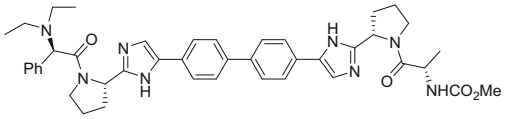
associated with the core alkynes in **36**–**40**. In **43** and **44**, a motif that was arrived at only after considerable experimentation, deannelation of the benzimidazole ring provided a structural arrangement that compensated for the reduced length of the core of the pharmacophore relative to **39** and **40**. The success of this design strategy is readily apparent since both **43** and **44** are exquisitely potent HCV antiviral agents with balanced GT-1a and GT-1b inhibition, with EC_{50} values ranging from 7 to 42 pM [4]. However, the oral bioavailability and systemic exposure of both **43** and **44** in the rat were poor, a result attributed, in part, to the high molecular weight (747 and 807 Da, respectively) and structural composition. This notion was reinforced by PK studies with the smaller (MW = 713) and unsymmetrical tetrahydrofuran **45** which divests of a H-bond donor. Although the oral bioavailability of **45** was low in rodents (F in mouse = 17%, F in rat = 6.8%), its exposure in the dog was much improved, with bioavailability measured at 45%. In an effort to reduce the molecular weight of the carbamate **44**, the two aromatic rings of the phenyl glycine moiety were curtailed to isopropyl substituents affording the D-valine derivative **46**. However, this structural modification resulted in a significant reduction in potency toward both HCV genotypes, with the GT-1a inhibition particularly sensitive, eroding by 44,000-fold. This SAR observation took some time to understand and was resolved only after further study of the chemotype, which revealed that the preferred absolute configuration of the alkyl-glycine caps was the opposite of that of the aryl-glycine caps. The initial observation in this direction was made when the tetrahydrofuran ring of **45** was replaced with L-alanine to provide **47**, which restored potency to sub-nanomolar levels. In an observation that proved to be pivotal, the symmetrical alanine derivative **48** performed similarly, and further optimization of the amino acid appendage led to the discovery of the *bis*-L-valine derivative **1**, an exercise that also included assessing the potential of unsymmetrical derivatives. The decision to advance **1** into IND-enabling toxicology evaluation was taken after a careful comparison with **49**, an analogue with two changes to the periphery that demonstrated similar antiviral properties to **1** (Table 2) but a different PK profile (Table 3). The decision to select **1** for development rather **49** was based on the observation of a twofold accumulation of the latter compound in the plasma, livers, and hearts of mice after 4 days of daily drug administration which occurred at all of the dose levels (15, 50, and 100 mg/kg) examined.

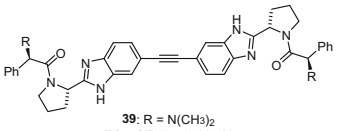
Table 2 Inhibition of replicons and hybrid replicons by **1** and **49**

Replicon genotype[a]	EC_{50} value for **1** (nM)	EC_{50} value for **49** (nM)
GT-1a H77	0.050	0.036
GT-1b Con1	0.009	0.012
GT-2a JFH-1	0.071	0.020
GT-3a	0.146	0.008
GT-4a	0.012	0.014
GT-5a	0.033	0.021
GT-6	0.054	ND

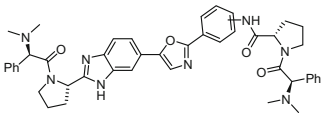
[a]With the exception of GT-2a JFH-1, all data are from hybrid replicons in either a GT-2a JFH-1 or GT-1b Con1 backbone: GT-3a (1–100 NS5A amino acid in Con1); GT-4a (full-length NS5A in Con1); GT-5a (1–110 NS5A amino acid in JFH-1); GT-6a (full-length NS5A in JFH-1)

Table 3 Pharmacokinetic profile of **1** and **49** in preclinical species

Species	Dose (mg/kg)	Plasma AUC (μM h)	Plasma concentration at 24 h (nM)	Oral bioavailability
1				
				
Rat	5.0	4.8	18	50%
Dog	2.3	11	26	108%
Cynomolgus monkey	2.8	1.93	6.5	38%
49				
				
Rat	5.0	0.17	Below detection	3.6%
Dog	3.5	1.2	9.0	66%
Cynomolgus monkey	3.0	0.5	4.2	21%

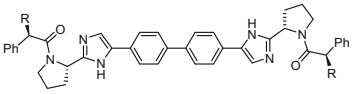


39: R = $N(CH_3)_2$
EC_{50} GT-1b = 0.09 nM
EC_{50} GT-1a = 260 nM
40: R = $NHCO_2CH_3$
EC_{50} GT-1b = 0.055 nM
EC_{50} GT-1a = 108 nM

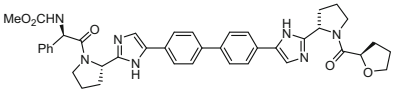


41 (para)
EC_{50} GT-1b = 0.077 nM
EC_{50} GT-1a = 251 nM

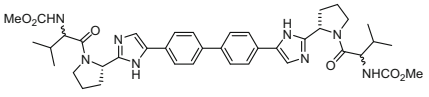
42 (meta)
EC_{50} GT-1b = 0.14 nM
EC_{50} GT-1a = 3.5 nM



43: R = $N(CH_3)_2$
EC_{50} GT-1b = 0.042 nM
EC_{50} GT-1a = 0.038 nM
44: R = $NHCO_2CH_3$
EC_{50} GT-1b = 0.007 nM
EC_{50} GT-1a = 0.028 nM

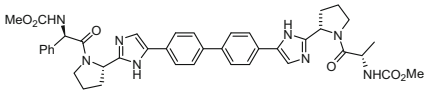


45
EC_{50} GT-1b = 0.015 nM
EC_{50} GT-1a = 138/ nM
F: rat (6.8%), mouse (17%), dog (45%)

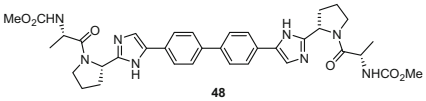


46 (R,R)
EC_{50} GT-1b = 1.22 nM
EC_{50} GT-1a = 1240 nM

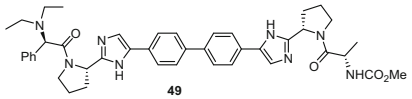
1 (S,S)
EC_{50} GT-1b = 0.009 nM
EC_{50} GT-1a = 0.050 nM



47
EC_{50} GT-1b = 0.013 nM
EC_{50} GT-1a = 0.112 nM



48
EC_{50} GT-1b = 0.090 nM
EC_{50} GT-1a = 0.282 nM



49
EC_{50} GT-1b = 0.012 nM
EC_{50} GT-1a = 0.036 nM

The antiviral profiles of **1** and **49** toward wild-type and hybrid replicons representing all of the genotypes and subtypes that were available at the time are summarized in Table 2 [1–4, 37–40]. Adding to confidence in the potential of **1** as it negotiated the development path toward clinical evaluation was the potent inhibition observed in a newly established GT-2a JFH-replicating virus assay. The EC_{50} value of 28 pM in this assay exhibited a good correlation with the inhibitory potency toward the GT-2a JFH replicon [1–4].

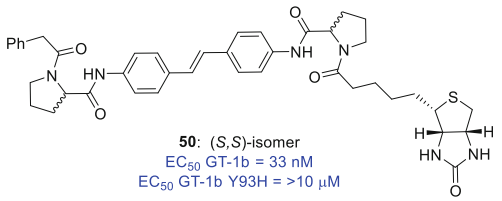
The pharmacokinetic parameters of **1** and **49** in rat, dog, and cynomolgus monkey are compiled in Table 3 and demonstrate good systemic exposure following oral administration, with the drug concentration measured at 24 h post-dose well in excess of the EC_{50} values for the GT-1a and GT-1b replicons and the protein-binding-adjusted EC_{90} value of 383 pM determined for the GT-1a replicon. More importantly, target organ exposure was also demonstrated, with liver levels of 103 nM measured in the rat 24 h following a 5 mg/kg dose of **1**, a concentration that was fivefold higher than that in plasma. The favorable absorption properties of **1** have been attributed, in part, to the formation of an intramolecular H-bond between the carbamate C=O and imidazole N-H moieties that enhances lipophilicity and reduces the apparent PSA of the molecule based on a chromatographic analysis and which is supported by modeling studies [41].

3 Mode of Action Studies with Daclatasvir

Despite its high potency and broad genotype inhibitory activity, the precise mode of inhibition of HCV replication by **1** remains as enigmatic as does the biochemical function of the NS5A protein [16, 18, 42–48]. HCV NS5A has no known enzymatic activity but is a critical element in the assembly and function of the replication complex on intracellular membranes and also in virion assembly [16, 42–48]. HCV NS5A is a 447-residue phosphoprotein that is comprised of three functional domains and an amphipathic helix at the amino terminus that associates with but does not traverse to biological membranes. Domain 1 contains a Zn^{2+} binding motif and several serine residues that are sites of basal phosphorylation and hyperphosphorylation. The phosphorylation state of NS5A may modulate its function in virus replication and assembly with the hyperphosphorylated form, which can be produced by the action of the host cell lipid kinase, phosphatidylinositol 4-kinase, involved in the assembly of virions. Domain 2 has been shown to bind to the NS5B RNA-dependent RNA polymerase and has been associated with the sensitivity of the virus to interferon therapy although that function is controversial. While domain 3 appears to play a role in virus replication, it has most prominently been associated with virion assembly. The mapping of resistance mutations arising in response to selective pressure exerted by **1** and related analogues to domain 1 of NS5A is consistent with the effect on virus replication, but studies with infectious virus have demonstrated that **1** also interferes with the assembly of virions [42–50]. The

latter effect has been postulated to explain the rapid decline in viremia observed in HCV-infected patients administered clinically effective doses of **1** (vide infra) [3, 51]. In addition to associating with all of the viral nonstructural proteins, HCV NS5A has also been shown to bind to an extensive repertoire of host cell proteins that exceeds 130 entities [52–57]. As a consequence, the NS5A protein is typically viewed as a master regulator of virus replication and virion production, orchestrating both viral proteins and the host cell environment to ensure the successful production and release of progeny virus [42–48].

An association of NS5A inhibitors with the viral protein was originally demonstrated by studies with the biotin-labeled derivative **50** which is an effective inhibitor of GT-1b replication [3]. The antiviral activity of **50** is highly sensitive to the absolute configuration of the proline moiety since the (*R*,*R*)-isomer **51** is inactive, while inhibition is substantially reduced by the Tyr93His resistance mutation that arises in response to virus passaging in the presence of **1**. This SAR profile is strictly analogous to that established for the stilbene chemotype, and **50** was thus viewed as a useful tool molecule with which to probe drug-target binding interactions. In an initial experiment, GT-1b replicon lysate was incubated with **50,** and the mixture passed over streptavidin immobilized on beads; however, this experimental protocol failed to pull down any viral protein. In contrast, incubating replicon cells with **50** for 18 h before lysing and passing the lysate over streptavidin beads identified only NS5A as a binding partner, while a control experiment with inactive diastereomer **50** failed to isolate any virial proteins. These results indicate that **50** binds to HCV NS5A and that binding is dependent on the absolute configuration of the proline element, an observation concordant with the SARs developed in the stilbene-based series.

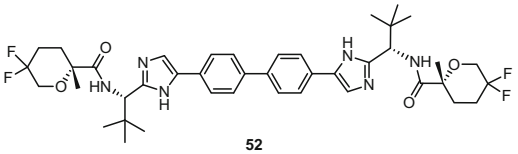


50: (*S*,*S*)-isomer
EC_{50} GT-1b = 33 nM
EC_{50} GT-1b Y93H = >10 μM

51: (*R*,*R*)-isomer
EC_{50} GT-1b = >10 μM
EC_{50} GT-1b Y93H = >10 μM

While the experiments conducted with **50** indicate that the binding of inhibitors to the NS5A is choreographically somewhat complex, the binding of inhibitors of NS5A to both domain 1 and the full-length viral protein was subsequently demonstrated in a series of independent biochemical experiments [58, 59]. These studies have suggested that the binding of inhibitors to NS5A interferes with the association of viral RNA with the protein, with the binding of compounds competed out by other NS5A inhibitors and demonstrating diminished affinity for the Tyr93His mutant protein [58, 59]. However, profiling of inhibitors in cell-based assays has indicated that disruption of RNA binding to NS5A does not appear to occur and that the introduction of key resistant mutations leads to only a modest reduction in the

binding of inhibitors [60, 61]. Studies with **50** in resistant GT-1b replicons indicated that while the Tyr93His-resistant mutation reduced inhibitory potency by 220-fold, an estimate of the amount of NS5A protein pulled down by the chemical probe, as determined by an analysis of Western blots, suggested similar levels of protein-drug association for the resistant and wild-type strains [60, 61]. Adding further to the complexity of the biochemistry was the observation that in a protein pull-down experiment, an attempt to outcompete the biotinylated tool compound **50** with a non-biotinylated analogue in a GT-1b Tyr93His-containing mutant ($EC_{50} = 290$ nM for stated analogue vs >7 μM for **50**) not only failed but, at low concentration, appeared to have incrementally enhanced the amount of NS5A pulled down. From these data, it was inferred that the development of resistance to **1** does not lead to exclusion of binding to the NS5A protein, as is often observed with other classes of antiviral agents. Rather, these observations suggested a scenario in which HCV NS5A develops resistance by accommodating rather than expelling inhibitors, with the mutations presumably allowing restoration of protein function in the presence of the bound inhibitor. Consistent with this suggestion, several of the resistant mutations incorporate smaller or more flexible amino acid side chains, exemplified by Tyr93His, Tyr93Cys, Leu31Met, and Leu31Val, which may restore conformational flexibility compromised by the binding of inhibitors. These observations stimulated an experiment designed to evaluate the effect of combining **1** with structurally related compounds on the function of HCV NS5A incorporating resistance mutations. Two possible outcomes were contemplated: in the first scenario, a molecule would simply compete with bound **1** and the observed effect would be one of silence. However, the alternative scenario speculated on the potential of a second molecule to act in conjunction with **1** to restore inhibition by binding to an adjacent site on the NS5A molecule. A screen of compounds selected from the library of HCV NS5A inhibitors assessed in the presence of **1** using the Tyr93Asn GT-1a-resistant replicon, followed by SAR optimization, identified Syn-395 (**52**) as a molecule capable of restoring the sensitivity of resistant virus to the inhibitory effects of **1**. For example, in a typical experiment, **1** exhibited EC_{50} values of 33 pM and 339 nM toward the wild type and Tyr93Asn mutant replicons, respectively, whereas **52** was poorly active in both replicons, with EC_{50} values of 214 and 215 nM, respectively. However, the EC_{50} of **1** toward the Tyr93Asn mutant replicon improved to 0.13 nM when titrated in the presence of a suboptimal concentration (40 nM) of **52**. This result represented a 2,600-fold increase in the sensitivity of the Tyr93Asn replicon to **1** in the presence of **52**. The synergistic relationship between **1** and **52** was confirmed in a reciprocal experiment where **52** was titrated in the presence of suboptimal amount of **1** affording a similar outcome [60, 61].



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These observations, taken together with the absence of structural data that holistically captures the HCV NS5A drug-binding sites in the context of the membrane-

bound replication complex, confer considerable complexity on the nature of drug-target interactions, the mode of inhibition, and the function of the NS5A protein. This has presented a significant challenge to developing a more coherent and detailed understanding of the mechanism of action of HCV NS5A inhibitors and the modeling of drug-target interactions of these potent antiviral agents and the allosteric modulators [60–67]. While the bivalent nature of NS5A inhibitors, including the allosteric modulators represented by **52**, complements the dimeric form of the protein observed in solid-state structures of elements of domain I, the binding interfaces between the proteins vary [26–28, 60, 61]. One interpretation of this observation anticipates an oligomeric form of HCV NS5A in cells that can bind to the viral RNA and protect it from chemical and enzymatic degradation while providing a platform for RNA presentation to the polymerase and translocation to the developing virion [68–70]. However, the biochemical pharmacological effects of NS5A inhibitors are multifaceted and complex and include altering the subcellular distribution of NS5A, modulating the phosphorylation state of the protein, interfering with the formation of the membranous factories where virus replication occurs, and blocking the transfer of the viral genome to assembly proteins, leading to a clustering of HCV proteins at endoplasmic reticulum membranes [71–76].

4 Clinical Trials with Daclatasvir

The phase I clinical trial with **1** comprised of a randomized, double-blind, placebo-controlled, single ascending dose study involving administration of 1, 10, 25, 50, 100, and 200 mg of the drug to normal healthy volunteers (NHVs) [3]. A dose-proportional increase in plasma exposure was observed over the dosing range, and the concentration of **1** in plasma 24 h after dosing exceeded the protein-binding-adjusted EC_{90} values of 49 pM (0.04 ng/mL) and 383 pM (0.28 ng/mL) recorded for the GT-1b and GT-1a replicons, respectively [3]. Compound **1** was quickly absorbed and exposure extended beyond 24 h, with plasma drug concentration maintained above the less sensitive GT-1a protein-binding-adjusted EC_{90} value of 383 pM at 72 h for all but the 1 mg dose, predicting the potential for once-daily dosing [51]. The absolute oral bioavailability of **1** in humans is 67%, and the compound is ~99% bound to plasma proteins [77–80]. In this trial, **1** was safe and well tolerated at all of the administered doses with no clinically significant adverse effects observed, a profile that set the stage for a proof-of-concept study in HCV GT-1-infected subjects. Doses of 1, 10, and 100 mg were administered in a randomized, double-blind, placebo-controlled, single ascending dose format similar to that used for the NHV study, and plasma HCV RNA levels were monitored until 172 h post-dose. The results of this study are compiled in Table 4 with mean plasma HCV RNA declining by 1.8 $\log_{10}$ IU/mL 24 h following the 1 mg dose, while the 10 and 100 mg doses provided increased efficacy, with viral load declines of 3.2 and 3.3 $\log_{10}$ IU/mL, respectively, measured at 24 h. The mean maximal viral load reduction in the 100 mg dose cohort was 3.6 $\log_{10}$ IU/mL with HCV RNA measured at 35 IU/mL in one of the GT-1b-infected subjects, while plasma RNA in another was below the

Table 4 Dose, HCV genotype distribution, and plasma HCV RNA levels following administration of **1** to HCV-infected subjects

Dose of **1**	1 mg	10 mg	100 mg
GT-1a/1b	6/0	3/2	2/3[a]
Mean viral load reduction at 24 h (range)	1.8 $\log_{10}$ (0.2–3.0 $\log_{10}$) IU/mL	3.2 $\log_{10}$ (2.9–4.0 $\log_{10}$) IU/mL	3.3 $\log_{10}$ (2.7–3.6 $\log_{10}$) IU/mL
Mean maximal reduction in viral load			3.6 $\log_{10}$ (3.0–4.1 $\log_{10}$) IU/mL

[a]One subject withdrew 8 h after dosing of **1**

lower limit of quantification (25 IU/mL) at 144 h post-dose. The decline in plasma viral RNA concentration following administration of the 10 and 100 mg doses of **1** was both rapid and profound in nature, with a mean reduction of 1.95 $\log_{10}$ IU/mL measured at 6 h post-dose for nine of the patients [49, 51]. The steep decline in viral load was subsequently explained after the development of a multiscale model of viral kinetics that took into account the effects of **1** on both viral replication and virus assembly and secretion, with the latter being the source of an immediate effect on virion production. The mean effectiveness of **1** on virus RNA production and virion assembly/secretion was estimated to be 99 and 99.8%, respectively, and yielded an estimate of plasma HCV half-life of 45 min, significantly shorter than the 2.7 h half-life estimated from an analysis of viral kinetics during treatment with older, interferon-based therapies [49].

The profile of **1** was further explored in a double-blind, placebo-controlled multiple ascending dose study in which the drug was administered for 14 consecutive days to GT-1-infected subjects at doses of 1, 10, 30, 60, and 100 mg once daily and 30 mg twice daily [51]. Each panel comprised of five patients randomized such that four received drug and one was administered a placebo control, with drug PK parameters determined on days 1 and 14. Median peak plasma concentrations of **1** occurred 1–2 h after dosing, and the PK profile was supportive of once-daily dosing with a mean terminal half-life of 12–15 h and steady state achieved after 3–4 days of drug administration. The mean maximal reduction in HCV RNA levels in plasma are compiled in Fig. 3 with 30 and 60 mg QD cohorts comprised solely of GT-1a-infected subjects. In the other dosing cohorts, those infected with GT-1b virus exhibited a greater response compared to those infected with GT-1a virus. However, most patients experienced viral rebound on or before day 7 of therapy with viral RNA levels below the mean maximal decline except in the 30 mg BID cohort (Fig. 4). Rebound was typically more rapid in the GT-1a-infected subjects which can be explained by a lower genetic barrier to resistance in this subtype [81–83]. In HCV GT-1a, only a single base pair change in the viral RNA is typically required to code for an alternative amino acid, while GT-1b frequently requires two base pair changes for coherent coding [81–83]. Population sequencing indicated the appearance of mutations at Met28, Gln30, Leu31, and Tyr93 all of which had been identified as resistance mutation hotspots in response to selective pressure by **1** in replicon studies in vitro [84, 85].

While the results of this trial further confirmed the potential of HCV NS5A as a therapeutic target, the rapid emergence of resistance to **1** anticipated that optimal

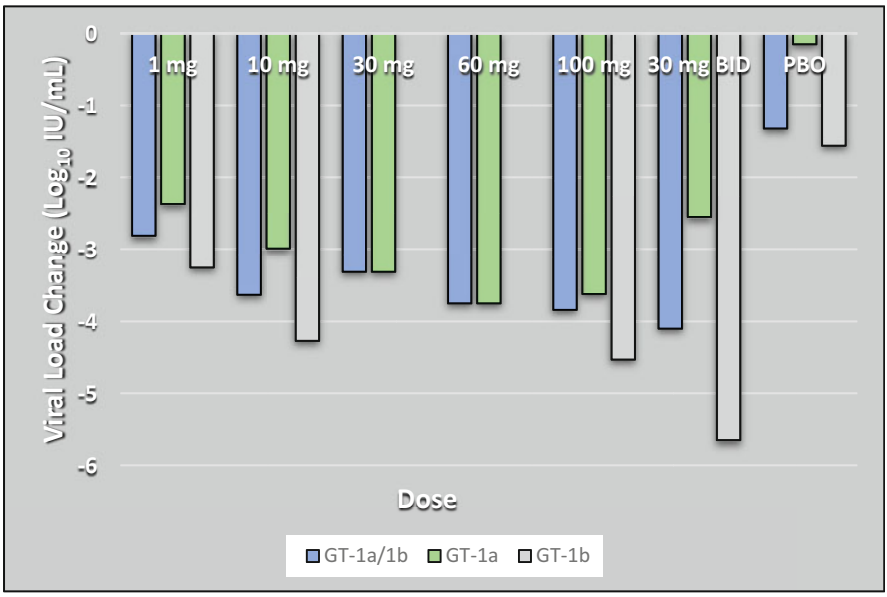


Fig. 3 Mean maximum decrease in plasma viral RNA in HCV GT-1-infected subjects following dosing of **1** for 14 days

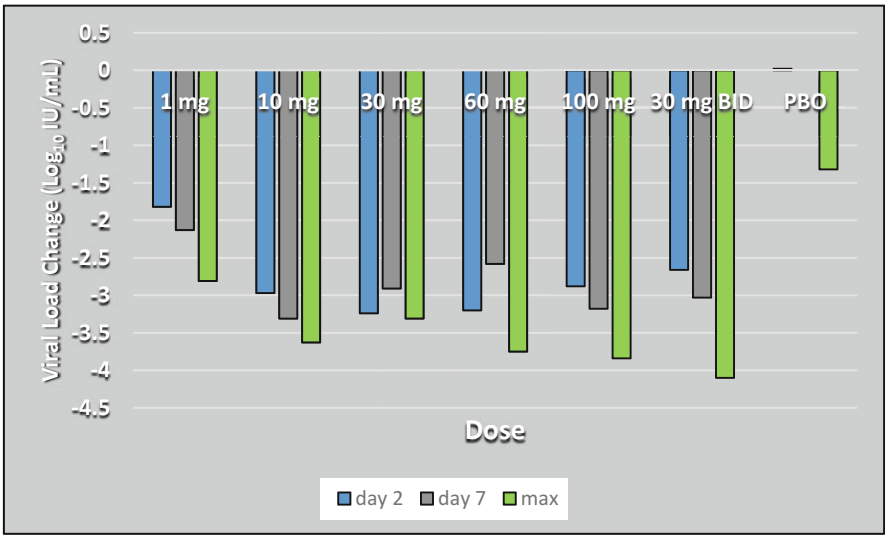
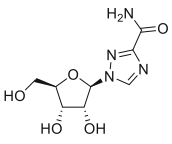
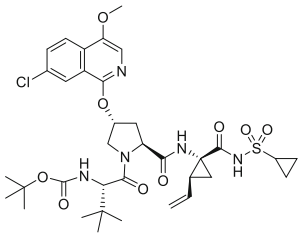


Fig. 4 Mean viral load reductions on days 2 and 7 compared with the maximal viral RNA decline following dosing of **1**

clinical application would be as part of combination therapy [79, 81–91]. The combination of **1** as add-on therapy to the extant standard of care, pegylated IFNα and ribavirin (**53**), was explored clinically in patients infected with HCV genotypes 1–4 and a subset of patients who were co-infected with HIV-1. The results indicated that sustained virologic responses could be achieved with shorter 24-week durations of therapy with a burden of side effects comparable to that of pegylated IFNα and **53** [92–96]. However, it was the opportunity availed by the contemporaneous development of the HCV NS3 protease inhibitor asunaprevir (**54**) that allowed the pursuit of a parallel clinical program that had a significant impact on the course of the clinical development of HCV therapeutic agents [97–102]. In a small open-label clinical trial conducted in HCV GT-1-infected subjects who had no evidence of cirrhosis and who had previously failed to respond to peg-IFN/**53** therapy (referred to as null responders), a combination of **1** (60 mg QD PO) and **54** (600 mg BID PO) with peg-interferon α2a (180 μg per week subcutaneously) and **53** (1,000 or 1,200 mg QD PO, depending on body weight) administered for 24 weeks was compared with a regimen comprised of only the two direct-acting antiviral agents (DAAs) [99, 102]. All of the ten patients receiving the quadruple drug regimen had undetectable levels of HCV RNA in plasma measured at 12 weeks following the last dose (SVR_{12}), while nine also achieved SVR_{24}. One patient in this group had detectable HCV RNA in plasma at week 24, but this was not quantifiable, and viral RNA was not detected in plasma 5 weeks later [99, 102]. Of the 11 patients receiving only the dual DAA combination, five had undetectable levels of HCV RNA in plasma at the end of therapy, and four maintained this status at weeks 4, 12, and 24 after the last drug dose. This cohort was comprised of nine subjects infected with GT-1a and two infected with GT-1b, with both GT-1b-infected patients achieving SVR_{24}, while six patients infected with GT-1a virus experienced virological breakthrough while on therapy and the remaining patient infected with GT-1a virus relapsed after completing drug therapy. This study, which was conducted in a very challenging patient population, provided the first indication that a chronic HCV infection could be cured solely by treatment with DAAs in the absence of the immune stimulation provided by peg-interferon α2a and **53** [100].



53 (ribavirin)



54 (asunaprevir)

The successful treatment of HCV GT-1b infections with **1** and **54** redirected the clinical development plan for this dual combination to Japan where GT-1b is the

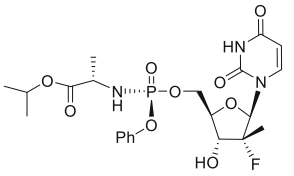
most prevalent, accounting for approximately 70% of the estimated two million infections at the time [103, 104]. The combination of **1** and **54** has been studied extensively in GT-1b-infected Japanese patients, leading to approval of the drug combination by the Japanese Pharmaceutical and Medical Devices Agency (PMDA) on July 4, 2014 [105–119]. The marketing authorization of **1** as Daklinza™ and **54** as Sunvepra® in Japan represented the first approval of a combination of DAAs for the treatment of HCV infection although the combination of sofosbuvir (**55**) and **53** had been approved by the FDA in December of 2013 [107]. The phase III Japanese clinical trials of **1** (60 mg QD) and **54** (100 mg BID) in GT-1b-infected subjects that subtended marketing approval evaluated 24 weeks of therapy and were associated with SVR_{12} rates of 81% in non-responders and 87% in those intolerant of or ineligible for pegylated interferon therapy. In a multinational study conducted in a broad GT-1b patient population, the SVR_{12} rates were 90% in those naïve to therapy and 82% in those intolerant of or ineligible for interferon-based therapies. The pre-existence of the Tyr93His polymorphism in the HCV NS5A gene was a predictor of lower clinical efficacy, with the SVR rates declining to 45 from 95% in those patients that harbored this mutation at baseline, which has a 15% prevalence in the Japanese patient population.

Broadening the utility of **1** and **54** to treat HCV GT-1a infections required the addition of a third agent, the allosteric RNA-dependent RNA polymerase inhibitor beclabuvir (**56**) which was developed as a fixed-dose combination comprising of 30 mg of **1**, 200 mg of **54**, and 75 mg of **56** administered as a BID regimen for 12 weeks [120–126]. In the UNITY 1 international study which was conducted in 415 non-cirrhotic patients with HCV GT-1 infection, 91% of the patients achieved SVR_{12}. SVR_{12} rates of 92% were observed in treatment-naive patients and 89% in treatment-experienced patients, while virologic failure occurred in 8% of the patients. In the UNITY-2 phase III study conducted in the United States, Canada, France, and Australia in 202 GT-1-infected patients with compensated cirrhosis, the SVR_{12} rates were 93% for patients in the treatment-naive group and 87% for those in the treatment-experienced group. SVR_{12} rates were improved to 98% for patients in the treatment-naive group and 93% for those in the treatment-experienced group when **53** was included in the regimen. In a phase III trial (UNITY 3) conducted in 217 Japanese patients infected with GT-1 HCV, SVR_{12} rates of $\geq$95% were achieved in both treatment-naive ($n = 152$) and interferon-experienced ($n = 65$) cohorts after 12 weeks of therapy. The SVR_{12} rates were similar across the patient subgroups evaluated that included patients with cirrhosis and those aged $\geq$65 years. These studies contributed to the approval of the fixed-dose combination of **1**, **54,** and **56** for marketing in Japan on December 20, 2016.

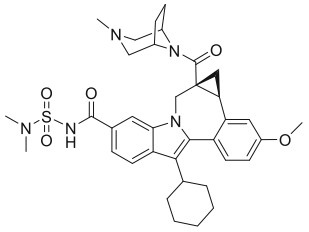
A number of clinical studies have demonstrated that co-dosing of **1** with the nucleoside-based NS5B RNA-dependent RNA polymerase inhibitor **55**, with and without **53**, achieves a high cure rate across HCV genotypes and patient population groups, including in those with comorbidities such as HIV-1 infection [127–129]. In a compassionate use program that reflected a real-world experience, including some subjects with advanced liver disease that would have been excluded from phase III studies, the combination of **1** and **55** (with and without **53**) demonstrated a high

efficacy [130]. In addition, long-term follow-up studies have demonstrated a 99% durability for the SVR_{12} associated with various regimens that include **1** [131].

Daclatasvir (**1**) has been approved in more than 60 countries for use in combination with **54**, **56,** or other HCV inhibitors, including **55** [132]. A combination of all four of these agents has been evaluated in GT-1-infected patients as a drug intensification approach to shortening the duration of therapy to 4 or 6 weeks [133]. However, while the majority (96%) of patients experienced undetectable levels of HCV RNA at the end of therapy, relapse occurred in 77% of those treated for 4 weeks and 43% of those subject to 6 weeks of treatment with quadruple therapy [133].



55 (sofosbuvir)



56 (beclabuvir)

5 Conclusion

The NS5A replication complex inhibitor class of HCV inhibitor has become established as a critical component of all of approved pan-genotypic DAA combination therapies [132]. The discovery of **1**, the prototype NS5A inhibitor that is the founding member of the class, was identified only after considerable optimization of a lead discovered by phenotypic screening, a powerful approach to drug discovery that has proven to be well-suited as a means of identifying mechanistically novel antiviral agents [134–138].

Compliance with Ethical Standards

Conflict of Interest The authors are employees of Bristol-Myers Squibb and own company stock.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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The Discovery of Ledipasvir (GS-5885): The Potent Once-Daily Oral HCV NS5A Inhibitor in the Single-Tablet Regimen Harvoni®

John O. Link

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Abstract The advent of direct-acting antiviral agents (DAAs) has revolutionized the treatment and cure of chronic hepatitis C virus (HCV) infection. Herein is described the discovery of ledipasvir (LDV), an orally available HCV nonstructural protein 5A inhibitor with picomolar antiviral potency and a long pharmacokinetic half-life. The combination of LDV with the nonstructural protein 5B inhibitor sofosbuvir (SOF) is Harvoni® and represents the first approved single-tablet regimen for the treatment of HCV infection. This safe simple and efficacious regimen affords clinical trial cure rates over 95% and comparable effectiveness in real-world studies and has treatment durations as short as 8 weeks. The approval of Harvoni® heralded a new era for the treatment of HCV infection.

Keywords Direct-acting antiviral, GS-5885, Harvoni®, HCV, LDV/SOF, Ledipasvir, NS5A inhibitor, NS5B nucleotide inhibitor, Single-tablet regimen

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1 Introduction

Prior to the direct-acting antiviral (DAA) era, the standard of care for HCV-infected individuals included toxic, poorly tolerated regimens based on ribavirin (RBV) and some form of interferon (IFN) and resulted in low SVR (cure) rates. Among the six major HCV genotypes, IFN-based regimens unfortunately resulted in the lowest cure rates for genotype 1 (GT1) patients, the most prevalent genotype worldwide (Fig. 1, US Veterans Administration) [1].

The complexity, poor response rates, and toxicity of IFN-based regimens are underrepresented by the SVR rates reported from clinical studies and the common description that these regimens engender "flu-like" symptoms. Real-world results present a more accurate picture of the patient's experience. The real-world efficacy of interferon regimens (cure rates outside of clinical trials) is vastly lower than even the ~30–50% SVR results reported from controlled clinical trials. Real-world efficacy late in the IFN era produced SVR as low as 3% in a report of 13,000 patients in 25 real-world studies across the United States (Fig. 2) [2]. This exceedingly low cure rate is a consequence of many factors, including a large number of exclusion criteria and significant toxicity and complexity of the regimen that precipitates patient discontinuation, hesitance to start therapy, and viral breakthrough and relapse [2].

Based on the poor results observed from the IFN-based standard of care, many patients deferred therapy in hope of newer treatment approaches while their liver

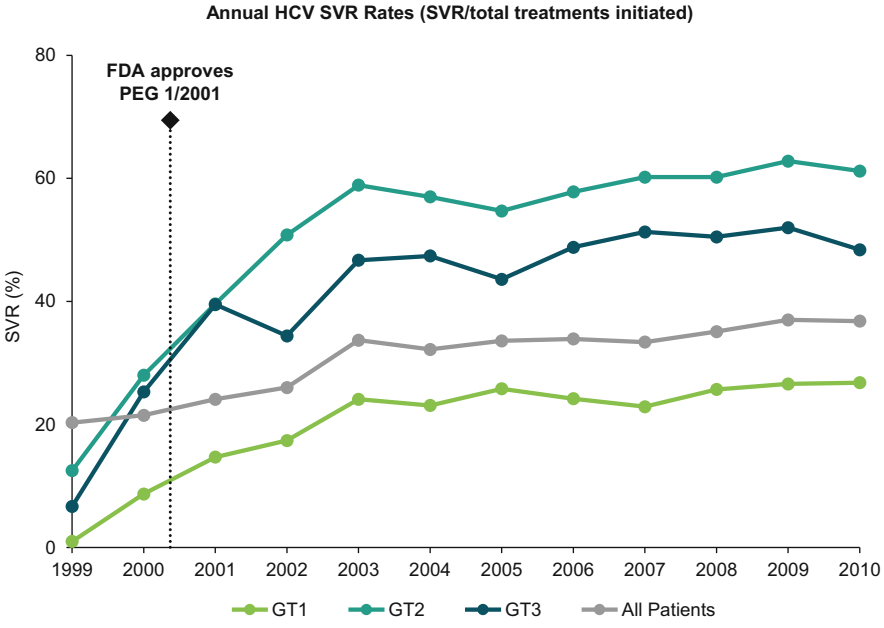


Fig. 1 SVR rates are the lowest for GT1 patients with IFN-based regimens

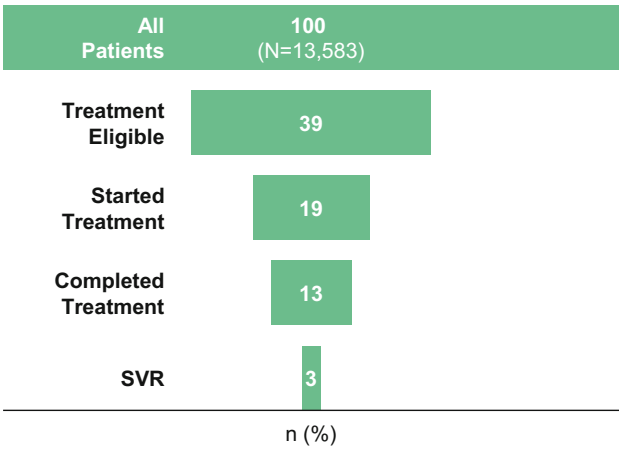


Fig. 2 Real-world IFN regimen results. 25 studies (2002–2012) show much lower SVR rates than those reported from clinical trials

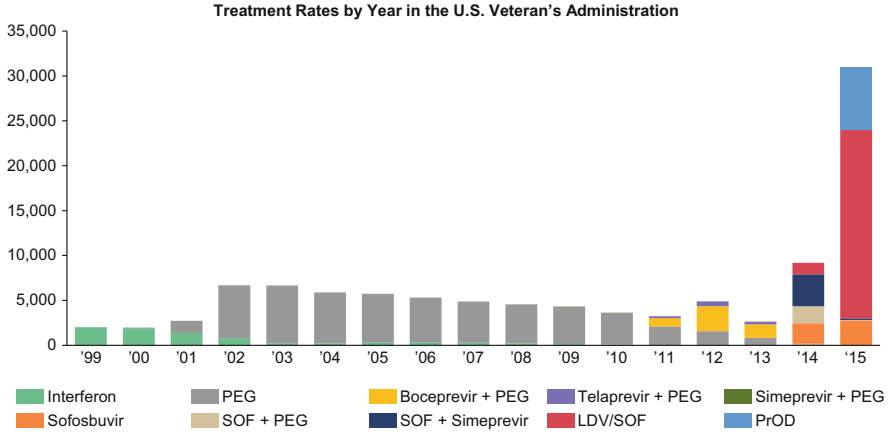


Fig. 3 Treatment rates increase sharply with the introduction of DAAs, particularly the STR LDV/SOF. *Interferon* beta-interferon, *PEG* pegylated interferon, *PrOD* paritaprevir, ritonavir, ombitasvir, dasabuvir

disease progressed to advanced stages of fibrosis or cirrhosis (Fig. 3) [1]. In 2013 as a consequence of the aging HCV-infected patient population progressing to liver cirrhosis, fibrosis, and hepatocellular carcinoma, the Center for Disease Control disclosed that deaths arising from HCV infection in the United States had surpassed those of all other notifiable infections combined in the United States (including human immunodeficiency virus [HIV], tuberculosis, and influenza) [3]. There was a critical need for improved therapies.

The advent of DAA therapies marked a revolution in the treatment and cure of HCV infection. The rapid uptake of DAA therapy underscores the high unmet need

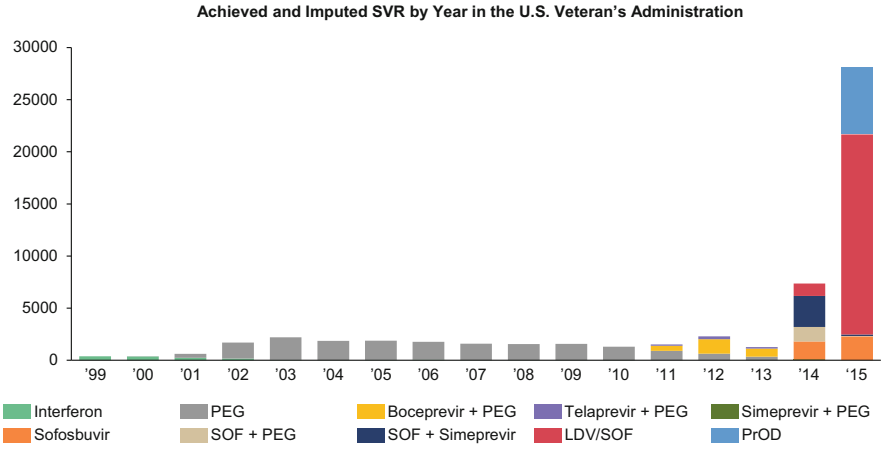


Fig. 4 The low SVR rates from the IFN era (compare to Fig. 3) are transformed during the DAA era. *Interferon* beta-interferon, *PEG* pegylated interferon, *PrOD* paritaprevir, ritonavir, ombitasvir, dasabuvir

that existed in HCV therapy, with telaprevir (2011) and then sofosbuvir (2013)[1,2] [4] and finally the combination drug ledipasvir/sofosbuvir (2014)[3] [5] each becoming the largest drug launches in history [6–9]. The benefit to patients in this progression of DAAs can be seen in Figs. 3 and 4 which show treatment rates and cure rates in the US Veterans Administration progressing from the IFN era to the approval and uptake of LDV/SOF [1].

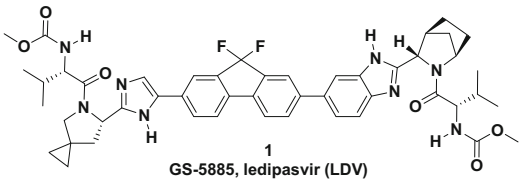
With the high level of unmet need for HCV patients in the IFN era as a backdrop, we pursued multiple viral and host targets for the treatment of HCV infection. At the time of initiating our efforts to discover an HCV nonstructural protein 5a (NS5A) inhibitor, we had over 20 HCV research programs ongoing, and several compounds undergoing, or selected to enter clinical trials. The more advanced agents, GS-9190 (NS5B non-nucleotide polymerase inhibitor), GS-9256, and GS-9451 (NS3/4a protease inhibitors), were directed at inhibiting GT1 HCV [10–12]. As a result of both the lower response rates to IFN therapy and high prevalence (with GT1 infection estimated to be as high as 60% of HCV-infected individuals worldwide [13]), there was a dominant unmet need for improved treatment of GT1 HCV infection. Based on this epidemiological and therapeutic landscape, we initiated our NS5A inhibitor program with primary potency assays directed toward GT1 HCV antiviral activity. This chapter details the discovery and early development of the potent HCV NS5A inhibitor ledipasvir (**1**, GS-5885, Table 1) and its clinical combination with sofosbuvir [14]. The discovery program toward the pan-genotypic NS5A inhibitor

[1] https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/204671s002lbl.pdf. Accessed 4 Dec 2018.

[2] Volume I, HCV: The Journey from Discovery to a Cure.

[3] https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/205834s001lbl.pdf. Accessed 10 June 2018.

Table 1 Potency of ledipasvir (**1**) against GT1–6 replicons and subtypes



1
GS-5885, ledipasvir (LDV)

	HCV GT EC_{50} (pM)[a]									
	1a[b]	1b[c]	2a[d]	2aJ6[e]	2b[f]	3a[g]	4a[h]	5a[i]	6a[j]	6e[k]
LDV	31	4	21,000	249,000	530,000	168,000	390	150	1,100	264,000

[a]Cheng G et al. J. Hepatol. 2013;58(suppl):S484. http://www.natap.org/2013/EASL/EASL_34.htm. Last accessed June 10, 2018
[b]GT1a (strain H77)
[c]GT1b Con-1
[d]GT2a JFH-1
[e]GT2a J6
[f]GT2b MD2b-1 NS5A
[g]GT3a S52 transiently transfected subgenomic HCV replicon
[h]GT4a ED43
[i]GT5a SA13 NS5A (9-184) transient chimeric replicons based on GT1b Rluc backbone
[j]GT6a HK6 stable subgenomic HCV replicon
[k]GT6e D88 NS5A (9-184) transient chimeric replicons based on GT1b Rluc backbone. In these replicons a–c, f, and g are stable subgenomic replicon cells; d and e are NS5A transient chimeric replicons based on GT2a JFH-1 Rluc backbone

velpatasvir initiated immediately following our ledipasvir discovery work. The discovery of our pan-genotypic NS5A inhibitor velpatasvir (VEL, GS-5816) is described in Volume II, HCV: The Journey from Discovery to a Cure and the following references: [15–17]. The discovery of our pan-genotypic NS3/4a protease inhibitor voxilaprevir (VOX, GS-9857) and its combination with SOF and VEL as Vosevi® is described in Volume I, HCV: The Journey from Discovery to a Cure and the following references: [18]. (https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/209195s000lbl.pdf. Accessed 10 June 2018).

We targeted our NS5A inhibitor to possess properties appropriate for incorporation with one or more other HCV antiviral agents in a single-tablet regimen (STR). Single-tablet regimens have proven beneficial for patient compliance and efficacy in the chronic treatment of HIV infection [19, 20]. We saw a similar utility for an STR in the treatment of HCV infection. Thus the attributes of our NS5A inhibitor required sufficient potency and metabolic stability to achieve a low dose, a long pharmacokinetic half-life compatible with once-daily dosing, and a drug interaction profile suitable for combination with other HCV antivirals of complementary mechanism. The research program focused on these principles to guide the optimization of potency and pharmacokinetic (PK) parameters in the discovery of LDV. LDV is combined with SOF as Harvoni®, and LDV was the first US Food and Drug Administration (FDA) approved NS5A inhibitor (October 10, 2014). Harvoni® was approved for the treatment and cure of GT1 HCV-infected individuals in as short as

8 weeks of therapy and was the first approved HCV STR. Based on the potent antiviral activity of LDV against GT4, GT5, and GT6 HCV (Table 1), and the concomitant high SVR rates for LDV/SOF in GT4–6 patients, Harvoni® was additionally approved for treatment of GT4–6 patients (https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/205834s001lbl.pdf. Accessed 10 June 2018) [5].

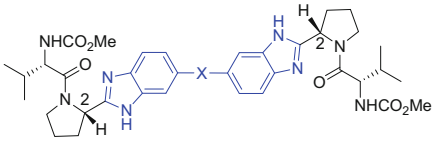
2 Discovery Work Leading to Ledipasvir

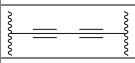
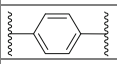
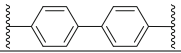
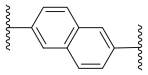
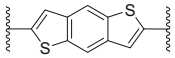
We focused on discovering an NS5A inhibitor that could be utilized in a single-tablet regimen in combination with other HCV agents. Further, we sought high antiviral potency and a long PK half-life to reduce the potential for emergence of viral breakthrough or resistance during treatment [14]. A large number of diverse cores were designed and synthesized in the discovery of LDV [21]. In early studies we investigated symmetric core inhibitors (where the core is the portion of the inhibitor structure that spans from between the C2 positions of each [modified] pyrrolidine – as colored in blue in Table 2). Potency proved challenging to attain against the genotype 1a subtype (GT1a) replicon but was more readily attained against the genotype 1b subtype (GT1b). Thus potency discussions herein most typically refer to GT1a potency, with GT1b potency provided in tables for reference. Throughout this manuscript, potency values represent effective concentration to reduce replication by 50% (EC_{50}) in cell lines with engineered replicons.

Table 2 outlines potency optimization for structural variation within the core between two benzimidazoles. With directly linked benzimidazoles, compound **2** does not achieve an EC_{50} against GT1a at the top concentration of 44,000 pM. Alkyne **3** is 138 pM against GT1b, but again is not active against GT1a. Bis-alkyne **4** improves GT1a potency to 11,000 pM. Incorporation of ring systems further improves potency. Thiophene- and phenyl-based cores improve potency from 1,700 to 500 pM, respectively. Biphenyl (**6**) loses activity relative to phenyl (**5**), while fused-ring systems provide highly potent inhibitors with naphthyl (**7**) and benzodithiophene (**8**) achieving 110 pM and 200 pM GT1a inhibition, respectively.

The inhibitors in Table 3 represent a shift in our thinking and provide the initiation of a fruitful path that we investigated throughout our NS5A inhibitor program. The inhibitors in Table 3 have unsymmetric cores, where one end of the core is a benzimidazole and the other end an imidazole. The use of unsymmetric cores has implications that will be discussed further (vide infra). Our unsymmetric approach afforded intriguing structural variation and properties to our inhibitors and afforded striking divergences from the results for the bis-benzimidazole cores in Table 2. In the imidazole/benzimidazole series, interestingly the phenyl-based core inhibitor **9** does not achieve 50% inhibition at the highest assay concentration and is >88-fold weaker in activity than in the bis-benzimidazole example (**5**). Most striking is that replacement of phenyl (**9**) by naphthyl (**10**) in this series affords an increase in potency by 620-fold. Despite significant divergence in potency for the phenyl inhibitors between these series, in both series the naphthyl-based core provides the

Table 2 GT1a and 1b replicon potency studies in symmetric core (core portion in blue) inhibitors



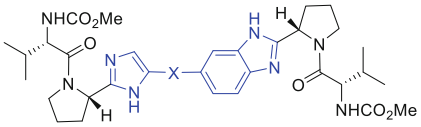
Compound	X	EC_{50} (pM) GT1a[a]	GT1b[b]
2	Bond	>44,000[c]	35,400
3		>44,000[c]	138
4		11,000	26
4		1,700	10
5		500	9
6		3,700	44
7		110	4
8		200	16

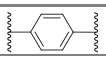
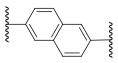
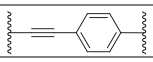
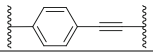
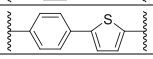
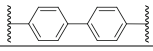
[a]GT1a (strain H77)
[b]GT1b Con-1; in this manuscript, these are the replicon strains for GT1a and GT1b
[c]A value of ">44,000 pM" means that the EC_{50} was not achieved at this top well concentration

most potent inhibitor against GT1a observed within each table of inhibitors (compounds **7** and **10**). It is also notable the unsymmetric core inhibitor **10** at 71 pM in GT1a is more potent than the symmetric inhibitor **7**. Phenyl-alkynyl inhibitors **11** and **12** reveal another important facet of these unsymmetric cores. With the core possessing unsymmetric ends (imidazole/benzimidazole), and with the presence of unsymmetric central "-X-" groups, there is a matched and mismatched combination within the core. The core with the phenyl attached to the imidazole (inhibitor **12**, 380 pM versus GT1a) affords 6.6-fold more potency than the core with the alkynyl attachment to the imidazole. Finally, replacement of the alkyne with an aryl group provides potent biaryl inhibitors. The biphenyl inhibitor **14** is 22-fold more potent at 170 pM in GT1a than the corresponding inhibitor in the bis-benzimidazole series.

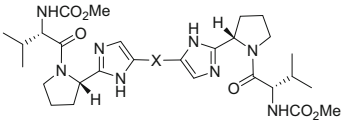
Fused-ring cores in Tables 2 and 3 afforded three out of the top four most potent inhibitors (**7**, **8**, and **10**, ranging from 70 to 110 pM versus GT1a). The biphenyl-based inhibitor **14** was the only non-fused-ring core that attained high potency (170 pM) comparable to the fused-ring cores. Importantly inhibitor **14** was stable at the lower measured limit of our routine human liver microsomes (HLM) stability

Table 3 GT1a and 1b replicon potency studies in unsymmetric core (portion in blue) inhibitors

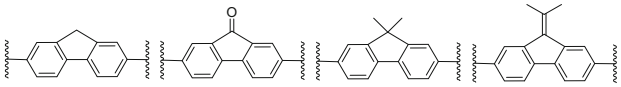


Compound	X	EC_{50} (pM) GT1a	GT1b
9		>44,000[a]	300
10		71	7
11		2,500	16
12		380	11
13		200	3
14		170	7

[a]A value of ">44,000 pM" means that the EC_{50} was not achieved at this top well concentration



X:

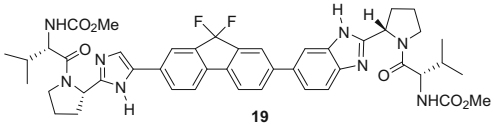


	15	16	17	18
GT1a EC_{50} (pM)	94	300	1200	7000
GT1b EC_{50} (pM)	13	18	14	14

Fig. 5 Fused tricyclic core-based inhibitor potency

assay (predicted clearance <0.16 L/h/kg), whereas the naphthyl inhibitor **10** demonstrated less metabolic stability in microsomes. The favorable potency and metabolic stability of the biaryl inhibitor **14**, along with our observation that fused-ring systems provide high potency, prompted us to combine these concepts through constraint of the biphenyl to afford a tricyclic fused-ring inhibitor series.

These tricycle-based core inhibitors (Fig. 5) were synthesized in a symmetric bis-imidazole core series allowing for an easier synthetic path than the unsymmetric series and a more rapid assessment of the tricyclic systems. Fluorene **15** showed good potency but was unstable even to air oxidation. The oxidation product, fluorenone **16**, was less potent, and dimethyl substitution to block the system from oxidation lost over 13-fold in potency. The exo-dimethylmethylene was synthesized



19

EC_{50} (pM)		HLM Pred CL (L/h/kg)
GT1a	GT1b	
40	3	< 0.16

Fig. 6 Difluorofluorene **14** affords improved potency and oxidative stability and has good PK half-lives

Table 4 Rat and dog pharmacokinetics[a]

Compound	Spec	CL (L/h/kg)	V_{ss} (L/kg)	$t_{1/2}$ (h)	MRT[a] (h)	%F
14	Rat	1.04 ± 0.17	1.76 ± 0.17	1.57 ± 0.19	1.71 ± 0.16	11.5 ± 8.7
	Dog	0.78 ± 0.29	2.31 ± 0.37	2.30 ± 0.28	3.00 ± 0.27	n.d.[c]
19	Rat	0.42 ± 0.04	0.93 ± 0.04	1.83 ± 0.22	2.21 ± 0.21	36.7 ± 3.2
	Dog	0.53 ± 0.04	1.96 ± 0.03	2.63 ± 0.18	3.69 ± 0.29	n.d.[c]
21	Rat	0.70 ± 0.02	0.98 ± 0.08	1.49 ± 0.02	1.40 ± 0.07	35.2 ± 10
	Dog	0.05 ± 0.007	0.31 ± 0.007	5.29 ± 0.60	6.60 ± 1.10	n.d.[c]
22	Rat	0.75 ± 0.04	1.81 ± 0.26	2.07 ± 0.19	2.42 ± 0.22	26.3 ± 9.0
	Dog	0.40 ± 0.26	2.03 ± 0.92	4.01 ± 0.82	5.47 ± 1.01	n.d.[c]

All parameters except %F are from intravenous dosing
[a]Mean residence time
[b]*n.d.* not determined

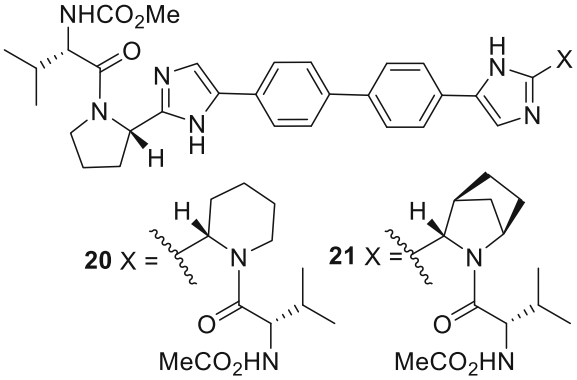
to avoid the out-of-plane bulk of **17** and the dipole of **16** but suffered a 78-fold potency loss relative to fluorene **15**. (It is intriguing that GT1b activity tightly ranges from 13 to 18 pM over this series.)

We sought to synthesize the difluorofluorene to block the fluorene methylene oxidation with a relatively small group – installation of two fluorines on the methylene proved challenging, and we incorporated them in the high value unsymmetric series (Fig. 6). The unsymmetric difluorofluorene-based core afforded inhibitor **19** with the highest potency in this series at 40 pM in GT1a and 3 pM in GT1b and was stable under the conditions of the HLM assay.

Unsymmetric biphenyl (**14**) and difluorofluorene (**19**) inhibitors were progressed into rat and dog PK (Table 4). Both inhibitors displayed good pharmacokinetic properties with moderate steady-state volumes of distribution (V_{ss}) higher than total body water. Difluorofluorene **19** had superior and low clearance in both rat and dog, longer half-lives, and greater bioavailability in rat than biphenyl inhibitor **14** (36.7% versus 11.5%). Thus difluorofluorene **19** showed improvements in potency and pharmacokinetic properties over biphenyl **14**.

Next a number of terminal heterocycle modifications were undertaken, again in a symmetric series for simplification of chemical synthesis. The azabicyclo[2.2.1] inhibitor **21** was more potent than piperidine **20** (Table 5). Although not as potent as the more advanced inhibitors such as **19**, azabicyclic inhibitor **21** displayed

Table 5 Potency of piperidine and 2.2.1 azabicyclic inhibitors

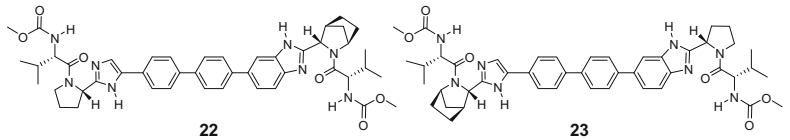


	20	**21**
GT1a EC_{50} (pM)	450	210
GT1b EC_{50} (pM)	5	9

favorable PK properties in dog as well as a very low clearance, a 5.29 h half-life and 35% oral bioavailability in rat (Table 4).

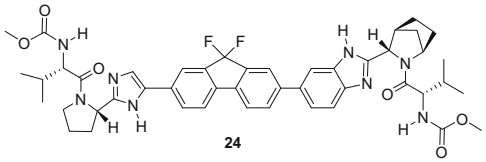
We sought to incorporate this pharmacokinetic enhancing azabicyclo[2.2.1] ring system into our unsymmetric imidazole/benzimidazole series. In this process we discovered a further important facet of structure-activity relationships in the unsymmetric core series. Earlier we found that the directionality of the central portion of the core (phenyl-alkyne) relative to the unsymmetric imidazole/benzimidazole groups produced a matched and mismatched pairing (compounds **11** and **12**). This core directionality again provided a matched and mismatched pairing but this time with positioning of the terminal azabicyclo[2.2.1] ring system relative to the unsymmetric core (i.e., positioning of the azabicyclo[2.2.1] system on either end of the core). When the bicyclic ring system is proximal to the benzimidazole (**22**), the inhibitor is more potent than when the bicyclic ring system is proximal to the imidazole (**23**) (Fig. 7). Further, the matched case inhibitor is more potent (**22**, GT1a = 160 pM) than the azabicyclic symmetric inhibitor (**21**, GT1a = 210 pM, Table 5), while the mismatched case inhibitor **23** is less potent (GT1a = 660 pM) than the symmetric case **21**. These results highlight a critical discovery; unsymmetric cores allow for diversification of structure that can provide beneficial properties (matched cases, with beneficial properties such as enhanced potency and/or pharmacokinetics) over the more limited symmetric cases. Finally, we had sought to determine if the beneficial PK properties imparted by the azabicyclo[2.2.1] ring in symmetric inhibitor **21** might translate in the unsymmetric series. Indeed, the half-lives in rat and dog are improved in azabicyclo[2.2.1] inhibitor **22** (unsymmetric "matched case") over the corresponding pyrrolidine **14** (Table 4).

Having improved the PK half-life with azabicyclic inhibitor **22**, we sought to enhance its potency. As demonstrated earlier, fused-ring systems within the inhibitor's core can provide potency enhancements. Accordingly, incorporation of the



	22	23
GT1a EC_{50} (pM)	160	660
GT1b EC_{50} (pM)	6	21

Fig. 7 Differential end-substitution with an unsymmetric core leads to divergence in potency. Bridged azabicyclic ring system is more potent on benzimidazole side of the core



GT1a EC_{50} (pM)	56
Protein-binding adjusted	784

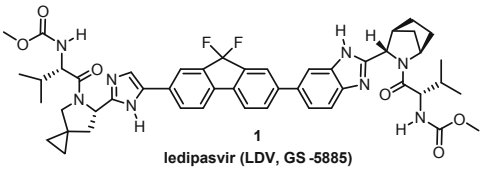
Fig. 8 Difluorofluorene core improves potency in combination with azabicyclic pyrrolidine

difluorofluorene ring system in inhibitor **24** (Fig. 8) afforded a GT1a potency of 56 pM, which is an ~3fold improvement over the biaryl core inhibitor **22**.

The inhibitors described herein are highly protein bound, even in the replicon cellular assay which includes 10% fetal bovine serum (FBS). We utilize a dialysis methodology to assess relative inhibitor free fraction based on plasma protein binding and then generate a value for the protein-binding-adjusted potency. In this method, the inhibitor of interest is dialyzed between 100% human plasma in one well and replicon cell culture medium (including 10% FBS) in a second well [22]. The measured concentration ratio between wells can be multiplied by the replicon potency to "cancel" the cell culture medium binding and provide a human plasma protein-binding-adjusted potency (PA EC_{50}). This methodology affords a GT1a PA EC_{50} for inhibitor **24** of 784 pM. We sought to improve upon this protein-binding-adjusted potency.

3 Ledipasvir (1, LDV, GS-5885)

During our final phase of discovery, one of the directions we undertook was modification of the pyrrolidine of inhibitor **24**. Incorporation of a spirocyclopropyl ring provided the most potent inhibitor (compound **1**) in the series with improvements in both the replicon potency and the plasma protein-binding-adjusted potency (GT1a $EC_{50} = 31$ pM, PA $EC_{50} = 208$ pM, Fig. 9) over inhibitor **24**; interestingly, the PA EC_{50} of **1** versus **24** was differentially improved (3.6-fold, 208 versus 740 pM) relative to the EC_{50} (1.8-fold, 31 versus 56 pM). The protein-binding ratio of human plasma versus cell culture medium of 6.7-fold for inhibitor **1** as



	GT1a	GT1b
EC_{50} (pM)	31	4
Intrinsic	0.31	0.04
Protein-binding adjusted	208	27
human $t_{1/2}$, (h), 90 mg dose	49.7	

Fig. 9 Ledipasvir structure, replicon cellular antiviral potency, intrinsic replicon potency, and human PK half-life

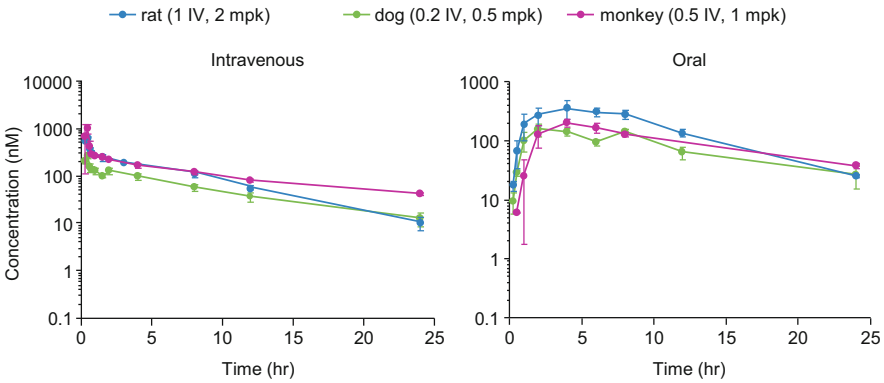


Fig. 10 Pharmacokinetic curves for ledipasvir (**1**) in rat, dog, and cynomolgus monkey. LDV has long half-lives in preclinical species

measured by dialysis was the lowest for any advanced inhibitor we had assessed. Compound **1** is highly protein bound, with only 1% free drug even in the replicon cell culture medium (containing 10% fetal bovine serum). Accounting for free drug, the intrinsic replicon GT1a and GT1b EC_{50} values for compound **1** are 310 femtomolar (fM) and 40 fM, respectively (Fig. 9).

Pharmacokinetic curves and PK parameters for inhibitor **1** are found in Fig. 10 and Table 6. Compound **1** has the longest half-lives (from 4.7 to 10.3 h in rat, dog, and monkey) among the compounds described herein and has high metabolic stability across preclinical species. Based on its exceptional replicon and PA potency, excellent pharmacokinetic half-lives, bioavailability, and low predicted clearance, compound **1** was selected for development and is now known as ledipasvir (LDV, GS-5885).

A synthesis of LDV is depicted in Scheme 1. The azabicyclo[2.2.1] ester **1a** [23] is debenzylated with palladium hydroxide and hydrogen, Boc protected, and the ester hydrolyzed to form bicyclic acid **1b**. Coupling of **1b** with 4-bromo-1,2-diaminobenzene and heating in ethanol affords benzimidazole **1c**, which is borylated to form pinacol boronate ester **1d**. Bromo-iodofluorene **1e** is difluorinated in a mild and novel "one-pot" procedure by treatment with *N*-fluorobenzenesulfonimide followed by KHDMS in THF (**1f**). The Grignard of **1f** is selectively formed and

Table 6 In vitro and in vivo PK parameters for ledipasvir in preclinical species and humans

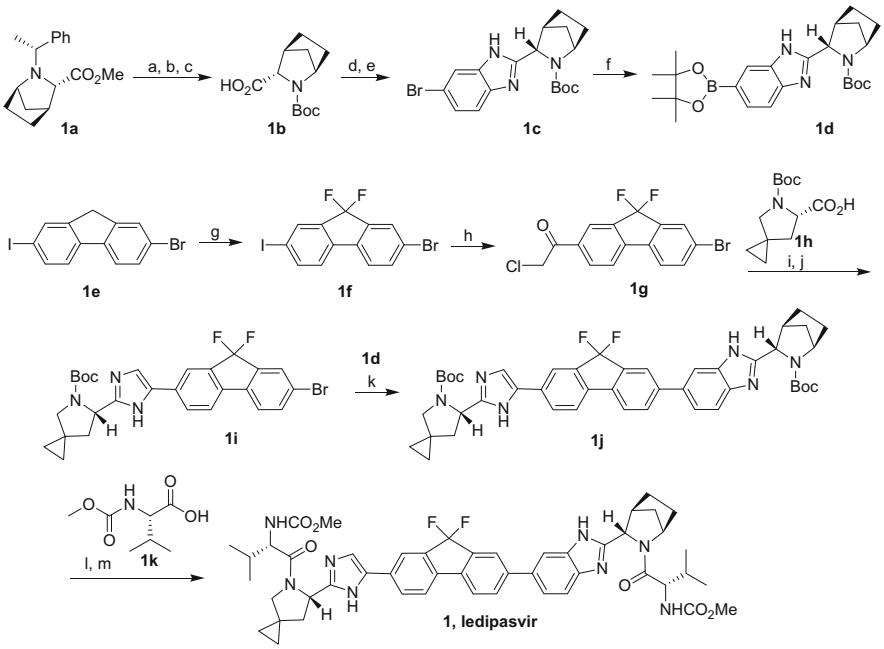
Species	Dose (route)	In vitro		In vivo				
		Percent free in plasma (%)	Pred CL microsomes (L/h/kg)	$t_{1/2}$ (h)[a]	CL (L h^{-1} kg^{-1})[a]	V_{ss} (L kg^{-1})[a]	MRT[b] (h)	%F
SD rat	1 mpk[c] (IV)	0.19	<0.34	4.67 ± 0.56	0.43 ± 0.04	2.66 ± 0.13	6.19 ± 0.28	32.5 ± 6.7
Beagle dog	0.2 mpk[c] (IV)	0.06	<0.18	7.41 ± 0.80	0.13 ± 0.02	1.19 ± 0.13	9.20 ± 1.35	53.0 ± 12.4
Cyno monkey	0.5 mpk[c] (IV)	3.85	<0.17	10.3 ± 1.2	0.17 ± 0.00	2.15 ± 0.42	12.9 ± 2.1	41.1 ± 3.6
Human	90 mg (PO)[d]	0.68	0.012[e]	49.7[d]	–	–	–	~50%[d,f]

[a]CL, V_{ss}, MRT, and $t_{1/2}$ are from IV dosing
[b]Mean residence time
[c]mpk = milligrams per kilogram
[d]Data in HCV-infected patients at approved dose in Harvoni®; see Tables 8 and 9 for results from other doses in humans
[e]Measured in human hepatocytes using ^{3}H-ledipasvir
[f]Human bioavailability estimated from CL/F from HCV-infected patients and CL predicted from preclinical studies

reacted with 2-chloro-*N*-methoxy-*N*-methylacetamide to generate the chloroketone **1g** which is alkylated with the potassium salt of spirocyclic acid **1h**. Heating of the resulting keto-ester with ammonium acetate in toluene affords cyclization to difluorofluorene-imidazole **1i**. Coupling of pinacol boronate **1d** with bromo-fluorene **1i** completes the core of ledipasvir **1j**. Double Boc deprotection of **1j** and HATU-mediated coupling with Moc-valine (**1k**) affords ledipasvir, **1** [14].

Further data supporting the decision to undertake the clinical development of ledipasvir follows. LDV showed no measurable instability at the lower limit of detection in our in vitro liver microsome assays in preclinical species and human (human predicted CL <0.16 L/h/kg). Therefore ^{3}H-LDV was incubated in hepatocytes to measure low-level metabolites for calculation of the predicted CL. This approach afforded an exceptionally low predicted human metabolic clearance of 0.012 L/h/kg (Table 6). In bile duct-cannulated dogs, LDV was slowly excreted, and over 24 h 65% of the dose was recovered as parent drug in bile consistent with the low hepatic oxidative metabolism measured across species in microsomes and human hepatocytes. (Less than 1% of LDV was recovered in urine.)

Despite its high protein binding, LDV displays moderate volumes of distribution (V_{ss}). It is interesting to note that although high serum protein binding often leads to a low volume of distribution [24], the V_{ss} of LDV in preclinical species (1.2–2.7 L/h/kg) is significantly higher than the plasma volume. The moderate V_{ss} of LDV in concert with its high metabolic stability contributes to its long PK half-life. As a consequence of its low oxidative metabolism in human hepatocytes, moderate V_{ss} in rat, dog, and monkey, and slow biliary excretion in dog, LDV was predicted to have a long human half-life. Based on its PK and potency, LDV was predicted to have a sufficiently low once-daily dose that would be compatible with dosing in a single-tablet regimen. As a result, LDV was progressed into clinical development. In our discovery of LDV, we targeted a long human half-life to



Scheme 1 ledipasvir synthesis. (**a**) $Pd(OH)_2/C$, H_2, EtOH; (**b**) Boc_2O, i-Pr_2NEt, CH_2Cl_2; (**c**) LiOH, THF/MeOH/H_2O; (**d**) 4-bromo-1,2-diaminobenzene, HATU, 4-methylmorpholine, DMF; (**e**) EtOH; (**f**) bis(pinacolato)diboron, $PdCl_2(dppf)_2$, KOAc, 1,4-dioxane; (**g**) *N*-fluorobenzenesulfonimide, KHMDS, THF; (**h**) *i*-PrMgCl, 2-chloro-*N*-methoxy-*N*-methylacetamide, THF; (**i**) **1h**, K_2CO_3, KI, acetone; (**j**) NH_4OAc, PhMe; (**k**) **1d**, $Pd(OAc)_2$, PPh_3, $NaHCO_3$, DME/H_2O; (**l**) HCl/dioxane/DCM; (**m**) **1k**, HATU, i-Pr_2NEt, DMF

enable once-daily dosing in an STR and to ensure that drug trough concentrations would remain sufficiently high to suppress viral breakthrough and the emergence of resistance potentially even in the event of patient non-compliance.

As described herein, multiple unique motifs make up the complex structure of ledipasvir and contribute to its picomolar cellular and PA potency, high metabolic stability, and excellent pharmacokinetic properties. The structure of LDV has captured the interest of a number of authors and has been cited in a range of publications focusing on some of the intriguing structural elements now becoming utilized in medicinal chemistry, including benzimidazoles [25]; spirocyclic ring systems [26]; cyclopropanes [27], bridged heterocyclic ring systems [28]; incorporation of stereocenters (LDV has six) [29]; and the use of fluorine in drug discovery (whereas most fluorinated drugs include aryl or heteroaryl fluorides, few bear aliphatic fluorine substitution as in LDV) [30]. With these and other elements taken together, the unique structure of ledipasvir is highly complex within known drug space. In a recent publication detailing a computational algorithm that defines structural complexity in drugs, ledipasvir is the most structurally complex orally bioavailable drug among the examples discussed [31]. The chemical complexity of ledipasvir based on

Table 7 Ledipasvir is not compliant with most contemporary medicinal chemistry rule-based bioavailability and "drug-likeness" metrics

Rule set	Parameter	Rule limit value	LDV value
Lipinski rule of 5 [36]	Molecular weight	≤500	889
Lipinski rule of 5	CLogP[a]	≤5	6.71
Lipinski rule of 5	H-bond donors	≤5	4
Lipinski rule of 5	H-bond acceptors[b]	≤10	14
Veber [37]	Rotatable bonds[c]	≤10	12
Veber	Polar surface area[c]	<140 Å^2	174 Å^2
Ring rule [38]	# of rings	≤5 is 95th percentile	10
Aromatic ring rule [39]	# Aromatic rings	≤3	5

[a]ChemBioDraw 14.0, CambridgeSoft Corporation
[b]Sum of N's and O's as defined by Lipinski et al. [36]
[c]Pipeline pilot

ring structure and calculated properties defines it as a "rulebreaker" drug by multiple metrics. LDV has been discussed in drugs "beyond the rule of 5" (bRo5) [32, 33], bRo5 "chameleonic" drugs [34] that display differential lipophilicity based on their conformational state, and has been provided as an example of the importance of organic synthesis in drug discovery [35].

Our discovery and implementation of the structural motifs incorporated in LDV followed a data-driven path that sought improvement of measured properties such as antiviral activity and in vitro and in vivo pharmacokinetic attributes and disregarded rule-based metrics. Indeed ledipasvir is a "rulebreaker" compound that defies much of the dogma dominating contemporary medicinal chemistry design that attempts to define "drug likeness" and potential for bioavailability. Rule limit values for molecular weight, calculated logP, the number of H-bond donors and acceptors [36], the number of rotatable bonds and polar surface area [37], the number of ring structures [38], and the number of aromatic rings [39] are provided in Table 7, along with the corresponding values for LDV. These rule-based metrics are provided here for reference and were not utilized in any way during the discovery of LDV. LDV lies outside most of these metric-based rules. Ledipasvir has proven to be a bioavailable, efficacious, safe, and well-tolerated drug.

4 Translation of Ledipasvir's Preclinical Potency and PK Properties in Phase 1 Clinical Trials

The preclinical pharmacokinetic optimization efforts in the discovery of LDV proved fruitful. In human healthy volunteers the exposure of a single dose of LDV increases dose proportionally from 3 to 100 mg, and gratifyingly the half-lives are typically over 40 h (Fig. 11 and Table 8) [14]. The LDV 24 h trough drug concentration is well over the PA EC_{50} at all doses (depicted with the red dotted line, Fig. 11), ranging from 12-fold at the 3 mg dose to 470-fold at the 100 mg dose.

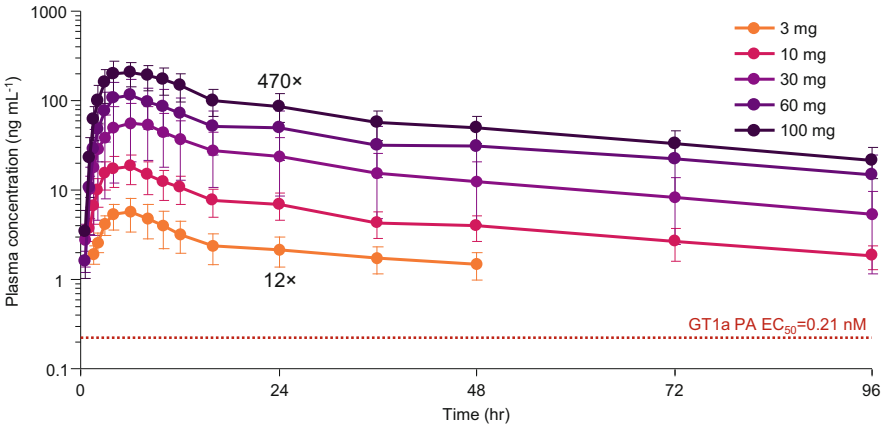


Fig. 11 LDV single oral dose pharmacokinetics in healthy volunteers

Table 8 LDV PK parameters after single oral dose administration, eight healthy volunteers per cohort

Mean parameter (%CV)	LDV oral dose				
	3 mg	10 mg	30 mg	60 mg	100 mg
$t_{1/2}$ (h)	45.2 (51)	42.4 (29)	37.2 (32)	44.2 (22)	39.5 (23)
C_{max} (nM)	6.75 (37)	21.3 (36)	82.2 (51)	133 (50)	242 (35)
AUC_{inf} (nM h)	245 (60.6)	695 (32.3)	2,717 (60.3)	5,299 (58.2)	8,658 (34.3)
C_{24hr} (nM)	2.45 (37)	7.94 (34)	31.4 (60)	56.4 (57)	98.8 (35)

For the 10–100 mg doses, the trough concentrations remain well over the PA EC_{50} even at 96 h post-dose.

The PK curves and PK parameters post the third-daily dose of LDV administered to GT1 HCV-infected individuals are depicted in Fig. 12 and Table 9, respectively [40]. Here the exposures are approximately dose proportional over five dose levels from 1 to 90 mg, and the half-life for the 90 mg dose of LDV (the dose utilized in the LDV/SOF STR Harvoni®) is 49.7 h. Although not yet at steady state 24 h post the third dose, the drug concentration is 610-fold over the GT1a PA EC_{50} and 4,730-fold over the GT1b PA EC_{50}. The 24 h drug concentration is above the GT1a PA EC_{50} even for the lowest total dose of 1 mg.

Accordingly, all monotherapy doses of LDV from 1 to 90 mgs displayed potent and rapid viral load reductions (VLR) in GT1-infected individuals (Fig. 13) [40]. Doses 3 mg and higher rapidly achieved VLR ~3 log10 within 24 h post the first dose and exceeded 3 log10 mean maximal viral load reductions during the dosing interval. Even the 1 mg total dose afforded a mean maximal VLR of 2.3 log10. The 30 and 90 mg doses maintained >2 log10 viral suppression at 144 h (4 days post the third and final dose), while the 10 mg dose in GT1b-infected

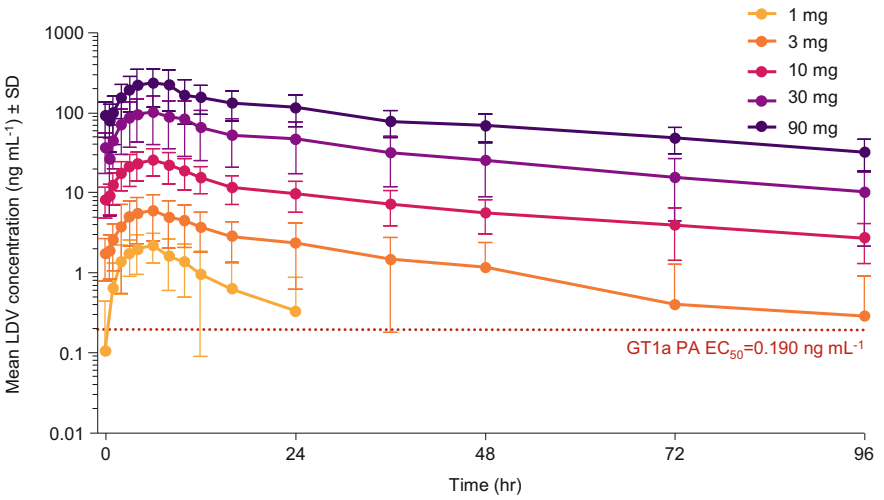


Fig. 12 LDV PK in patients post the third and final dose. Depicted as mean with standard deviation. Day 3 dose administered at time zero

Table 9 LDV pharmacokinetic parameters post third dose in HCV GT1-infected patients

	LDV oral dose				
	1 mg	3 mg	10 mg	30 mg	90 mg
$t_{1/2}$ (h)	13.0 (7.7, 17.8)	22.8 (13.1, 36.8)	39.9 (28.5, 47.2)	41.7 (25.8, 53.4)	49.7 (37.8, 54.3)
C_{max} (ng mL^{-1})	2.2 (39.7)	6.1 (56.6)	25.3 (40.0)	103.3 (57.5)	247.7 (45.4)
C_{tau} (ng mL^{-1})	0.3 (161.0)	2.4 (73.6)	9.7 (41.5)	46.5 (62.7)	115.9 (42.6)
AUC_{tau} (ng h mL^{-1})	34.0 (29.8)	89.7 (54.6)	368.8 (39.0)	1,592.4 (59.5)	3,815.5 (42.1)

Data are presented as mean values (coefficient of variability %); $t_{1/2}$ are median (quartile 1, quartile 3); Tau values are at 24 h post-dose; 10 mg cohort ($n = 19$) includes GT1a,b patients, others are GT1a ($n = 10$)

individuals afforded a mean maximal VLR of 3.3 log10 and maintained ~2.5 log10 viral suppression 4 days post the final dose.

The NS5B polymerase is highly error-prone. Based on the polymerase error rate, the HCV replication rate in vivo (10^{12} viral particles per day per patient), and the size of the HCV genome, it is estimated that every single, double, and some triple viral mutants are produced every day in a single patient [41]. In the NS5A gene sequence, variants present at gene positions 28, 30, 31, and 93 have shown reduced susceptibility to inhibitors [42]. Substitutions in the NS5A sequence are termed resistance-associated substitutions (RAS, plural RASs) and noted with the amino acid in the wild-type (WT) sequence first, the position next, and substituted amino acid last;

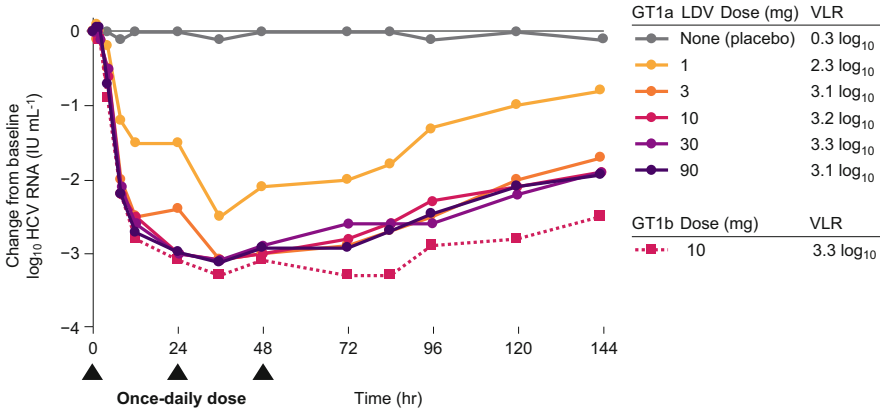


Fig. 13 Viral load reduction, 3-day LDV dosing in monotherapy in GT1a (1–90 mg) or GT1b patients (10 mg). All cohorts included ten patients except the GT1a 10 mg dose (**9**) and the placebo (**11**)

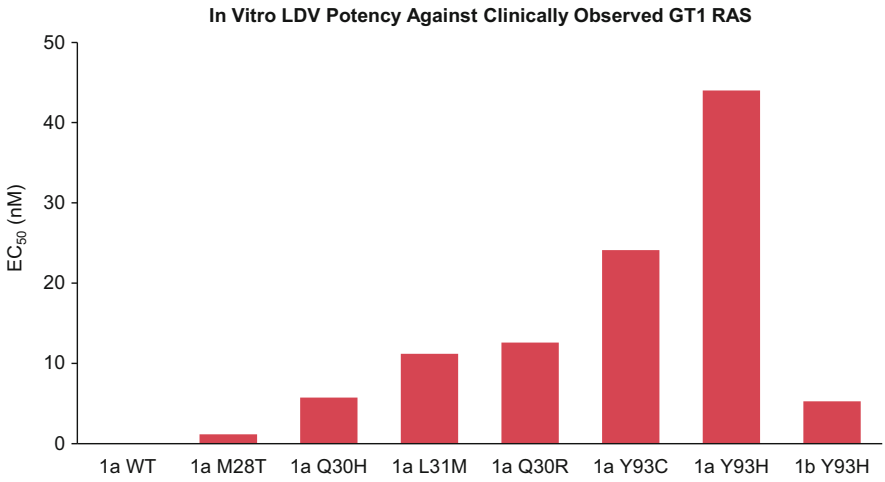


Fig. 14 Ledipasvir potency against clinically observed GT1 RAS (transient transfection in wild-type replicon)

e.g., Y93H denotes the RAS where a histidine is substituted for the WT tyrosine at position 93. Figure 14 and Table 10 depict the potency of LDV for a range of GT1a and GT1b RASs [43]. These RASs are present at low levels in most patients prior to treatment, but in some cases one or more RASs may be present at higher levels or may even represent dominant virus. The mean maximal viral load reduction for a given patient in monotherapy is defined by the titer of viruses with these RASs along with the inhibitor activity against these RASs.

It is interesting to note that there does not appear to be a dose-response for LDV in monotherapy for doses from 3 to 90 mg in the Phase 1b monotherapy study shown in Fig. 13 (VLR ranges from 3.1 to 3.3 log10 over this dose range) [40]. Assessment of baseline RASs helps to understand the apparent absence of dose-response. By chance, RASs were not present (at detectible levels) in any patient at baseline in the 1 and 3 mg GT1a cohorts (Fig. 15, baseline RAS assessments were determined post cohort randomization) [42]. Thus the patients in the 1 and 3 mg dosing cohorts had a disproportionately strong mean responses relative to the 30 and 90 mg cohorts which had relatively weaker responses in two patients showing high levels of the RAS Q30E/Q (30 mg cohort) or L31M (90 mg cohort). The weaker response of LDV against these RASs in vivo is consistent with the reduced susceptibility of the L31M and Q30E replicons to LDV in vitro (Table 10, Fig. 14). In our work to

Table 10 GT1 resistance profile of ledipasvir against clinically relevant resistance-associated substitutions (RAS)

	GT1a EC_{50} (nM)								GT1b EC_{50} (nM)	
	WT	M28T	Q30H	Q30R	L31M	Y93C	Q30E	Y93H	WT	Y93H
LDV	0.031	1.9	5.7	19.6	17	49.6	169	52.0	0.004	7.2

All RASs are transiently transfected GT1a or GT1b subgenomic HCV replicons

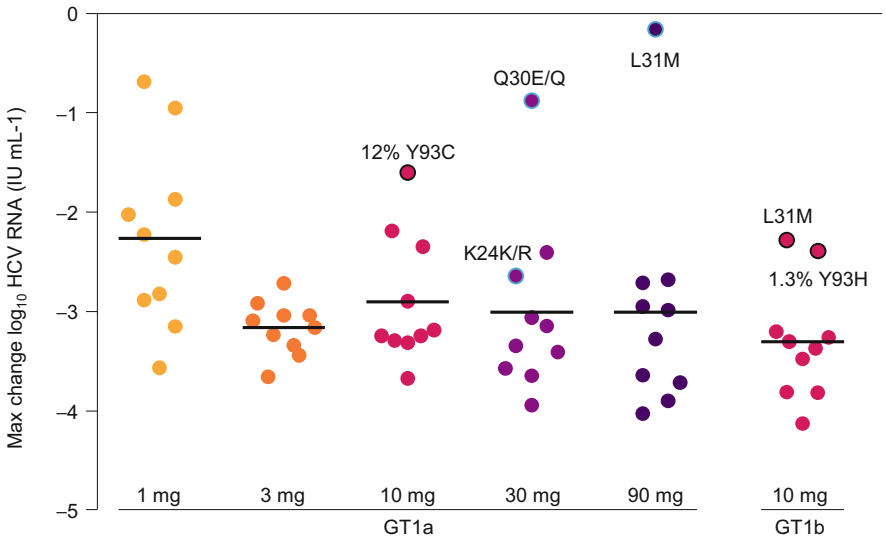


Fig. 15 LDV clinical VLR by individual patient. Clinical RASs found at baseline are noted. RASs are measured by two methods, either by population sequencing (detectable as >25% of total viral population which are labeled and outlined in blue) or by deep sequencing (limit of detection ~1% of total population are labeled and outlined in black). The percentage of the resistant population is noted for RAS detected by deep sequencing. The horizontal lines represent the mean viral load reduction

discover a pan-genotypic NS5A inhibitor, we improved activity across genotypes along with improved potency against NS5A RASs. The culmination of those efforts resulted in the discovery of velpatasvir [15–17], a potent pan-genotypic NS5A inhibitor with a high barrier to resistance that is combined with sofosbuvir in the first pan-genotypic STR Epclusa® and with sofosbuvir and voxilaprevir in the pan-genotypic STR Vosevi®. For both Harvoni® and Epclusa®, the presence of RASs detected in patients at baseline does not diminish the SVR relative to the SVR in patients with no detectible RASs [16, 44–48].

5 LDV/SOF Approval and Real-World Data

In October of 2014 LDV (90 mg) combined with sofosbuvir (400 mg) was approved under the name Harvoni®, for the treatment of GT1 HCV-infected non-cirrhotic and compensated cirrhotic patients based on the ION 1–4 Phase 3 clinical trials [44–47]. Administration of a single pill, once-daily for 8 or 12 weeks affords cure rates from 94 to 97%. In subsequent studies, Harvoni® was shown to afford high SVR rates in genotype 4, 5, and 6 patients (LDV displays potent GT4a–6a replicon potency, Table 1), and the prescribing label was accordingly expanded for treatment of these patients (https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/205834s001lbl.pdf. Accessed 10 June 2018) [5].

A measure of the practicality of a treatment regimen can be assessed by studies outside of the controlled environment of clinical trials in "real-world" "effectiveness" studies. There is no more extreme disparity between efficacy (risk-benefit in a clinical setting) in Phase 3 trials and real-world effectiveness (risk-benefit in real-world healthcare practice) than has been observed for SVR rates for IFN-based regimens. The poor tolerability, high complexity, and low efficacy of IFN-based therapy all conspire to afford real-world SVR rates that are dramatically lower than the ~60% [49, 50] achieved in later Phase 3 clinical studies. Strikingly, the real-world SVR in the US Veterans Administration (VA) is as low as 3.5%, with only 35.9% of the 99,156 HCV-infected veterans having no contraindications to this poorly tolerated regimen [51]. The attrition leading to this low SVR rate is outlined in Fig. 16 and includes patients unable or unwilling to undergo treatment, patients unable to complete treatment, and a high failure rate for those completing treatment. In contrast, a recent study of Harvoni® in this US VA HCV-infected patient population showed that 90% of patients had no contraindications, and of those patients initiating therapy the real-world SVR was 92–94% (4,365 patients, 8- and 12-week regimens, respectively). The high SVR of SOF/LDV in this population is even more notable since the authors of the study posited that advanced age, higher body mass index, ethnicity, and the prevalence of advanced liver disease (fibrosis and cirrhosis, including decompensated cirrhosis) are all factors defining this VA population as more difficult to treat than a typical cohort of HCV-infected individuals [51]. Accordingly, elimination of HCV within the 200,000 US VA HCV-infected individuals is projected for the end of the year 2018 [52].

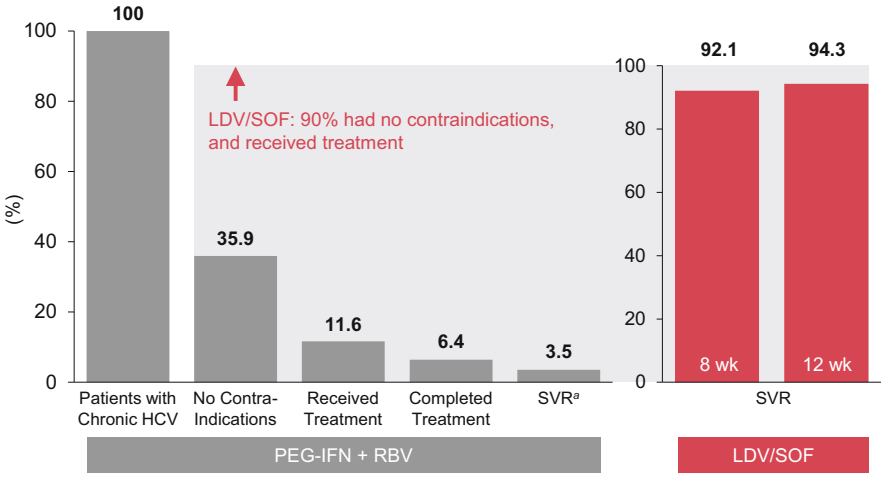


Fig. 16 Real-world SVR rates in the US Veterans Administration: IFN-based effectiveness contrasted with LDV/SOF. Tolerability and simplicity of LDV/SOF therapy present a striking real-world effectiveness advantage over IFN-based therapies. [a]VA calculated IFN-based SVR by including attrition from contraindications and those who did not receive treatment

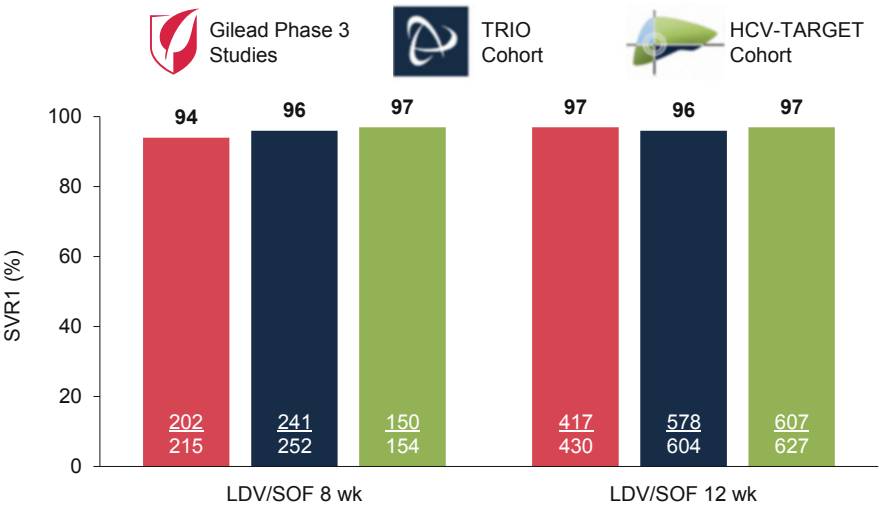


Fig. 17 Comparison of overall SVR rates from Phase 3 clinical studies relative to "real-world" studies (treatment-naïve patients). Bars colored as follows: red = Gilead Phase 3 clinical studies; dark blue = Trio Real-World Cohort; green = HCV Target Real-World Cohort

Additionally, two large real-world cohorts showed comparable results to those of the LDV/SOF Phase 3 ION-1 and ION-3 clinical trials as depicted in Fig. 17 ranging from 94 to 97% SVR. The Hepatitis C Therapeutic Registry and Research Network (HCV-TARGET) is a study comprised of North American and European academic

and community medical centers. Trio Health Innervation Platform (TRIO) is a disease management cloud-based platform with data collection from specialty pharmacies [53]. It is probable that the simplicity, safety, and potency of the SOF/LDV single-tablet regimen are important attributes leading to this high level of translation from clinical trials to the real world [54].

6 Conclusion

Ledipasvir is a highly potent NS5A (GT1a and GT1b EC_{50} values are 31 and 4 pM, respectively) inhibitor with a long pharmacokinetic half-life of 49.7 h in HCV-infected individuals. These attributes were critical aspects of the discovery LDV, making it a drug favorable for combination in a single-tablet regimen. Ledipasvir is the first FDA-approved NS5A inhibitor (October 10, 2014). LDV combined with sofosbuvir as Harvoni® is the first STR for the treatment and cure of HCV infection and the first HCV therapy to provide cure rates of 94–97% in as little as 8 weeks of treatment. Subsequent to approval for GT1 HCV infection, the label of Harvoni® was expanded to include treatment of GT4–6-infected individuals. The real-world effectiveness of Harvoni® is comparable to that achieved in controlled clinical trials, making it a valuable regimen for application in resource-limited settings and an important drug for HCV eradication programs [51, 52].

Acknowledgment The author would like to thank the Gilead research and development colleagues that discovered and developed ledipasvir and the clinical collaborators and patients who made this work possible.

Compliance with Ethical Standards

Conflict of Interest: John O. Link is an employee of Gilead Sciences, Inc.

Ethical Approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent: Informed consent was obtained from all individual participants included in the study.

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The Discovery of Velpatasvir (GS-5816): The Potent Pan-Genotypic Once-Daily Oral HCV NS5A Inhibitor in the Single-Tablet Regimens Epclusa® and Vosevi®

John O. Link

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Abstract The initial approval of the single-tablet regimen (STR) Harvoni®, containing the hepatitis C virus (HCV) nonstructural 5A protein (NS5A) inhibitor ledipasvir and the nonstructural 5B protein (NS5B) nucleotide inhibitor sofosbuvir (SOF), provided a major advancement in the treatment of individuals with chronic genotype 1 (GT1) HCV infection. Herein is described the discovery of velpatasvir (VEL, GS-5816), a pan-genotypic NS5A inhibitor with low picomolar activity against GT1–6 HCV and a high resistance barrier. The combinations of SOF/VEL as Epclusa® and SOF/VEL/voxilaprevir (VOX, NS3/4a protease inhibitor) as Vosevi® are the only pan-genotypic STRs for the treatment and cure of HCV infection. Epclusa® is the first approved pan-genotypic STR and affords high cure rates with a single 12-week treatment duration regardless of genotype, cirrhosis status, or the presence of baseline resistance variants. Vosevi® provides high cure rates for GT1–6-infected individuals who have previously failed therapy (96% cure rates for GT1–6 patients who had failed regimens with an NS5A inhibitor or 98% for those who had failed regimens without an NS5A inhibitor). With pan-genotype

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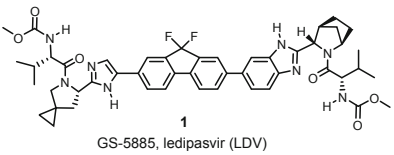
activity, no need for on-treatment monitoring, and real-world effectiveness comparable to that observed in clinical trials, the safe, simple, and effective STR Epclusa® is an important agent for eradication of HCV infection worldwide.

Keywords Direct-acting antiviral, Epclusa®, Vosevi®, GS-5816, HCV, NS5A inhibitor, Pan-genotypic, SOF/VEL, SOF/VEL/VOX, Velpatasvir

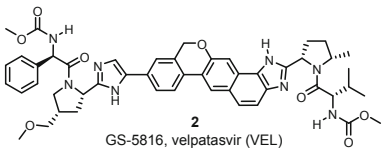
1 Introduction

Our initial work in nonstructural protein 5A (NS5A) inhibitor discovery led to ledipasvir (LDV, **1**, GS-5885, Table 1)[1] [2, 3]. LDV is the first NS5A inhibitor to be approved by the US Food and Drug Administration (FDA, October 10, 2014) and is combined with sofosbuvir in the single-tablet regimen (STR) Harvoni® [4–6].[2] The work toward LDV targeted the high unmet need for an effective treatment of HCV genotype 1 (GT1)-infected patients. This patient population had suffered high unmet need because (1) GT1 infection is the most prevalent worldwide among the six major HCV genotypes [7] and (2) among HCV genotypes, treatment of GT1 infection with the interferon (IFN)/ribavirin (RBV) standard of care resulted in lower sustained viral response (SVR, cure) rates [8–10]. As the first STR for HCV therapy, Harvoni provided a safe, simple, and effective treatment option for GT1 HCV-infected patients. The discovery work resulting in the high GT1 antiviral potency and long

Table 1 Replicon cellular potency of ledipasvir and velpatasvir (GT1–6 replicons and subtypes) [1]



1
GS-5885, ledipasvir (LDV)



2
GS-5816, velpatasvir (VEL)

	GT EC_{50} (pM) [1]									
	1a	1b	2aJFH1	2aJ6	2b	3a	4a	5a	6a	6e
LDV	31	4	21,000	249,000	530,000	168,000	390	150	1,100	264,000
VEL	14	16	8	16	6	4	9	54	6	130

[1]Unless otherwise noted, the EC50 values are based on the following replicon constructs for each genotype: 1a = GT1a (strain H77). 1b = GT1b Con-1. 2b = GT2b MD2b-1 NS5A. 3a = GT3a S52 transiently transfected subgenomic HCV replicon. 4a = GT4a ED43. 5a = GT5a SA13 NS5A (9-184) transient chimeric replicons based on GT1b Rluc backbone. 6a = GT6a HK6 stable subgenomic HCV replicon. In these replicons 1a, 1b, 2a, 3a and 4a are stable subgenomic replicon cells; 2aJ6 and 2b are NS5A transient chimeric replicons based on GT2a JFH-1 Rluc. backbone.

[2]https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/205834s001lbl.pdf. Accessed 10 June 2018.

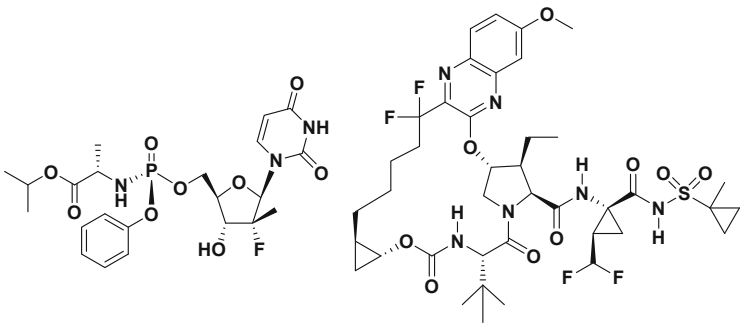
human pharmacokinetic half-life of LDV [2, 3, 6] (https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/205834s001lbl.pdf. Accessed 10 June 2018) can be also be found in this volume of "HCV: The Journey from Discovery to a Cure."

After completing our research effort toward LDV, our next step was to discover an NS5A inhibitor with the potential to effectively treat all HCV-infected patients worldwide, regardless of genotype. The discovery of the pan-genotypic NS5A inhibitor velpatasvir (VEL, **2**, GS-5816, Table 1) described herein represents the culmination of these efforts [11, 12]. As guiding principles, we sought to discover an NS5A inhibitor with high potency across HCV genotypes 1–6, a low projected human dose, and once-daily pharmacokinetics sufficient for combination in an STR with one or more yet to be identified pan-genotypic agents of complementary antiviral mechanism. Although interferon (IFN) and ribavirin (RBV) treatment afforded higher SVR for GT2 and GT3 patients than for GT1 patients, the SVR was still far from optimal, and the therapy was complex and toxic leading many patients to defer treatment while their disease progressed to fibrosis and cirrhosis. Additionally, the real-world effectiveness (efficacy outside of a controlled clinical trial setting) of IFN-based therapies is far lower than reported clinical trial results arising from their complexity, toxicity, poor tolerability, and low efficacy [13]. As we envisioned with Harvoni for GT1 infection, we saw a parallel need for an STR for the treatment of GT1–6 HCV infection. STRs have been shown to improve patient compliance and concomitant efficacy in the chronic treatment of HIV infection [4, 5]. We envisioned that a single, simple, safe, and effective STR for HCV patients regardless of genotype would provide broad applicability for cure and ultimately eradication of HCV infection worldwide.

Toward this end, velpatasvir (100 mg) combined with sofosbuvir [14] (400 mg, Fig. 1) is the single-tablet regimen Epclusa®, a pan-genotypic and pan-cirrhotic 12-week treatment for HCV with overall cure rates of 98% [15–19] (https://www.gilead.com/~/media/files/pdfs/medicines/liver-disease/epclusa/epclusa_pi.pdf. Accessed 24 June 2018). The improvements in GT1–6 replicon potency for VEL over LDV are shown in Table 1. Further, the co-formulation of the pan-genotypic NS3/4a protease inhibitor voxilaprevir (100 mg, Fig. 1) with SOF and VEL is Vosevi®, an STR for the treatment of GT1–6 patients who have previously failed therapy with another regimen (96% SVR for GT1–6 patients who had failed with an NS5A inhibitor or 98% for those who had failed on a regimen without an NS5A inhibitor) [20, 21] (https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/209195s000lbl.pdf. Accessed 10 June 2018). Epclusa® and Vosevi® are the only approved pan-genotypic STRs for the treatment of HCV infection.

2 Genetic Variability of the HCV Genome

The high mutability of HCV forms the basis for the chronicity of the virus [22]. It has been estimated that each possible single, double, and some triple viral mutants are produced daily within a single HCV-infected patient based on the high viral replication (10^{12} viral particles produced daily) and the error-prone nature of the HCV



GS-7977, sofosbuvir (SOF) GS-9857, voxilaprevir (VOX)

	SOF	LDV	VEL	VOX	
Sovaldi®	+				
Harvoni®	+	+			STR
Epclusa®	+		+		STR
Vosevi®	+		+	+	STR

Fig. 1 Structures of sofosbuvir and voxilaprevir. Compositions of the single-tablet regimens (STRs) containing sofosbuvir

NS5B polymerase [23]. Further complicating the genetic landscape of HCV, there are eight known genotypes and 86 subtypes (another consequence of the high mutability of HCV) (https://talk.ictvonline.org/ictv_wikis/flaviviridae/w/sg_flavi/56/hcv-classification. Accessed 18 Dec 2018). Genotype prevalence is geographically heterogeneous. Of the six major genotypes (percent of worldwide infection noted in parentheses), GT1 (49%) is dominant in the USA, Asia, Japan, and Europe. GT2 (11%) and GT3 (18%) are prevalent in Europe and GT3 is dominant in India and Pakistan. Egypt, where GT4 (17%) is dominant, has the highest prevalence of HCV of any country worldwide at 8–15% of its population infected. GT4 is also prevalent in central Africa, and GT5 is almost exclusively found in South Africa. GT6 is found in Southern Asia. GT5 and GT6 infections total 5% worldwide [7]. There is high sequence variability in the coding region for the HCV NS5A protein, with many viral sequence variants arising from the high mutability of the virus in addition to the sequence variations among the genotypes and subtypes. This high variability presents a confounding challenge for discovering a pan-genotypic NS5A inhibitor. The viral sequence coding the NS5A region is among the most variable in the HCV genome; the basis for the variability in the NS5A region is unknown but may be derived from the finding that the NS5A protein has no known enzymatic activity [24] and therefore does not have an active site with typical obligate conserved residues. Many HCV variants have reduced susceptibility to inhibitors. The dominant sequence for each subtype is defined as the "wild-type" (WT) sequence, whereas variants that produce reduced susceptibility to inhibitors are defined as resistance-associated substitutions (RAS, plural RASs). These

substitutions are annotated as "WT amino acid/residue position/substituted amino acid," e.g., Y93H (WT tyrosine at position 93 replaced by histidine). RASs in NS5A are commonly present at residue positions 28, 30, 31, 32, 58, 92, and 93 [25, 26]. Position 31 provides a useful example of the complexity of these substitutions: In GT1 L31 is WT, and L31M is a RAS. In GT2 L31M is a RAS that is more prevalent than in GT1 and is dominant in more than half of the GT2a and GT2b sequences. In GT4 M31 is present as WT and interestingly does not show reduced susceptibility to most inhibitors.

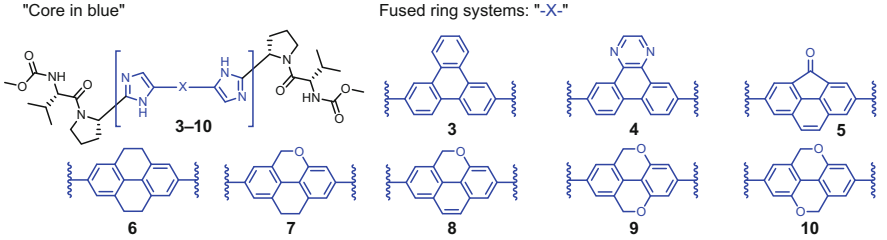
The structurally diverse set of NS5A proteins that would need to be potently inhibited by an effective pan-genotypic drug is daunting; similarly challenging is defining an assay paradigm representative of the viral species present in patients worldwide. To this end we analyzed patient-derived baseline (pre-treatment) NS5A gene sequences from public databases, along with sequences from our in-house clinical trial database to help understand the range of variation in the NS5A coding region. Central to our assay paradigm, we produced over 60 diverse and viable GT1–6 replicons to represent the diversity of HCV NS5A in infected individuals [1]. Consistent with published reports, we found that the prevalent GT2a and GT2b RAS L31M resulted in weaker potency against a range of NS5A inhibitor chemotypes [25, 26]. Additionally, subsequent to our discovery of VEL, it was reported that during monotherapy Phase 1 studies, an NS5A inhibitor produced a significantly weaker response in patients with GT2 L31M RAS present pre-treatment [27] (http://www.natap.org/2013/HCV/013113_03.htm. Accessed 14 Oct 2018).

Although we assayed for a range of genotypes and RASs, the structure-activity relationships (SAR) for inhibitor classes against GT2 L31M RASs provide instructive examples of our early studies to discover a pan-genotypic inhibitor (vide infra). We assayed for an increasing number of genotypes, subtypes, and RAS as our inhibitors evolved. Our inhibitors increased in structural complexity and size as we gained pan-genotypic activity; acceptable bioavailability and high metabolic stability proved increasingly difficult to obtain as our inhibitors gained broader activity against viral variants. High pan-genotypic potency, high metabolic stability, and good bioavailability were elements requiring significant parallel optimization to achieve our aim of discovering a pan-genotypic NS5A inhibitor with a sufficiently low dose for inclusion in an STR [11, 12, 28, 29].

3 Fused-Tetracyclic Core Inhibitors

Table 2 (see footnote 1) shows GT1 and GT2 replicon potency (potency values herein are reported as the effective concentration to inhibit replication in replicon cells by 50%, "EC_{50}") and predicted clearance (Pred CL) from human liver microsomes (HLM) for a number of inhibitors bearing fused tetracycle-based cores. Herein we define the "core" of the molecule as the moiety spanning between the C2 positions of the substituted pyrrolidines (core denoted in blue, Table 2). The GT2a JFH1 replicon in Table 1 is generated from a patient isolate derived clone

Table 2 Cores with fused-ring systems provide high potency: replicon potency data and human liver microsomal metabolic stability values



Cpd	GT EC_{50} (pM)						Pred CL (L/h/kg) HLM
	1a	1b	2a JFH1 (L31)	2a JFH1-L31M[a]	2a J6 (L31M)	2b (L31M)	
3	92	5	20	6,790	–	–	<0.16[b]
4	528	18	223	–	–	–	<0.16
5	220	13	73	–	–	–	0.43
6	156	21	131	13,300	–	–	0.19
7	65	28	16	–	23,700	–	0.39
8	63	22	14	–	19,700	10,200	0.29
9	87	21	19	9,850	–	–	–
10[c]	29	22	7	5,900	8,810	1,770	0.18

[a]GT2a JFH-1 subgenomic transient replicon cells with L31M mutation
[b]A value of <0.016 L/h/kg is the lowest predicted clearance value measurable in the routine HLM assay
[c]Additional replicon EC_{50} values for compound **10**, GT(EC_{50} value): 3a (GT3a S52 NS5A transient chimeric replicon based on GT1b Rluc backbone) (55 pM), 4a (GT4a ED43 NS5A transient chimeric replicons based on GT1b Rluc backbone) (29 pM), 5a (GT5a SA13 NS5A (9–184) transient chimeric replicons based on GT1b Rluc backbone) (56 pM), 6a (GT6a HK6 stable subgenomic HCV replicon) (16 pM), and 6e (GT6e D88 NS5A (9–184) transient chimeric replicons based on GT1b Rluc backbone. In these replicons a–c, g, and i are stable subgenomic replicon cells; d and f are NS5A transient chimeric replicons based on GT2a JFH-1 Rluc backbone) (1,160 pM)

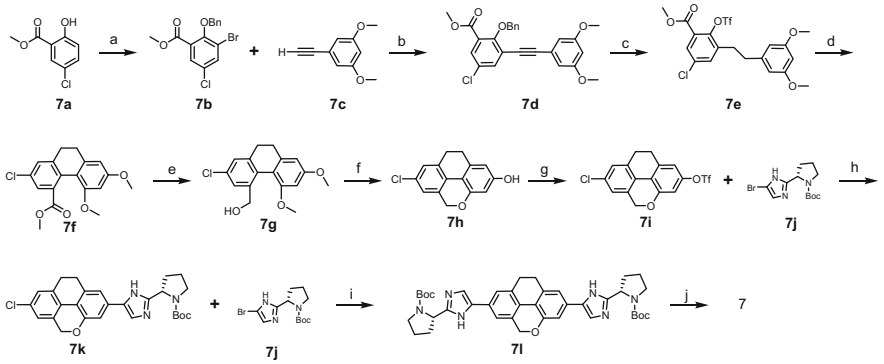
bearing L31. Site-directed mutagenesis (SDM) was used to generate the GT2a JFH1-L31M replicon that served as our surrogate for the L31M RAS in early studies. Subsequently we utilized the patient-derived GT2a J6 and GT2b replicons that have L31M in their native sequence. We regard potency data against the L31M RAS from these patient-derived sequence replicons with greater weight than the surrogate JFH1-L31M; underlying sequence differences among these replicons are additional bases for their differing susceptibilities to inhibitors. We typically found that the GT2a J6 replicon was the most difficult to inhibit among these GT2 L31M replicons.

In our NS5A inhibitor program, we synthesized over 100 unique core systems with differing lengths, shapes, numbers of rings and ring topologies, flexibility or rigidity, degrees of saturation, and heteroatom count that were elaborated into full inhibitors [2, 3, 28, 29]. Throughout our studies one of the fruitful directions we undertook included utilization of fused-ring systems in the core of the inhibitor. As described in our discovery of ledipasvir, we found that fused-ring systems present in

the core of inhibitors could enhance GT1 potency [2, 6]. As we pursued pan-genotypic inhibitors, we found complex patterns of inhibition across genotypes where potency could sometimes be enhanced in certain inhibitors bearing fused-ring cores. We hypothesized that a highly evolved core structure could provide the base on which to build broad potency across genotypes.

Many of the complex core ring systems we designed were of unprecedented structure and were accessed through novel multistep synthetic paths. These core explorations required a significant resource commitment to chemical synthesis. To provide an example of such synthetic complexity, the route to the tetracyclic core inhibitor 7 is detailed in Scheme 1: Phenol **7a** was brominated with NBS, benzyl protected, and underwent Sonogoshira coupling with arylalkyne **7c**. Hydrogenation/hydrogenolysis of diarylalkyne **7c** and intramolecular biaryl coupling mediated by palladium acetate afforded dihydrophanthrene **7f**. Reduction with LAH afforded alcohol **7g** which was deprotected and cyclized to dihydronaphthochromene **7h** with boron tribromide. Triflation of 7h and formation of the boronate ester were followed by Suzuki coupling with bromo imidazole **7j**, followed again by boronate formation and Suzuki coupling with **7j** to complete the bis-imidazole dihydronaphthochromene core system (**7l**). Boc-deprotection of **7l** and HATU-mediated coupling with methoxycarbonyl valine gave tetracyclic pyran inhibitor **7** [11, 28, 29].

In our efforts to discover pan-genotypic inhibitors, we initially sought to enhance potency against the GT2 L31M RAS while maintaining high potency against the GT1a replicon. In one avenue we pursued a series of tetracyclic core inhibitors (Table 2) [11]. Relative to LDV, triphenylene core inhibitor **3** exhibits a ~threefold loss in GT1a potency along with a 1,050-fold gain in GT2a JFH1. Compound **3** was



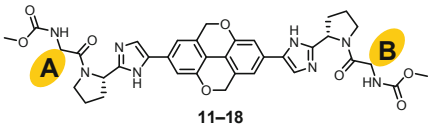
Scheme 1 The synthesis of 7. (**a**) (i) N-bromosuccinimide, DMF (ii) BnBr, K_2CO_3, DMF. (**b**) $Pd(PPh_3)_4$, CuI, Et_3N, DMF, 80°C. (**c**) (i) H_2, Pd/C, 60 PSI, and (ii) Tf_2O, Py, CH_2Cl_2. (**d**) $Pd(OAc)_2$, PPh_3, Cy_2NMe, DMF, 110 °C. (**e**) LAH, THF. (**f**) BBr_3. (**g**) Tf_2O, Py, CH_2Cl_2. (**h**) (i) KOAc, $Pd(dppf)Cl_2$, $(Bpin)_2$, dioxane, 110 °C, and (ii) 7j, $Pd(PPh_3)_4$, K_2CO_3, DMSO, 100 °C. (**i**) (i) KOAc, $Pd(dppf)Cl_2$, X-Phos, $(Bpin)_2$, dioxane, 110 °C, and (ii) 7j, $Pd(PPh_3)_4$, K_2CO_3, DMSO, 100 °C. (**j**) (i) HCl, dioxane, CH_2Cl_2, and (ii) methoxycarbonyl valine, 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU), i-Pr_2NEt

stable at the lower measurable limit of our HLM assay (Pred CL < 0.16 L/h/kg). The heterocyclic dibenzoquinoxaline system in **4** suffered losses in GT1a potency and GT2a JFH1 potency relative to the triphenylene inhibitor. We decided to change the tetracyclic ring topology. The cyclopentaphenanthrenone inhibitor **5** showed unacceptable losses in GT1 and GT2 activity. Again we changed the ring topology. Among the tetracycles **6**–**10**, incorporation of oxygen within the ring system proved beneficial. Proceeding from tetrahydropyrene **6** to tetracyclic pyran-based cores, **7** and **8** afforded enhanced GT1a and GT2 potency, although **6** proved more stable in HLM. Replacing the ethylene in **8** with methylenoxy afforded slight losses in GT1a and GT2a JFH1 potency. Importantly, the methylenoxy isomer **10** afforded the highest potency among the inhibitors in Table 2 for GT1a, GT2a JFH1, and the L31M GT2 variants. Although **10** had stability near the lower limit of our routine HLM assay (0.18 L/h/kg), improvements would need to be made to meet the criteria of once-daily dosing. Overall we considered the benzopyrano-benzopyran core system in **10** to provide the best balance of potency and stability within Table 2. Inhibitor **10** also showed potent activity against genotypes beyond GT1 and GT2: GT(EC_{50} value): 3a (55 pM), 4a (29 pM), 5a (56 pM), 6a (16 pM), and 6e (1,160 pM) (see footnote 1). The attributes of the core in benzopyrano-benzopyran inhibitor **10** warranted further study.

We investigated amino acid side-chain variants of inhibitor **10** (Table 3) (see footnote 1). Alkyl or cycloalkyl variants (**11**–**13**) showed losses in GT2a JFH1-L31M relative to valine. Intriguingly, the symmetric tetrahydropyranyl glycine (THP-Gly) inhibitor **14** displayed GT1a, GT2a J6, and GT2b EC_{50} values that only varied by twofold (330–600 pM). The symmetric D-phenyl-glycine (D-PhGly) inhibitor **15** improved the profile further with EC_{50} values of 93, 240, and 22 pM against GT1a, GT2a J6, and GT2b, respectively.

We became interested in understanding the pharmacokinetic properties of bis-D-PhGly inhibitor **15**. We have found that in high MW, low solubility, high lipophilicity "rulebreaker" [30] chemical space, some in vitro assay systems can produce artefactual results. This has been particularly true where systems have high surface area in the measurement apparatus – such as Caco2 permeability assays – potentially due to surface binding of the inhibitors. We have been unable to produce reliable Caco2 values for the inhibitors herein, including LDV and VEL. We prefer in vivo measurement of percent fraction absorbed (F_a%) from non-precipitating solution dosing to gauge the permeability of these compounds. The calculation of fraction absorbed removes the hepatic clearance component from the measured bioavailability [bioavailability (F%) includes the percent fraction absorbed (F_a%), hepatic clearance, and gut metabolism (herein our calculation of F_a% assumes no gut metabolism)]. We employ solution dosing from a non-precipitating formulation to remove the dissolution component from these pharmacokinetic studies. The calculation of F_a% removes the hepatic CL component of F% and therefore represents the gut absorption component [31]. The F_a% of bis-valine **10** and bis-D-PhGly **15** are shown in Table 4 in rat, dog, and cyno-monkey. The F_a% in dog for both compounds is moderate to high, but the values in rat and cyno tell a different story. The F_a% of compound **15** is exceedingly low in both rat and cyno (each are 2% F_a), while

Table 3 Potency and metabolic stability derived from terminal amino acid modifications in benzypyrano-benzopyran series



11–18

Cpd	A	B	GT EC_{50} (pM)						Pred CL (L/h/kg) HLM
			1a	1b	2a JFH1 (L31)	2a JFH1-L31M[a]	2a J6 (M31)	2b (M31)	
11			31	19	9	12,100	–	1,850	0.18
12			53	20	20	31,300	–	–	0.21
13			55	22	22	>44,000[b]	–	–	–
14			380	800	74	–	330	600	–
15			93	48	7	145	240	22	0.19
16			185	172	14	1,330	1,430	–	0.23
17			87	79	10	3,670	–	–	0.21
18			50	31	4	600	1,820	76	0.24

[a]GT2a JFH-1 subgenomic transient replicon cells with L31M mutation
[b]Value of >44,000 means that the EC_{50} was not achieved at this highest concentration tested

Table 4 Fraction absorbed (F_a%) for benzopyrano-benzopyran series; bis-D-PhGly inhibitor 15 has low F_a% in rat and cyno

Cpd	Fraction absorbed (F_a%)		
	Rat	Dog	Cyno-monkey
10	23	70	15
15	2	34	2

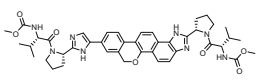
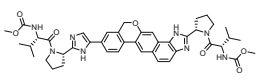
compound **10** shows moderate F_a% of 15–23%. It has been noted that drugs can undergo pericellular absorption in dogs due to the presence of "loose junctions" in their gut wall [32]. With our NS5A inhibitors, and other "rulebreaker" compounds, we have sometimes observed the trend of high F_a% in dog and low F_a% in rat and cyno. In these cases we therefore consider the high F_a% value for dog to be a species-specific value (for further examples, vide infra) and rely on rat and/or cyno values. Thus although compound **15** possesses important potency improvements, its low permeability presents a significant risk for progression. Since bis-valine compound

10 afforded moderate F_a% in rat and cyno, and the bis-D-PhGly inhibitor possessed beneficial potency, we next pursued mixed amino acid inhibitors maintaining one valine in hopes of retaining permeability in inhibitors **16**–**18**. In this context, valine/D-PhGly inhibitor **18** produced a good potency profile that was intermediate between the profiles of symmetric inhibitors **10** and **15**. Although all compounds measured in the benzopyrano-benzopyran series in Table 2 had low Pred CL values in HLM, these values are not low enough to project to pharmacokinetic half-lives sufficient for once-daily dosing in humans since they range from 0.18 to 0.24 L/h/kg (the steady-state volumes of distribution [V_{ss}] for these compounds are typically 1–2 L/kg). We therefore sought to investigate further core discovery with the hope of improving human metabolic stability and further improve pan-genotypic potency.

4 Fused-Pentacyclic Inhibitors and the Discovery of Velpatasvir (VEL)

We decided to push further to even more complex fused-ring systems within the core of the inhibitors [11, 28, 29]. Pyranyl ring systems had proven beneficial (inhibitors **7**–**10**) so one design direction we undertook focused on the pyran-containing fused pentacyclic ring inhibitors **19** and **20** (Table 5, see footnote 1). We envisioned two alternate ring systems. In one system the methyleneoxy of the pyran is vicinal to the central ring of the embedded naphthimidazole (**19**) and an alternate ring system where the methylenoxy is distal to the middle ring of the embedded naphthimidazole (**20**). These unprecedented ring systems and a number of their precursors proved difficult to synthesize and isolate. As luck would have it, inhibitor **19** was synthesized and isolated first and presented a devastating setback for the team – where the significant effort required to produce this compound was met with potency and HLM stability results inferior to that of the tetracyclic benzopyrano-benzopyran **10**. Nonetheless we persevered with the synthesis and isolation of target **20**, and

Table 5 Potency and metabolic stability of benzopyran-naphthimidazoles

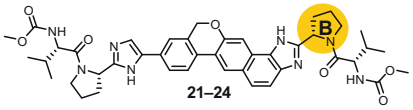
		GT EC_{50} (pM)								Fraction abs. (F_a%)	
	Cpd	1a	1b	2a JFH1 (L31)	2a J6 (M31)	2b (M31)	3a	4a	Pred CL (L/h/kg) HLM	Rat	Dog
	**19**	41	17	16	20,900	32,300	12	130	0.22	31	54
	**20**	16	17	4	1,160	162	4	8	<0.16	15	50

The metabolic stability and improved broad genotype potency of **20** is an important discovery

unexpectedly it showed a dramatic reversal in trend relative to its isomer **19**. *The core within inhibitor **20** contributes the greatest pan-genotype potency of any core system that we have synthesized.* For inhibitor **20** the replicon EC_{50} values for GT1, 3 and 4 range from 4 to 17 pM, while the challenging GT2a J6 EC_{50} is 1,160 pM and GT2b is 162 pM. The fold improvements for inhibitor **20** versus **19** are 18-fold for GT2a J6 and 199-fold for GT2b. Importantly, the pentacyclic benzopyrano-naphthimidazole **20** is stable within the conditions of the HLM assay (Pred CL < 0.16 L/h/kg), while the isomer **19** (along with the tetracyclic benzopyrano-benzopyran series) is less stable in HLM. The F_a% for **20** was 15% in rat showing potential for absorption. Interestingly, the pentacyclic benzopyrano-naphthimidazole would represent a new ring system in drug space; it has been noted that it is relatively rare to find newly applied ring systems in drugs [33]. We continued with investigation of inhibitors with the core system from pentacyclic benzopyrano-naphthimidazole **20**.

During our discovery of LDV, we found that modifications to the pyrrolidine rings could modulate GT1 potency and pharmacokinetic half-life [2, 3]. Thus in the pentacyclic benzopyrano-naphthimidazole core series, we pursued a number of pyrrolidine modifications, and important sets are represented in Tables 6 and 8.

Table 6 Single pyrrolidine modifications in the benzopyran-naphthimidazole series



B	Cpd	GT EC_{50} (pM)							Pred CL (L/h/kg) HLM	Fraction absorbed (F_a%)		
		1a	1b	2a JFH1	2aJ6 (M31)	2b (M31)	3a[a]	4a[b]		Rat	Dog	Cyno
	21[c]	11	18	19	234	–	77[d]	20[e]	–	–	–	–
	22	11	18	7	443	132	18	11	<0.16	10	–	–
	23	15	18	7	378	265	48	20	<0.16	–	–	–
	24	21	26	8	280	135	24	14	<0.16	16	56	–

[a]GT3a S52 NS5A transient chimeric replicon based on GT1b Rluc backbone
[b]GT4a ED43 NS5A transient chimeric replicons based on GT1b Rluc backbone
[c]The side chain of the valine on the methyl-pyrrolidine side is perdeutrated in compound **21**
[d]GT3a S52
[e]GT4a ED43

Table 6 (see footnote 1) shows a range of 4- and 5-position pyrrolidine substituents that each provides improved GT2a J6 potency relative to inhibitor **20** (**20** is unsubstituted at the 4- and 5-positions of the pyrrolidine). The 5-methyl inhibitor **21** afforded a GT2a J6 $EC_{50} = 234$ pM, although the GT3a potency eroded to 77 pM (relative to 4 pM in **20**). The 4-methyl inhibitor **22** was nearly as potent in GT 2a J6 (443 pM) as inhibitor **21** and had good GT3a potency at 18 pM. The 4-ethoxy inhibitor **23** had a relatively similar potency profile to that of the 4-methyl inhibitor. The 4-methoxymethyl substitution in inhibitor **24** improves on the 4-methyl and 4-ethoxy in GT2a J6 at 280 pM and is comparable to the 4-methyl inhibitor in GT3a affording the best overall potency profile we had yet seen. The 4-methoxymethyl emerged as an important pyrrolidine substituent. Importantly all inhibitors measured in HLM in the series are stable at the lower limit of the assay.

As has been apparent throughout the foregoing discussion, structural changes that improve potency against one genotype can lead to losses in potency against another. But as inhibitors trend toward improved potency across genotypes, we also noted that inhibitors can trend toward improved potency against RASs in various genotypes, although this remains a complex SAR. This trend is apparent in Table 7. We tested 4-methoxymethyl-substituted inhibitor **24** (best overall potency to this point) against clinically relevant GT1a RASs and show the results alongside those for LDV in Table 7. Inhibitor **24** displays improved potency against all GT1 RASs in Table 7 relative to LDV, with sub-nanomolar potency against most of these RASs. The complexity in the pattern of improvement is apparent from the observation that the fold improvement for each RAS from LDV to **24** is not constant and further that **24** *loses* potency relative to LDV versus GT1a and GT1b WT virus.

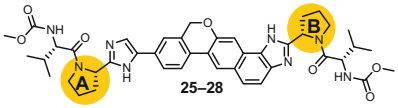
We continued with these modifications by pursuing substitutions on both pyrrolidines while keeping the beneficial 4-methoxymethyl substituent constant on pyrrolidine "B" for inhibitors **25**–**27** (Table 8, see footnote 1). Using 4-methoxymethyl substitution on both sides (**25**) drove the GT2a J6 potency to 61 pM (greatest potency for this subtype in Table 8) while being detrimental to GT3a potency (206 pM). Changing to the 4-methyl substituent lost some GT2a J6 potency (106 pM) while gaining GT3a potency (58 pM). Substitution of pyrrolidine "A" with a 5-methyl provided the best balance of potency in Table 8, with GT2a J6 and GT3a

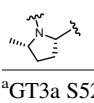
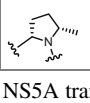
Table 7 Compound **24** has improved GT1 resistance profile

	GT1a EC_{50} (pM)								GT1b EC_{50} (pM)	
	WT	M28T	Q30H	Q30R	L31M	Y93C	Q30E	Y93H	WT	Y93H
LDV	31	1,900	5,700	19,600	17,000	49,600	169,000	52,000	4	7,200
24	37	190	150	220	370	220	10,400	4,700	26	86
Fold improvement of **24** over LDV	0.84X	10X	38X	89X	46X	225X	16X	11X	0.15X	84X

All RAS are transiently transfected GT1a or GT1b subgenomic HCV replicons

Table 8 Data for compounds with modifications to both pyrrolidines in the benzopyran-naphthimidazole series



A	B	Cpd	GT EC_{50} (pM) 1a	1b	2a JFH1	2aJ6 (M31)	2b (M31)	3a[a]	4a[b]	Pred CL (L/h/kg) HLM	Fraction absorbed (F_a%) Rat	Dog	Cyno
		**25**	24	34	10	61	180	206	36	<0.16	10	–	–
		**26**	14	19	9	106	166	58	22	<0.16	11	–	–
		**27**	11	17	12	67	157	61	15	<0.16	13	–	64
		**28**	8	12	14	280	63	20	8	<0.16	27	100	53

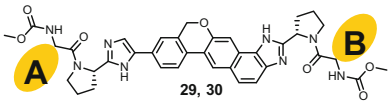
[a]GT3a S52 NS5A transient chimeric replicon based on GT1b Rluc backbone
[b]GT4a ED43 NS5A transient chimeric replicons based on GT1b Rluc backbone

nearly equipotent (67 and 61 pM, respectively), 11 pM GT1a activity, and GT2b potency at 157 pM. Assessing the 5-methyl substitution on both pyrrolidines (**28**) led to a less balanced profile than that of **27** with GT2a J6 potency at 280 pM. Thus the 4-methoxymethyl and 5-methyl work well in combination for potency. Notably, all compounds measured in Tables 6 and 8 have HLM Pred CL < 0.16 L/h/kg and low but double-digit F_a% in rat. Compounds **27** and **28** have good cyno F_a%.

Combining D-PhGly with valine in the benzopyrano-naphthimidazole series afforded inhibitors with potency below 134 pM (**29**) or 73 pM (**30**) for all genotypes tested (Table 9, see footnote 1). The concept (vide supra) that combining a valine on one side of the inhibitor with a D-PhGly on the other could provide a boost in permeability (relative to a bis-D-PhGly compound) is supported by the F_a% of 13% and 16% in rat and cyno, respectively, for inhibitor **29**.

We sought to combine the beneficial pan-genotypic potency discoveries of the pyrrolidine substitutions with those of the D-PhGly. We approached this by determining if there was a matched or mismatched pairing of the 4- or 5-pyrrolidine with the D-PhGly. We pursued this in the symmetric tetracyclic benzopyrano-benzopyran inhibitor series in Table 10 (see footnote 1) for ease of synthesis. Indeed, the 5-methyl-pyrrolidine inhibitor provides a mismatch with the D-PhGly (less potent than the parallel unsubstituted pyrrolidine inhibitor **15** for the critical GT1a and

Table 9 D-PhGly improves overall potency profile

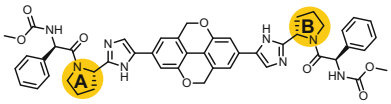


29, 30

A	B	Cpd	GT EC_{50} (pM) 1a	1b	2a JFH1	2aJ6 (M31)	2b (M31)	3a[a]	4a[b]	Pred CL (L/h/kg) HLM	Fraction absorbed (F_a%) Rat	Dog	Cyno
		**29**	22	21	4	134	12	5	8	<0.16	13	56	16
		**30**	35	33	6	73	16	6	11	<0.16	–	–	–

[a]GT3a S52 NS5A transient chimeric replicon based on GT1b Rluc backbone
[b]GT4a ED43 NS5A transient chimeric replicons based on GT1b Rluc backbone

Table 10 5-Methyl-pyrrolidine generally antagonizes, and 4-methyl-pyrrolidine generally synergizes, with D-PhGly relative to the des-methyl-pyrrolidine inhibitor **15**



15, 31, 32

A	B	Cpd	GT EC_{50} (pM) 1a	1b	2a JFH1	2aJ6 (M31)	2b (M31)	3a[a]	4a[b]	Pred CL (L/h/kg) HLM	Fraction absorbed (F_a%) Rat	Dog
		**15**	93	48	7	240	22	–	–	0.19	2	34
		**31**	150	20	8	1,835	94	4	15	<0.16	3	43
		**32**	31	20	5	58	26	6	13	<0.16	2	30

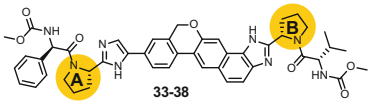
[a]GT3a S52 NS5A transient chimeric replicon based on GT1b Rluc backbone
[b]GT4a ED43 NS5A transient chimeric replicons based on GT1b Rluc backbone

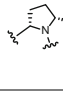
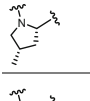
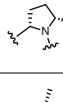
GT2a J6). In contrast, the 4-methyl-pyrrolidine **32** provides a matched pair with the D-PhGly and is similar or improved in potency against all genotypes relative to **15**, with GT1a and GT2a J6 showing threefold and fourfold improvements, respectively. Again, like inhibitor **15**, these bis-D-PhGly inhibitors **31** and **32** have very low F_a% in rat (3 and 2%, respectively) and the apparently species-specific high permeability

in dog. Thus despite the excellent pan-genotypic potency of inhibitor **32**, its low permeability renders it a high risk for development.

We intended to exploit the finding of potency synergy between 4-pyrrolidine substitution and D-PhGly (Table 10) in the pentacyclic benzopyrano-naphthimidazole series (Table 11, see footnote 1). Indeed combination of the 4-methoxymethyl-pyrrolidine with the D-PhGly in inhibitor **33** provided our most potent pan-genotypic inhibitor to this point, with potencies for all genotypes $\leq$19 pM. Unfortunately the F_a% for this inhibitor is low in rat (12%) and very low in cyno (3%). Contrary to prevailing thought, we have found that small structural changes in "rulebreaker" chemical space (changes that make no significant change in the rule-based calculated metric values) can provide significant shifts in measured

Table 11 Pyrrolidine "B" modification improves potency and fraction absorbed resulting in velpatasvir (**2**)



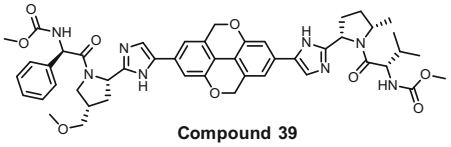
A	B	Cpd	GT EC_{50} (pM)							Pred CL (L/h/kg) HLM	Fraction absorbed (F_a%)		
			1a	1b	2aJFH1	2aJ6 (M31)	2b	3a[a]	4a[b]		Rat	Dog	Cyno
		**33**	11	19	6	13	9	17	7	<0.16	12	–	3
		**34**	13	17	6	14	15	25	11	<0.16	9	–	4
		**35**	10	11	6	113	46	18	9	0.24	–	–	–
		**36**	13	20	7	21	28	25	9	<0.16	13	–	–
		**2 VEL**	**14**	**16**	**8**	**16**	**6**	**4[c]**	**9**	**<0.16**	**36**	**29**	**37**
		**37**	8	5	4	53	15	20		<0.16	4	–	–
		**38**	33	30	18	45	27	50	63	<0.16	4	–	–

[a]GT3a S52 NS5A transient chimeric replicon based on GT1b Rluc backbone
[b]GT4a ED43
[c]GT3a S52 transiently transfected subgenomic HCV replicon

pharmacokinetic parameters (such as fraction absorbed or bioavailability). Thus we held the matched pairing of methoxymethyl and D-PhGly constant while making modifications to the pyrrolidine "B" seeking to improve the F_a%. Substitution with 4-methyl in (**34**) did not provide F_a% improvement, and cyclopropyl ring fusions (which can be considered an interpolation of the 4- and 5-methyl substitution in isomers **35** and **36**) resulted in a loss of GT2a J6 potency in **35** and no benefit in F_a% in **36**. Strikingly, moving to a 5-methyl substitution in inhibitor **2** resulted in a positive frameshift in F_a% in both rat and cyno to 36 and 37%, respectively. Further inhibitor **2** maintained high pan-genotypic activity at $\leq$16 pM for the genotypes detailed in Table 11. This standout inhibitor **2** was selected for development and is now known as velpatasvir [11, 12, 28, 29].

Further close analogs are inferior to VEL; **37** (where the 4-methoxymethyl of VEL is replaced by methyl) and **38,** which is the isomer of **37,** display low fractions absorbed in rat (4%). Continuing with the theme of variations related to VEL, the inhibitor where the pentacyclic benzopyrano-naphthimidazole of VEL is "swapped" for the tetracyclic benzopyrano-benzopyran imidazole-based core (compound **39**, Table 12, see footnote 1) was synthesized and tested. Although **39** displays the most advanced overall potency and PK profile in the tetracyclic benzopyrano-benzopyran series, it falls short of VEL in potency for GT2 and GT3, HLM stability, and F_a% in rat. Other inhibitors with similar structures to VEL (depicted in Fig. 2) were synthesized and also have inferior characteristics: The dihydro analog **40** has threefold higher HLM Pred CL, the oxo inhibitor **41** loses 2- to 12-fold in potency across GT1–4, and "flipping" the core results in compound **42** that has a 13% F_a% in rat (Fig. 2).

Table 12 Replacing the benzopyrano-naphthimidazole core of VEL with the benzopyrano-benzopyran core results in an inferior profile



Compound 39

GT EC_{50} (pM)										Fraction abs. (F_a%)	
1a	1b	2aJ6	2b	3a[a]	4a[b]	5a	6a	6e	Pred CL (L/h/kg) HLM	Rat	Monkey
16	16	480	290	120	13	81	22	110	0.20	29	26

[a]GT3a S52 NS5A transient chimeric replicon based on GT1b Rluc backbone
[b]GT4a ED43

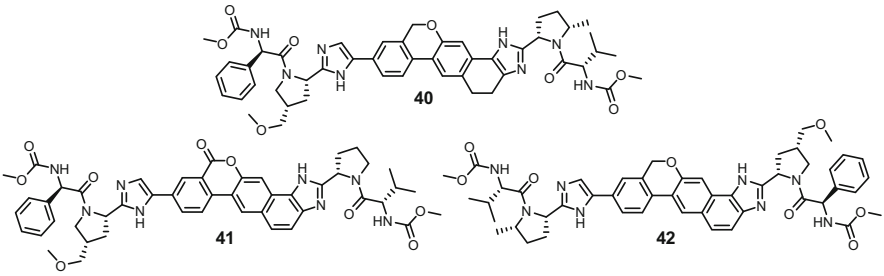
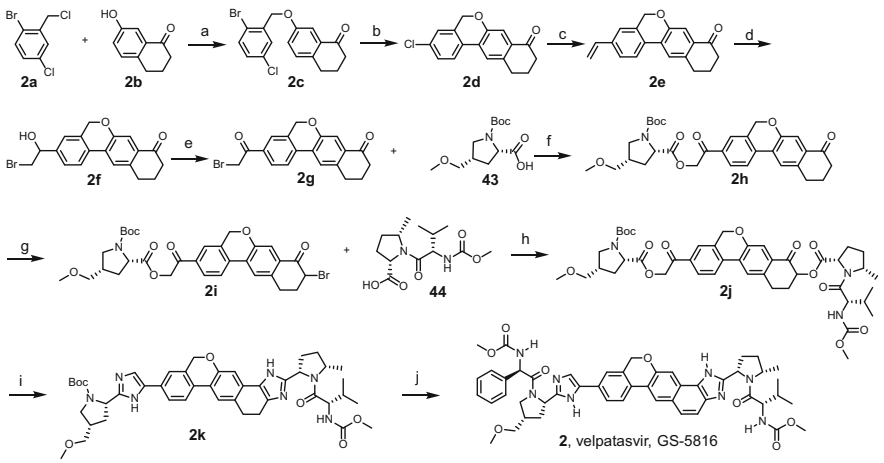


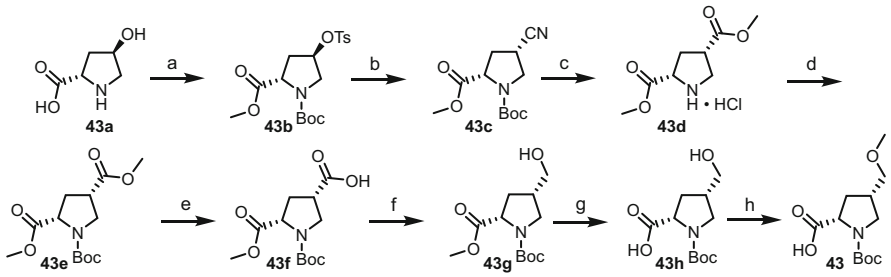
Fig. 2 Comparator structures to velpatasvir

5 Velpatasvir Synthesis

A synthesis of velpatasvir and the intermediate Boc-4-methoxymethylproline (**43**) are outlined in Schemes 2 and 3 [12, 28, 29]. Treatment of phenol **2b** with benzylic chloride **2a** under basic conditions forms ether **2c**, which undergoes palladium-mediated biaryl coupling to form tetracyclic ketone **2d**. Vinylation of **2d** forms styrene **2e** which is converted to the bromohydrin **2f** with NBS under hydrolytic conditions. The bromoketone **2g** resulting from oxidation of **2f** is used to alkylate the acid of Boc-protected methoxymethylproline **43** to form ester **2h**. Bromoketone **2i** results from bromination of **2h** and is then alkylated with dipeptide acid **44** to form diketo-diester **2j**. Heating **2j** in the presence of ammonium acetate effects double imidazole formation to form the fused pentacycle in **2k**. Oxidation completes the



Scheme 2 Synthesis of VEL. (**a**) K_2CO_3, DMAc. (**b**) $Pd_2(dba)_3$, P(4-F-Ph)$_3$, PivOH, K_2CO_3. (**c**) $CH_2CH_2BF_3K$, $Pd(OAc)_2$, S-Phos, n-PrOH. (**d**) NBS, THF/DMSO/H_2O. (**e**) MnO_2, CH_2Cl_2. (**f**) **43**, K_2CO_3, CH_2Cl_2. (**g**) $PyHBr_3$, CH_2Cl_2, MeOH. (**h**) **44**, Cs_2CO_3, MeTHF. (**i**) NH_4OAc, toluene, 2-methoxyethanol. (**j**) (i) MnO_2, CH_2Cl_2 (ii) HCl, dioxane, CH_2Cl_2, and (iii) methoxycarbonyl-D-phenylglycine, COMU, i-Pr_2NEt, DMF



Scheme 3 Synthesis of Boc-methoxymethylproline. (**a**) (i) $SOCl_2$, CH_2Cl_2, (ii) Boc_2O, $NaHCO_3$, CH_2Cl_2, H_2O, and (iii) TosCl, Et_3N, DMAP, CH_2Cl_2. (**b**) NaCN, DMSO. (**c**) AcCl, MeOH. (**d**) Boc_2O, $NaHCO_3$, EtOAc, H_2O. (**e**) NaOH, MeOH. (**f**) BH_3•THF, MeTHF. (**g**) NaOH, MTBE. (**h**) MeI, NaOtBu, THF

elaborated core, and Boc-deprotection followed by coupling with methoxycarbonyl-D-phenylglycine provides velpatasvir (**2**).

Boc-protected methoxymethylproline (**43**) is synthesized starting with hydroxyproline (**43a**) (Scheme 3). Esterification, Boc-protection, and tosylation provide **43b**. Tosylate displacement forms cyano-proline **43c,** and its treatment with methanolic HCl effects cyano conversion to the methyl ester along with Boc-deprotection (**43d**). Reprotection and selective ester hydrolysis provide acid **43f** which is reduced with borane to alcohol **43g**. Ester hydrolysis and alcohol methylation afford Boc-protected methoxymethylproline (**43**).

Our commitment of extensive resources to the discovery of new cores was a fundamental aspect of our NS5A program. It has been posited that chemists resort to known ring systems due to the chemical synthesis resource cost of novel ring systems [34]. The pentacyclic benzopyrano-naphthimidizole is a novel ring system, and its discovery, despite its chemical and synthetic complexity, proved important.

6 Velpatasvir Nonclinical Data and Clinical Antiviral Activity

In vitro protein binding and metabolism and in vivo pharmacokinetic parameters for VEL are shown in Table 13. VEL is highly protein-bound with 0.2–0.4% free drug in rat, dog, and monkey plasma; the human plasma value is intermediate in this range at 0.3% free. The predicted clearance from microsomes is low across nonclinical species, and the values are similar to the clearance observed in vivo, suggesting that the main route of clearance is hepatic oxidative metabolism. Since VEL did not show measurable instability in our routine HLM assay (Table 11, Pred CL < 0.16 L/h/kg), ^{3}H-VEL was assayed in human hepatocytes with quantitation of metabolites. This method affords accurate measurement of metabolism at lower levels than in the routine assay and resulted in a low Pred CL for VEL of 0.06 L/h/kg. In rat, dog, and

Table 13 VEL in vitro protein binding and metabolism and in vivo PK parameters in preclinical species and healthy volunteers

		In vitro		In vivo			
	Dose (route)	% Free plasma	Pred CL microsomes (L/h/kg)	CL (L/h/kg)	V_{ss} (L/kg)	$t_{½}$ (h)	F%
Rat	(IV)	0.2	0.74	0.94	1.61	2.25	28
Dog	(IV)	0.2	0.37	0.25	1.43	5.20	25
Monkey	(IV)	0.4	<0.17	0.30	1.60	5.03	30
Human	100 mg (PO)	0.3	0.06[a]	–	–	15.7	~50[b]

[a]Data generated in hepatocytes using ^{3}H-VEL
[b]Calculated from human oral CL/F and human predicted clearance from nonclinical data

cyno, the steady-state volumes of distribution (V_{ss}) are higher than total body water and range from 1.4 to 1.6 L/kg. Half-lives and bioavailabilities range from 2.2 to 5.2 h and 25–30%, respectively [11]. Taken together the nonclinical data are consistent with the potential for VEL to be dosed once-daily with low clearance and low dose in human and were the basis for the progression of VEL into clinical studies.

Gratifyingly the human half-life of VEL is 15.7 h at the 100 mg dose used in Epclusa®, consistent with once-daily dosing (Table 13) [35]. Estimation of the human bioavailability from the clinical solid dosage form is 50% and exceeds the solution-dosed bioavailability values in rat, dog, and cyno. The plasma exposure curves after three oral doses in healthy volunteers are shown in Fig. 3. The concentration of VEL at 24 h post the third dose exceeds its GT1–6 average protein adjusted EC_{50} at all dose levels from 5 to 450 mg total dose (dotted line, Fig. 3).

Sets of rules seeking to predict orally bioavailable and "drug-likeness" chemical space have proliferated in the medicinal chemistry literature since the seminal 1996 publication by Lipinski on the "Rule of 5" [36]. Rule-based dogma now pervades the medicinal chemistry literature. A subset of such rules is noted in Table 14, along with the "rule limit value" and the values calculated for LDV and VEL. These rule-based approaches typically define a count or calculation and then set limit values at 90th or 95th percentile of known oral drugs falling below the limit. LDV and VEL are "rulebreakers" [30], violating three-out-of-four of Lipinski's Rule of 5 (two violations are needed to "break" the Rule of 5) and breaking each of Veber's rotatable bond and polar surface area rules [37, 38], the number of aromatic ring rule [38], and the number of total ring rules [33]. Both LDV and VEL values fall significantly beyond the majority of the rule limit values. The values in Table 14 are provided for reference and discussion; we did not calculate, consider, nor abide by these limiting rule-based concepts in the discovery of the orally bioavailable inhibitors LDV or VEL. Instead, as discussed herein, we utilized in vitro and in vivo data along with hypothesis generation and testing to guide our design.

As we improved the pan-genotypic activity of our inhibitors, we trended toward improvement of their inhibitory activity against RASs; the structure-activity

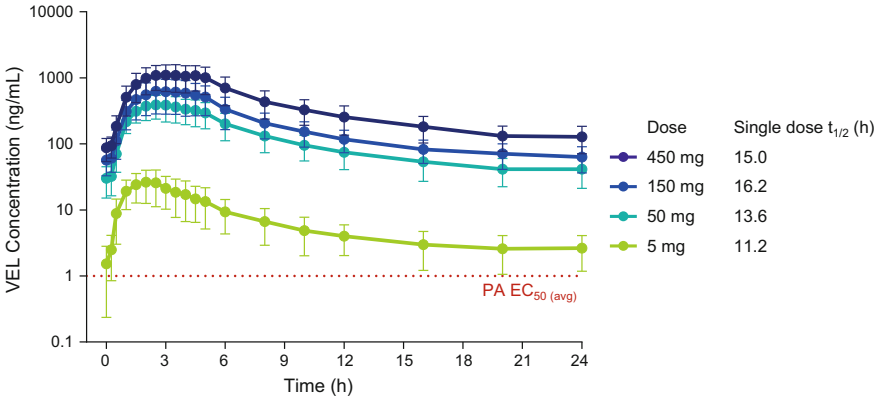


Fig. 3 VEL PK in healthy volunteers after third daily dose

Table 14 Ledipasvir and velpatasvir are rulebreakers: bioavailability and "drug-likeness" rule-based metrics

Rule	Parameter	Rule limit value	LDV value	VEL value
Lipinski rule of 5 [36]	Molecular weight	≤500	889	883
Lipinski rule of 5	CLogP[a]	≤5	6.71	5.70
Lipinski rule of 5	H-bond donors	≤5	4	4
Lipinski rule of 5	H-bond acceptors[b] (sum N + O)	≤10	14	16
Veber [37, 38]	Rotatable bonds[c]	≤10	12	13
Veber	Polar surface area[c]	<140 Å	174 Å	193 Å
Ring rule [33]	# of rings	≤5 is 95th percentile	10	9
Aromatic ring rule [38]	# aromatic rings	≤3	5	6

[a]ChemBioDraw 14.0, CambridgeSoft Corporation
[b]Sum of N's and O's as defined by Lipinski et al.
[c]Pipeline pilot

relationships of our various NS5A inhibitors against differing genotypes and their subtypes and RASs are complex. During our discovery of velpatasvir, clinical trial data [39] afforded information regarding GT1 RASs in the NS5A coding region. VEL displays improved replicon potency against a selection of GT1a RASs where LDV shows reduced activity. VEL affords sub-nanomolar activity against all RASs in Table 15 except GT1a Y93H where its EC_{50} is 8.5 nM [1].

As noted (vide supra), we utilized published databases and in-house sequences to identify prevalent sequence variants from patient isolates for non-GT1 sequences. We then generated replicons bearing these variants to determine if they had reduced

Table 15 LDV and VEL potency against clinically relevant GT1 resistance-associated substitutions (RAS)

	GT1a EC_{50} (nM)								GT1b EC_{50} (nM)	
	WT	M28T	Q30H	Q30R	L31M	Y93C	Q30E	Y93H	WT	Y93H
LDV	0.031	1.9	5.7	19.6	17	49.6	169	52.0	0.004	7.2
VEL	0.014	0.105	0.032	0.031	0.22	0.053	0.25	8.5	0.016	0.011

All RASs are transiently transfected GT1a or GT1b subgenomic HCV replicons

susceptibility to our inhibitors. Thus we worked to define clinically relevant non-GT1 RASs to guide our NS5A inhibitor discovery process. While a number of these variants that were produced did not exhibit significantly reduced susceptibility to our inhibitors, there were others that did. Table 16 shows the activity of LDV and VEL against a number of these variants that proved challenging to inhibit [1]. These replicons helped guide our discovery process. For example, our selection of VEL for clinical studies was based in part on its high activity against GT3a A30K. Indeed, after initiating clinical studies with VEL, a report of an NS5A inhibitor in combination with sofosbuvir noted relapse with the presence of GT3a A30K virus [40]. VEL is potent with an EC_{50} of 210 pM against GT3a A30K. VEL has improved potency over LDV for all GT2 and GT3 RASs in Table 16.

Velpatasvir produced rapid and sustained viral suppression at all monotherapy dose levels (5–150 mg total dose) in GT1–4-infected individuals [26]. Median viral load reduction (VLR) results versus time for three once-daily oral monotherapy doses (50 or 150 mg) of VEL is plotted in Fig. 4 (three total doses per patient). The VLR for three once-daily oral monotherapy 90 mg doses of LDV (from an independent study) is also plotted in Fig. 4 for reference. The median maximal VLR for each dose is also provided in Fig. 4 [41]. The GT1a median maximal VLR for VEL is 4.2 log10 for both the 50 and 150 mg doses, while it is 3.2 log10 for LDV (90 mg). At all doses the inhibitory quotient (IQ or concentration-fold above the protein adjusted EC_{50} 24 h post-dose, e.g., IQ > 470 for 90 mg LDV, data not shown) for WT virus is very high. The greater VLR for VEL over LDV is a probable consequence of the increased potency of VEL against pre-existing (baseline) RASs (Table 15). A wide range of viral species is present in each patient's sera, and suppression of viral load achieves a maximal value when the drug IQ is insufficient to suppress given RASs that are present in the patient. The pharmacokinetic half-life, WT IQ, and dose are all greater for LDV at the 90 mg dose than for VEL at the 50 mg dose, and therefore it can be seen that these factors are not the drivers of the maximal VLR. VEL encounters viral species with reduced susceptibility prevalent at ~4.2 log10 below baseline, while LDV encounters such species ~3.2 log below baseline. As a further consequence of VEL's potent activity against RASs, VEL maintains nearly maximal suppression of virus in GT1a patients 3 days post the last dose, and in GT1b and GT2 patients, maximal suppression is maintained 5 days post the last dose. GT3 and GT4 patients remain suppressed ~1 and ~1.5 log10 at 5 days post the last dose, respectively (Fig. 4).

Table 16 LDV and VEL potency against GT2 and GT3 RAS

	GT2a EC_{50} (nM)				GT2b EC_{50} (nM)				GT3a EC_{50} (nM)		
	WT	K44R	N62V	N62S	WT[a](L31M)	WT[b](L31M)	R44K	P58S	WT	A30K	Y93H
LDV	209	164	222	123	865	211	943	292	>44.8	>88.8	>88.8
VEL	0.009	0.005	0.01	0.003	0.004	0.007	0.011	0.01	0.013	0.21	3.2

GT2a WT is JFH-1 Renilla luciferase (Rluc) subgenomic replicon. GT2a RASs are GT2a backbone which encode the full-length NS5A gene from various GT2a clinical isolates carrying the indicated RAS

GT2b WT are chimeric replicons based on GT2a JFH-1 Rluc subgenomic replicon, encoding the full-length gene from GT2b

GT3a WT is GT3a S52 chimeric replicon based on GT1b Rluc subgenomic replicon backbone. GT3a RASs were produced by site-directed mutagenesis in the WT replicon. ">" indicates the highest concentration tested and EC_{50} was not achieved

[a]MD2b-1 strain or [b]AY232738. GT2b RASs were constructed similarly using GT2b clinical isolates carrying the indicated RAS

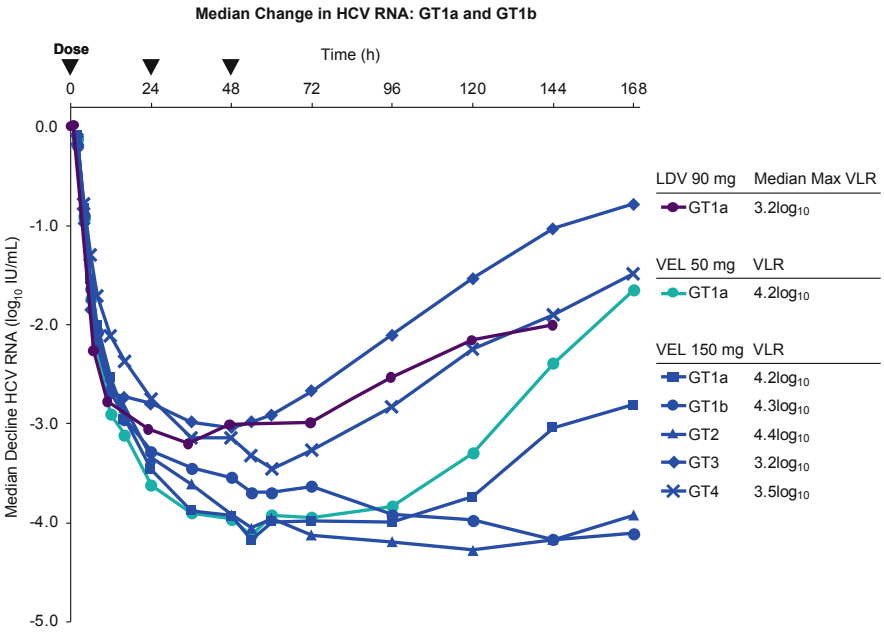


Fig. 4 Viral load reduction, three daily doses in monotherapy: LDV or VEL. LDV data from a different study

Data points in Fig. 5 depict the maximal viral load attained for each treated GT1a patient in the 5–150 mg cohorts (three once-daily monotherapy doses) [26]. Viral deep sequencing data for GT1a at baseline (prior to treatment, 1% prevalence is the lower limit of detection) are notated in Fig. 5, with some infected individuals displaying extensive pre-existing RASs present at $\geq$1% (limit of detection). All individuals experienced viral load reductions despite the presence of measurable baseline RASs. All RASs assessed in replicons in Table 15 except Q30R are represented in Fig. 5, and the replicon potencies of VEL against GT1a M28T, Q30H, L31M, Y93C, Q30E, and Y93H are all validated through observed viral load reductions in vivo. At the 5 mg dose, the mean maximal VLR was greater than 4 log10, although by happenstance in this cohort no RAS was detected at baseline (1% limit of detection). In contrast one patient in the 50 mg cohort had seven pre-existing RASs, with individual RAS percentages totaling >100%, suggesting that some of these RASs exist at least as double mutants in this patient. Even this individual experienced a >1 log10 viral load reduction.

Maximal VLR are plotted, and the measurable baseline RASs are noted for non-GT1a individual patients in the 150 mg cohort (three once-daily monotherapy doses) in Fig. 6. All individuals with measurable RASs achieved >3 log10 VLR, validating the replicon potency of VEL against GT1b Y93H (>5 log VLR); GT2b L31M (two individuals with >99% prevalence, both >4.5 log10 VLR); and GT3a

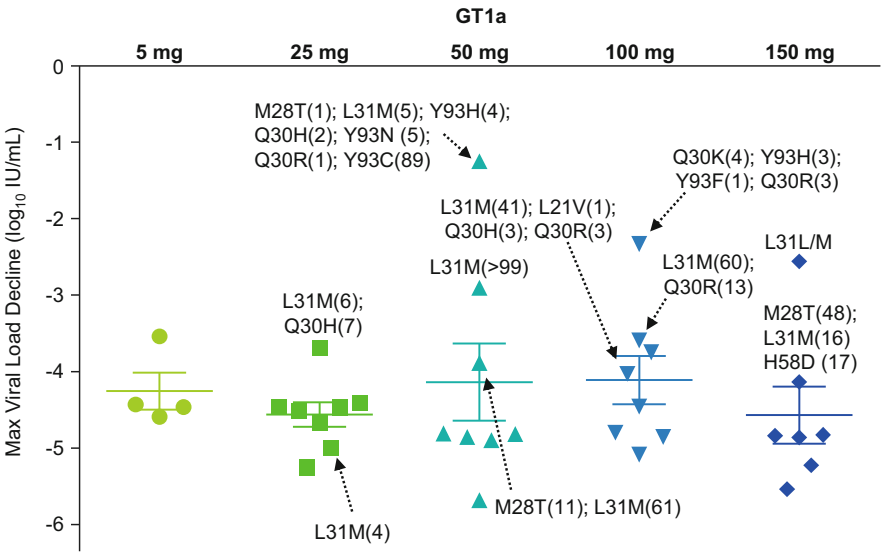


Fig. 5 GT1a VEL 3-day dosing monotherapy, maximum VLR by individual patient. Mean VLR and standard deviation are provided with the horizontal lines. Individuals with baseline RAS are annotated (measured by deep sequencing, 1% limit of detection) with percent prevalence in parentheses

Y93H and A30K (each >3 log10 VLR). Each of the foregoing RASs is susceptible to VEL in replicon assays in Table 16.

The potent VLR in GT1–4 patients (Fig. 4) and against the wide range of pre-existing GT1–4 RAS in patients (Figs. 5 and 6) validates the replicon assay strategy and inhibitor design approach described herein leading to the discovery of VEL.

7 Approval of Epclusa® (SOF/VEL) and Vosevi®(SOF/VEL/VOX)

With velpatasvir we achieved our goal of discovering an NS5A inhibitor with pan-genotypic potency and pharmacokinetics favorable for inclusion in STRs for the treatment of HCV infection. Epclusa® and Vosevi® are the only approved pan-genotypic STRs for the treatment of HCV infection.

In order to simplify therapy, clinical trial design with SOF/VEL focused on defining a single 12-week treatment duration for all patients regardless of genotype, prior treatment experience, cirrhosis status, or the presence of baseline RAS. (A review of the SOF/VEL clinical development can be found in this volume of HCV: The Journey from Discovery to a Cure.) Simplicity of therapy has been achieved with the STR Epclusa® which provides an overall SVR of 98% for

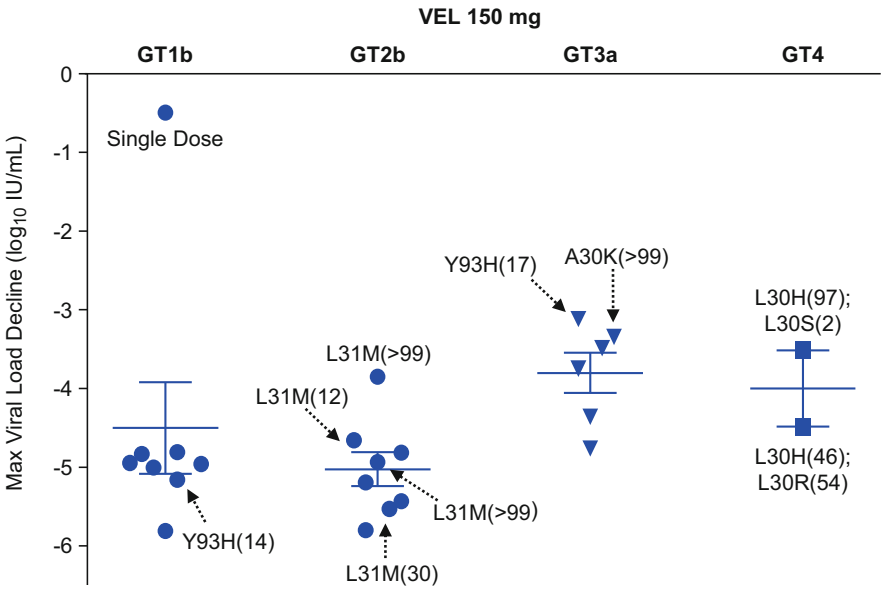


Fig. 6 GT1–4 VEL 3-day dosing monotherapy, maximum VLR by individual patient. Individuals with baseline RAS noted (measured by deep sequencing) with prevalence in parentheses

GT1–6 patients without cirrhosis or with compensated cirrhosis (ASTRAL Phase 3 clinical trials) [15–19] (https://www.gilead.com/~/media/files/pdfs/medicines/liver-disease/epclusa/epclusa_pi.pdf. Accessed 24 June 2018). The high SVR rates in the ASTRAL trials were achieved including a wide range of genotypic subtypes: multiple baseline RASs were present in every treatment arm, and 35 subtypes and 13 new or mixed subtypes were represented in these trials. Epclusa® is the first regimen approved for treatment of patients with GT1–6 HCV infection. Epclusa®'s simplification of the HCV treatment landscape upon FDA approval (6/28/16) is apparent in Table 17. Complexities or limitations associated with other approved regimens included contraindication for treatment of some genotypes 1–6; multiple daily pills; twice-daily dosing including differing pill count during day and evening dosing; differing treatment durations (in some cases inclusion of RBV) depending on baseline viral load, presence of baseline RASs, or the presence of compensated cirrhosis; contraindication for the presence of cirrhosis; differing dosages of NS5A inhibitor depending on co-medications (victim drug interactions); and regimen-dependent requirement for pre-treatment testing of HCV genotype, level of cirrhosis, viral load, or baseline RAS (Table 17).

Additionally Vosevi® (SOF 400 mg, VEL 100 mg, VOX 100 mg) has been approved for GT1–6 treatment-experienced patients and affords 96% SVR for patients who failed previous treatment that included an NS5A inhibitor or 98% SVR for patients who failed a treatment not including an NS5A inhibitor (FDA approval July 18, 2017) [20] (https://www.accessdata.fda.gov/drugsatfda_docs/

Table 17 American Association for the Study of Liver Diseases recommended regimens at the time of SOF/VEL FDA approval

GT	± CC	LDV/SOF	SIM+SOF	PTV/RTV/ OBV+DSV	PTV/RTV/OBV +DSV+RBV	PTV/RTV/ OBV+RBV	DCV+SOF	EBR/GZB	SOF/VEL
Approval Date		10/10/14	11/5/14	12/19/14	12/19/14	7/24/15	7/24/15	1/28/16	6/28/16
1a	–	8 or 12 weeks	12		12		12 (30/60/90 mg)[a]	12 or 16+RBV (12 if no RAS)	12
	+	12 weeks						12 or 16+RBV (12 if no RAS)	12
1b	–	8 or 12	12	12			12 (30/60/90 mg)[a]	12	12
	+	12		12				12	12
2	–								12
	+								12
3	–						12 (30/60/90 mg)[a]		12
	+						24[a] ± RBV		12
4	–	12				12		12	12
	+	12						12	12
5	–	12							12
	+	12							12
6	–	12							12
	+	12							12

Nucleotide NS5A Protease NS5B RBV PK Booster ✷☾ Morning and evening dose if 2x daily 1 pill Not recommended Recommended

Number in green box represents treatment time in weeks
LDV/SOF 8 weeks for patients with VL < 6 million IU. EBR/GZB GT1a pre-treatment NS5A RAS testing is recommended
Date corresponds to the first FDA approval for each drug combination (some combinations added further indications at later dates)
[a]DCV dose depends on victim drug interactions with certain co-dosed HIV drugs
+CC compensated cirrhosis, *−CC* non-cirrhotic, *DCV* daclatasvir, *DSV* dasabuvir, *EBR* elbasvir, *GZB* grazoprevir, *OBV* ombitasvir, *PTV* paritaprevir, *RBV* ribavirin, *RTV* ritonavir, *SIM* simeprevir

label/2017/209195s000lbl.pdf. Accessed 10 June 2018); Vosevi® is also approved for 8-week therapy in GT1–6 treatment-naïve patients affording 95% SVR [21] (European Medicines Agency).

8 Epclusa® (Sofosbuvir/Velpatasvir) Real-World Effectiveness

The real-world effectiveness [42, 43] comparing Harvoni® (LDV 90 mg, SOF 400 mg) to the earlier IFN-based standard of care is discussed in the ledipasvir discovery chapter in this volume and represented a major advance for the treatment

of patients with HCV infection. Real-world SVR rates for IFN-based therapy are as low as 3% reflecting the complexity, toxicity, and poor efficacy of these regimens, and IFN-based regimens are therefore untenable for widespread therapeutic use [13]. In contrast, Harvoni affords real-world SVR rates comparable to the ≥95% SVR achieved in controlled clinical trial settings [44] and accordingly has played a major role in the eradication of the virus within the 200,000 US veteran's administration HCV-infected patients, with eradication targeted for the end of 2018 [45].

Simplification of therapy has further advanced with the safety and efficacy of the pan-genotypic Epclusa® 12-week regimen and translates to high cure rates in real-world settings (Table 18). In three real-world efficacy studies, Epclusa® afforded high SVR rates: 97% (91/94 GT2 patients and 66/68 GT3 patients; TRIO network) [46] and 99.5% (213/214 GT3 patients, German hepatitis C cohort [GECCO]) [19]. The results from the GECCO study are even more striking considering that GT3 infection had been considered the most difficult genotype to cure early in the DAA era [47].

Epclusa®'s practicality as a pan-genotypic single duration treatment regimen has been applied to a minimal monitoring study with no on-treatment study assessments in a resource-limited setting. Broad enrollment criteria allowed inclusion of patients

Table 18 SOF/VEL or SOF/VEL/VOX clinical trial (Phase III) and real-world effectiveness SVR results

Study	GT	Study characteristics	Overall SVR%	
ASTRAL-1	1, 2, 4–6	Treatment-naïve/treatment-experienced cirrhotic and non-cirrhotic	99%	(618/624)
ASTRAL-2	2	Treatment-naïve/treatment-experienced cirrhotic and non-cirrhotic	99%	(133/134)
ASTRAL-3	3	Treatment-naïve/treatment-experienced cirrhotic and non-cirrhotic	95%	(264/277)
ASTRAL-4	1–4	Decompensated cirrhosis	83%	(75/90)
ASTRAL-5	1–4	HIV/HCV coinfected; treatment-naïve/treatment-experienced cirrhotic and non-cirrhotic	95%	(99/104)
Trio GT2	2	Real-world effectiveness	97%	(91/94)
Trio GT3	3	Real-world effectiveness	97%	(66/68)
GECCO	3	Real-world effectiveness	99.5%	(213/214)
POLARIS-1	1–6	NS5A-experienced	96%	(253/263)
POLARIS-2	1–6	8 week, DAA-naïve, 18% with cirrhosis	95%	(477/501)
POLARIS-3	3	8 week, DAA-naïve, 100% with cirrhosis	96%	(106/110)
POLARIS-4	1–6	DAA-experienced, no NS5A	98%	(178/182)

ASTRAL and POLARIS are Phase III clinical trials
SOF/VEL/VOX in POLARIS 1–4. All others are SOF/VEL

with any HCV genotype, with or without cirrhosis, and with treatment status as naïve or experienced. The overall SVR rate was 93% (including five patients lost to follow-up and one who withdrew consent) and 97.5% when based on virologic failure in this study in India [48].

9 Conclusion

The treatment of HCV-infected individuals with direct-acting antivirals represents the first time that a widespread, life-threatening chronic disease can be cured. The simple, safe, and effective pan-genotypic STRs Epclusa® and Vosevi® provide a means for broad treatment and cure of HCV-infected individuals worldwide.

Acknowledgment The author would like to thank the Gilead research and development teams and external clinical investigators who contributed to the discovery and development work described in this chapter and patients and healthy volunteers who have participated in the clinical trials described here.

Compliance with Ethical Standards

Conflict of Interest: John O. Link is an employee of Gilead Sciences, Inc.

Ethical approval: All procedures performed in the studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent: Informed consent was obtained from all individual participants included in the study.

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Discovery of Elbasvir

Craig Coburn

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Abstract On January 28, 2016, the US Food and Drug Administration approved Zepatier™ (a fixed-dose two-drug combination containing the NS5A inhibitor elbasvir and the NS3/4A protease inhibitor grazoprevir) for the treatment of adult patients with chronic hepatitis C virus genotype 1 or genotype 4 infection. The discovery of elbasvir (EBR) was the result of a concerted research effort within Merck's newly formed External Basic Research (also, EBR) group and a team of scientists from WuXi AppTec. Lead ID efforts combined components from both internal and literature compounds to generate a first-generation benzofuran-based NS5A inhibitor (MK-4882) that demonstrated preclinical proof of concept before entering phase 1 clinical trials. Lead optimization efforts and refinement of the core structure ultimately led to the identification of elbasvir, a ring-constrained tetracyclic indole-based analogue of MK-4882 which showed increased potency against clinical resistance variants and an improved resistance profile.

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Keywords EBR, Elbasvir, External research, HCV, MK-4882, MK-8742, NS5A inhibitor, Zepatier

1 Background

A series of breakthrough cures for hepatitis C began earning approval from the US FDA in 2014 of which inhibitors targeting the viral nonstructural protein 5A (NS5A) emerged as a key component of the direct-acting antiviral (DAA) regimens. As of March 2018, these included daclatasvir, ombitasvir, ledipasvir, elbasvir, pibrentasvir, and velpatasvir – each of which shows remarkable potency against a wide variety of genotypes and resistance mutations. Combination of these agents with other DAAs has demonstrated sustained virologic response rates >90% after only 8–12 weeks of therapy.

NS5A inhibitors were originally identified by phenotypic screening campaigns using HCV sub-genomic replicons, and these efforts produced a number of structural chemotypes that displayed low nanomolar EC_{50} values against genotype 1b but weaker activity against other genotypes. Pioneering work by the BMS team on a series of symmetric bis-pyrrolidines catalyzed discovery programs across pharma with a seminal report in 2010 that showed clinical validation of a highly potent and pan genotypic inhibitor of HCV NS5A [1]. Structural features of this compound can be found in each of the six currently marketed NS5A inhibitors.

For nearly 30 years, HCV discovery research teams at Merck pursued multiple molecular targets in the search of a cure. The NS5A project took root at its IRBM site where the team discovered a series of piperazine-based small molecules that inhibited HCV replication but did not show activity in any of the typical HCV enzyme assays. The partial mode of action was subsequently characterized and linked to their ability to alter NS5A biogenesis which resulted in a reduction of p56–p58 conversion, and the resistance mutations identified were mapped to NS5A [2].

Early in 2008 the NS5A project was transferred to the newly formed External Basic Research (EBR) group whose chemistry team was headed by Joseph Vacca and then later by Peter Meinke. EBR was comprised of a small group of senior medicinal chemistry leaders from several sites across the Merck network whose mandate was to carry out medicinal chemistry projects using ex-US resources for all early discovery activities including synthetic chemistry, assay screening, and routine PK-ADME work.

I assumed leadership of the HCV NS5A project (called EBR-8) and initiated lead identification work with a team of 25 chemists from WuXi AppTec who were based in Shanghai, China. Chemists on the team were, at first, inexperienced in drug discovery but extremely productive and eager to learn medicinal chemistry concepts in order to participate in SAR development and target generation. All project data was shared in real time, and weekly teleconferences and regular site visits formed the basis of a cohesive team. The WuXi EBR-8 chemistry team under the local

leadership of Hao Wu, Bin Hu, Bin Zhong, and Richard Soll would become instrumental in the discovery of elbasvir (ironically abbreviated as EBR).

2 Lead Identification

With the IRBM compounds as a starting point, initial efforts focused on the synthesis and SAR development of these *N*-arylpiperazines (**1**) (Fig. 1). The incorporation of a cyclic constraint within these structures afforded indole **2**, which showed similar potency in the replicon assay (GT1b EC_{50} ~150 nM) and was an attractive entry toward modulating this target. Concurrent to these efforts, other NS5A inhibitors began to appear in the patent literature, but their SAR had not been fully delineated. Because of the structural similarity between compound **1** and the stilbene inhibitors **3** [3], a lead-hopping effort was initiated with the goal of applying a similar cyclization strategy in order to explore the SAR of the pseudosymmetric isosteres **4**. Subsequent reports revealed that compound **3** was also pivotal in the design of daclatasvir [4, 5].

Several heterocyclic scaffolds (**4a–4f**) (Table 1) were synthesized and incorporated into the final inhibitor structures. The cellular activity of each new compound was determined using the replicon-system-expressing genotypes 1b, 2a, and 1a. Initial results showed that benzimidazole **4a**, benzothiazole **4b**, and benzoxazole **4c** each had in vitro profiles worse than the parent stilbene inhibitor **3**, whereas

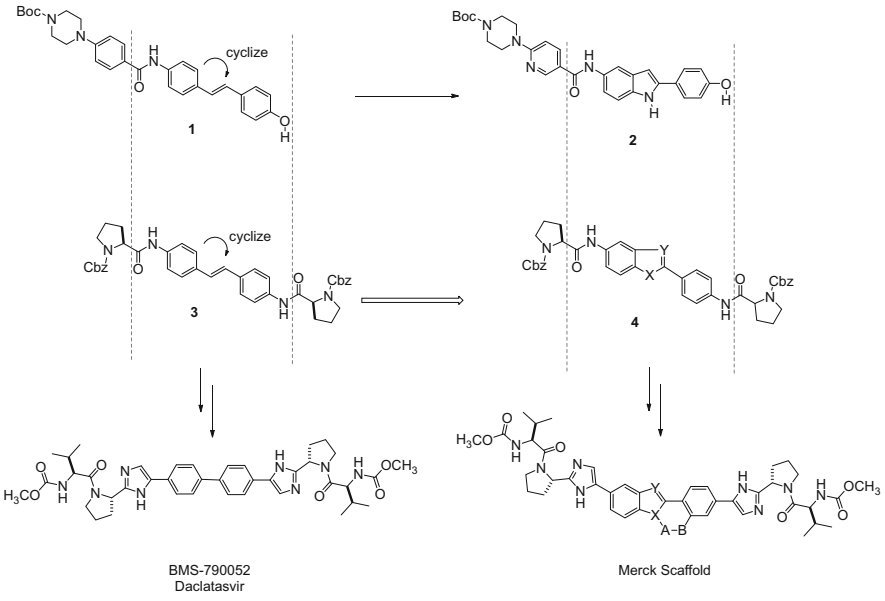
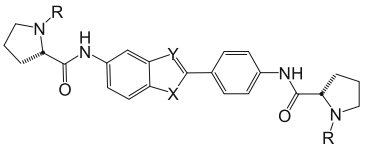


Fig. 1 Strategy for the development of NS5A inhibitors

Table 1 Central scaffold SAR



Compound	Substituent		Genotype, EC_{50} (nM)		
	R	X, Y	1b	2a	1a
3			5	60	>20,000
4a	Cbz	NH, N	>20,000	>20,000	>20,000
4b	Cbz	S, N	>20,000	>20,000	>20,000
4c	Cbz	O, N	160	1,500	>20,000
4d	Cbz	O, CH	5	230	>20,000
4e	Cbz	NH, CH	16	290	1,600
4f	Cbz	NCH_3, CH	170	990	>20,000
4g	Cbz	CH, NH	>20,000	>20,000	>20,000
5	$PhCH_2CH_2$	NH, CH	4	290	2,100
6	(*S*)-Boc-Phe	NH, CH	14	nd	nd
7	(*S*)-Boc-Phg	NH, CH	0.4	20	1,500
8	(*R*)-Boc-Phg	NH, CH	0.004	0.05	70

benzofuran **4d** and indole **4e** had profiles similar to the reference compound. Alkylation of the indole nitrogen atom resulted in an ~tenfold loss in replicon activities (**4f**), whereas the isomeric 2,6-disubstituted indole **4g** resulted in a substantial loss in potency relative to **4e**. Within the context of the indole-based scaffold, replacement of the proline *N*-Cbz group by either the isosteric hydrocinnamate ligand (**5**) or an (*S*)-*N*-Boc-phenylalanine group (**6**) did not have a significant influence on potency. Synthesis and evaluation of the (*S*)-*N*-Boc-phenylglycine (Phg) homolog **7** showed a tenfold increase in genotype 1b and 2a potencies, while the epimeric (*R*)-*N*-Boc-Phg-containing diastereomer **8** afforded a 100- to 400-fold increase in GT1b and GT2a potencies and a 20-fold improvement in GT1a potency (Table 1).

Additional profiling of inhibitor **8** showed bioavailability to be low (<2%) in preclinical animal models with poor oral absorption attributable to the high peptidic nature of the compound. To address this limitation, the C2'-phenyl amide bond was replaced by a variety of amide isosteres (Fig. 2; Y = NH).

These modifications, however, resulted in compounds that displayed inferior replicon profiles relative to the parent amide **8**. Pyrazole analogue **11** appeared to have the best profile, and it was speculated that the NH group was important for maintaining genotype 1a and 1b potencies. As such, imidazole **13** was synthesized, and whereas EC_{50} values versus GT1b and 2a were similar to those of amide **8**, this modification resulted in an ~20-fold improvement in genotype 1a potency (EC_{50} = 3 nM). Further exploration into the SAR of NH-containing heterocyclic amide isosteres resulted in the synthesis of the isomeric 2-prolyl-5-phenylimidazole analogue **14**. This modification gave an additional ~20-fold increase in potency

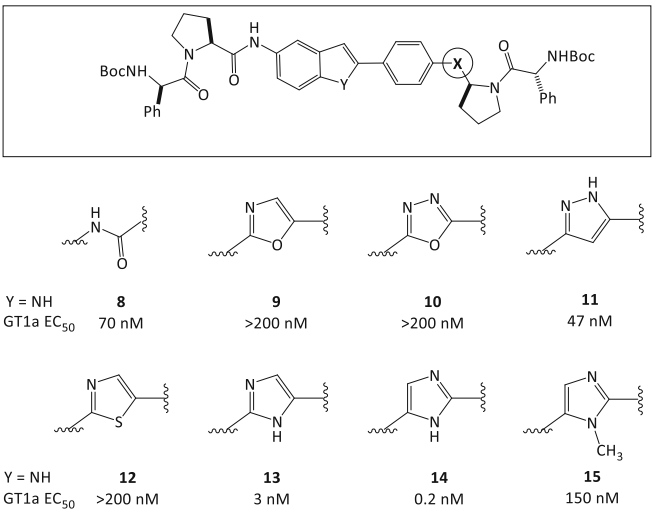
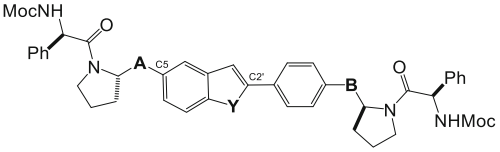


Fig. 2 Amide isosteres and genotype 1a potency

Table 2 Imidazole SAR



	Substituent		Genotype, EC_{50} (nM)[a]			C_{max}[b]
Compound (Y = O)	A	B	1b	2a	**1a**	uM
16	Amide	Amide	0.01	0.01	**27**	0.03
17	Imidazole	Amide	0.002	0.004	**2**	0.01
18	Amide	Imidazole	0.006	0.002	**0.3**	0.03
19	Imidazole	Imidazole	0.004	0.004	**0.015**	0.02

[a] $n = 3$
[b] 10 mpk PO dosed in 10% Tween to fasted male SD rats

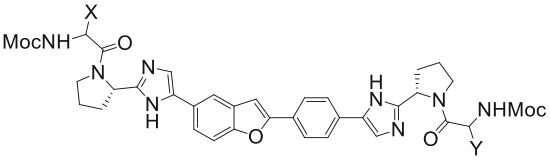
against GT1a while maintaining low-picomolar EC_{50} values in the GT1b and GT2a replicon assays. The importance of the heterocyclic NH substituent was further proven by the loss in replicon potency of the *N*-methylated analogue **15**. Altogether, the incorporation of the imidazole amide isostere maintained both GT1b and 2a potency while improving GT1a potency by ~400-fold relative to the amide **8**.

3 Lead Optimization

Both single and double imidazole amide isosteres in the benzofuran series (Table 2; Y = O) were prepared to further explore the effects of the 2-prolyl-5-arylimidazole substitution on both potency and pharmacokinetics. Table 2 shows the results from imidazole incorporation first on the C5 benzofuran side (**17**; A = imidazole) and then the C2' phenyl side (**18**; B = imidazole). In each case, genotype 1b and 2a potencies remained the same, while genotype 1a potency improved by a factor of 10. Incorporation of both imidazole amide isosteres (**19**; A = B = imidazole) resulted in an additional 20-fold improvement in GT1a EC_{50} values. On the basis of the low-picomolar EC_{50} values in GT1a, 1b, and 2a replicon assays, compound **19** became an important lead. Subsequent research efforts focused on the SAR of the terminal amino acid groups in order to address the problematic oral absorption profile without perturbing the virologic profile. Table 3 shows the area under the curve values after 10 mg/kg oral dosing to fasted male Sprague–Dawley rats for some of the amino acids surveyed. With the exception of compound **21**, little difference in GT1a potency was observed upon incorporation of various alkyl and cycloalkyl substituents.

Conversely, plasma drug exposure depended heavily on the nature of the amino acid substituent. For example, replacement of the C2' phenyl side D-phenylglycine residue with an L-valine subunit (**20**; Y = (*S*)-^{i}Pr) resulted in 20-fold higher compound exposure than analogue **19** (Y = (*R*)-Ph). The addition of a second L-valine group (X = (*S*)-^{i}Pr) **21** resulted in even higher plasma drug levels after 10 mg/kg oral dosing. Additionally, compound **22**, which has an *S,S,S,S* configuration, showed 420-fold higher plasma AUC values than its *R,S,S,S* diastereomer **21**. Further SAR on a variety of homologated valine analogues (i.e., cyclopropyl glycine **23**, tert-butyl glycine **24**, isoleucine **25**, homoalanine **26**) showed better exposure than **19**, although each was inferior to **22**. Cyclopropyl glycine analogue **23** displayed similar plasma drug exposure and oral bioavailability in the rat while maintaining good potency in the GT1a and GT1b replicon assays. However,

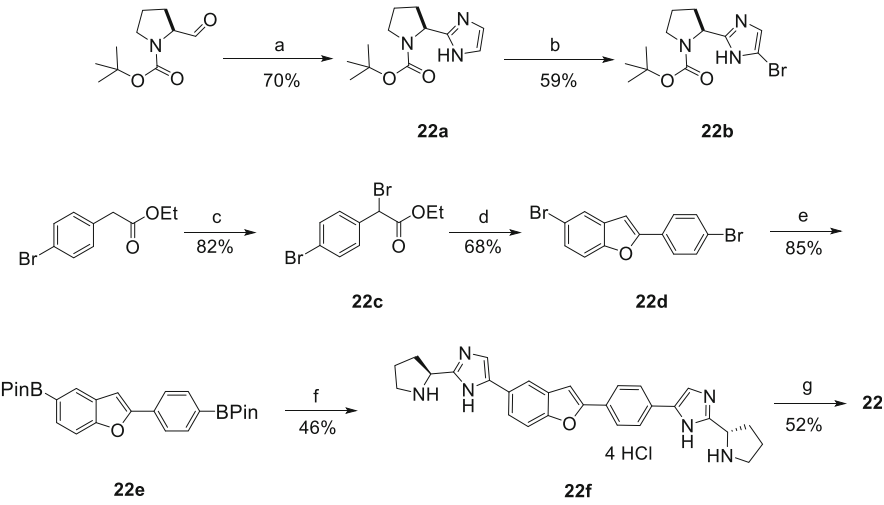
Table 3 Amino acid SAR



Cmpd	X	Y	AUC (uM*h)
19	*R*-phenyl	*R*-phenyl	0.5
20	*R*-phenyl	*S*-isopropyl	10
21	*R*-isopropyl	*S*-isopropyl	0.1
22	*S*-isopropyl	*S*-isopropyl	42
23	*S*-cyclopropyl	*S*-cyclopropyl	38
24	*S*-tert-butyl	*S*-tert-butyl	10
25	*S*-sec-butyl	*S*-sec-butyl	2
26	*S*-ethyl	*S*-ethyl	7

Table 4 Profiles of compound **22** (MK-4882) and **23**

	Genotype, EC_{50} (nM)				10 mpk PO rat	
Cmpd	1b	2a	1a	1aY93H	AUC (uM*h)	%F
22	0.001	0.05	0.01	27	42	38
23	0.003	6.2	0.07	230	38	45



Scheme 1 Synthesis of MK-4882. (**a**) Glyoxal. 7N NH_3 in MeOH. (**b**) i. NBS, THF; ii. Na_2SO_3, EtOH, water, reflux. (**c**) NBS, HBr, CCl_4. (**d**) 5-Bromosalicylaldehyde, Cs_2CO_3, DMF, reflux. (**e**) Bis(pinacolato)diboron, KOAc, Pd(dppf)Cl_2, dioxane, reflux. (**f**) i. **22b**, Na_2CO_3, Pd(dppf)Cl_2, THF, water, reflux; ii. HCl, MeOH. (**g**) *N*-Moc-L-valine, BOP, DIPEA, DMF

examination of the overall potency profiles of **22** versus **23** showed a significant loss in potency against both GT2a and the key genotype 1a Y93H mutants. As such, compound **22** was selected for early clinical development as MK-4882 (Table 4).

The synthesis of MK-4882 is straightforward and is illustrated in Scheme 1. *N*-Boc-L-proline aldehyde was converted into the 2-substituted imidazole **22a** using a Radziszewski imidazole synthesis in good yield. Imidazole **22a** was subsequently treated with excess NBS in THF to give the C4,C5-dibrominated intermediate, which was reduced with sodium sulfite to provide the key mono-brominated imidazole intermediate **22b**. The 2-phenylbenzofuran scaffold was easily prepared via one-pot Cs_2CO_3-mediated alkylation/intramolecular cyclocondensation between 5-bromosalicylaldehyde and ethyl-2-bromo-(4-bromophenyl)acetate (**22c**). Metal–halogen exchange of 5-bromo-2-(4-bromophenyl)benzofuran (**22d**) afforded the bis-pinacol boronate ester, which was subsequently coupled to bromoimidazole **22b**. Deprotection of the proline Boc groups afforded penultimate compound **22f**, which was subjected to an amide coupling protocol using the BOP reagent and two equivalents of *N*-methoxycarbonyl-L-valine.

MK-4882 was found to be highly potent against both genotypes 1a and 1b, with EC_{50} values in the low-picomolar range, and showed only a three- to fourfold shift in

the presence of 40% normal human serum. A number of clinical, in vivo, and in vitro studies have identified key mutations that confer resistance to NS5A inhibitors [6, 7]. These mutations arise principally at residues 28 (1a and 1b), 30 (GT1a), 31 (GT1a and GT1b), and 93 (GT1a and GT1b). MK-4882 potency is shifted to low nanomolar against many mutations at the key NS5A residues 30, 31, and 93. Typically, the magnitude of the potency shift was greater in the GT1a background. For example, MK-4882 potencies against L31V and Y93H mutants in the GT1b background were 0.5 and 3.0 nM, respectively, whereas in the genotype 1a background, they were 2 and 27 nM. Data are summarized in Table 5.

The pharmacokinetic properties of MK-4882 were studied in Sprague–Dawley rats, beagle dogs, and rhesus monkeys. MK-4882 demonstrated low clearance and moderate half-life (2–5 h) in the three species. The oral bioavailability was 26% in dog and 38% in rat, demonstrating that the compound was moderately absorbed in preclinical species. The T_{max} in both rat and dog was somewhat long at 4–6 h. MK-4882 demonstrated slightly greater than dose-proportional exposures in rat when dosed at 2 and 100 mpk and also in dog between oral doses of 1 and 50 mg/kg. Unlike many of the HCV protease inhibitors, MK-4882 did not appear to undergo transport-mediated uptake into the liver, as the liver-to-plasma ratio averaged <10 after oral dosing to rats. The oral absorption profile in chimpanzees was also evaluated in preparation for in vivo efficacy studies. As such, two male chimpanzees were dosed orally with MK-4882 at 1 mpk as a suspension in Tang, and both plasma and liver levels were determined. Twelve-hour average plasma and liver concentrations were 0.19 and 1.3 μM, respectively. Total free drug concentration in plasma at C_{24h} (~3 nM) was greater than the genotype 1b wild-type and genotype 1b mutant EC_{50} values (Table 6).

Table 5 MK-4882 in vitro potency profile vs. GT1 and key mutants

Genotype	EC_{50} (nM)	Genotype	EC_{50} (nM)
1a WT	0.01	**1b WT**	0.003
1a Q30H	5	**1b L28V**	0.03
1a Q30R	8	**1b R30Q**	0.001
1a L31F	2	**1b L31F**	0.17
1a L31V	6	**1b L31V**	0.5
1a Y93C	9	**1b Y93C**	0.02
1a Y93H	24	**1b Y93H**	2

Table 6 MK-4882 pharmacokinetics[a,b]

Species	Cl (mL/min/kg)	$T_{1/2}$ (h)	PO C_{max} (uM)	PO AUC (uM*h)	%F
Rat	9.3 ± 0.4	2.0 ± 0.1	0.31 ± 0.11	1.74 ± 0.27	38
Dog	6.4 ± 4.0	4.7 ± 0.5	0.19 ± 0.03	1.06 ± 0.06	26
Rhesus	9.1	3.0	0.18	1.44	12
Chimp	nd	nd	0.45	5.1	nd

[a]Rat, dog, and monkey iv (1 mg/kg, $n = 3$, 30% captisol + 2 equiv. HCl)
[b]Rat po (2 mg/kg, $n = 3$, 0.5% methylcellulose), dog po (1 mg/kg, $n = 3$, 0.5% methylcellulose), rhesus po (5 mg/kg, $n = 2$, 0.5% methylcellulose), and chimpanzee po (1 mg/kg, $n = 2$, Tang)

4 Pharmacological Activity

Both single-dose and multiple-dose studies in chimpanzees chronically infected with HCV were conducted to determine the antiviral efficacy of MK-4882. A single dose of MK-4882 was orally administered at 1 mpk as a suspension in Tang to three chronically infected chimpanzees. Two carried high viral load infections (~106 IU/mL) of GT1a or GT1b. The third had a GT1a viral load of 104 IU/mL that was homogeneous for the NS3 protease R155K mutation.

All three animals responded rapidly after the single dose; viral load was suppressed an average 2.15 log units within 12 h, with continued suppression to an average nadir of 2.91 log units at 48 h before rebounding. Initial 12 h viral load decreases were similar for both the GT1a and GT1b infections, but an additional one-log suppression was observed with the GT1b infection by 24 h. Suppression of the GT1a infection was maintained through this time but did not increase further. Plasma concentrations of MK-4882 in this study were similar to those found in the satellite study and ranged from 0.06 to 0.17 uM at 12 h, diminishing approximately by half at 24 h. Drug was cleared from plasma and was below the level of quantification (LOQ ~25 nM) by 48 h. The potency of the drug was sufficient to maintain viral suppression through at least 48 h. Drug concentration in the liver, as determined from liver biopsy samples, ranged between 0.77 and 1.56 μM at 12 h. The results are consistent with the satellite PK study in uninfected chimps. Resistance analysis was conducted by population sequencing of the NS5A gene of viral RNA isolated from serially collected plasma samples. The GT1b-infected chimp became homogeneous for the Y93H mutation after 24 h (sequence could not be generated for the 12 h time point, as no additional sample was available at this time). Viral load was further suppressed another 0.6 log units by 48 h, which suggests suppression of mutant virus. Early rebound virus at day 4 was also homogeneous for Y93H, but wild-type virus became the predominant population by day 7. An additional K44R polymorphism, co-encoded with the Y93H virus, was no longer observed at day 10 upon reemergence of wild-type virus, suggesting that mutant and wild-type viruses are two distinct populations. Sequencing of a sample collected on day 28 (4 weeks post-dose) showed the emergence of a new mixture of L31V/L virus. A similar late emergence of apparently distinct resistant virus was also observed in the GT1a (wild-type)-infected chimpanzee (see below). The reason for these phenomena is currently not understood, and the timing of the emergence of L31L/V virus cannot be further pinpointed, as plasma samples were not collected between days 10 and 28. Rebound virus from the GT1a (wild-type)-infected chimpanzee was heterogenous for both Q30E and Y93H. By day 7, Y93H was the only mutation detectable and as a mixed population with wild-type virus. By day 10 this evolved to a mixed population of Y93C and wild-type virus. The shift from Y93H to Y93C coincides with diminishing drug plasma levels and is consistent with the greater loss of potency observed with Y93H than Y93C in vitro. However, by day 28, virus evolved further to an L31M/V mixture. For both the GT1a and 1b infections, wild-type eventually re-emerged as the principle viral population (data

not shown). For the chimpanzee encoding the GT1a NS3 R155K infection, L31M/L was detected as a mixed population at day 10, and only wild-type virus was detected on day 28. Although MK-4882 exhibited a robust virologic response after a single 1 mpk dose, the emergence of mutant virus in the rebound phase warranted further evaluation of efficacy following multi-dose administration. Two different HCV-infected chimpanzees (GT1a and GT1b), both treatment-naïve to NS5A inhibitors, were dosed orally at 1 mpk once daily for 7 days. Liver biopsies were collected 12 h following the final dose; drug concentrations in the liver were 7–15-fold higher than plasma levels, consistent with the findings in the Sprague–Dawley study and suggested that MK-4882 was not selectively retained in liver tissue.

The results from the study showed a rapid and robust response immediately following the initial dose, with an average maximal decrease in viral load by ~3.5 log units. The GT1b-infected chimpanzee showed a further decline in viral titer through the duration of the study, reaching a maximal 3.8-log suppression of virus by day 7 before rebounding. Virus was mixed with Y93H/Y population at day 10 (3 days post-dosing) and eventually became homogeneous for wild type (data not shown). Although the initial response in the GT1a-infected chimpanzee was robust, viral breakthrough was noted beginning at day 2. This eventually led to a 2-log increase in viral titer during dosing, although viral load was still suppressed greater than 1 log from pre-dose levels. Sequence analysis showed that at day 6 virus collected from this animal was heterogenous for both Q30R and L31M/L. A similar viral mixture was observed at day 10 (3 days post-dosing). Eventually wild-type virus emerged as the principle population.

MK-4882 entered the clinic supported by an eCTA preclinical safety paradigm with a single rising dose study in healthy male volunteers in October 2010. MK-4882 was generally safe and well tolerated following single doses as high as 400 mg. The prespecified PK target ($C_{24hr} \geq 20$ nM) was reached at doses of MK-4882 higher than 25 mg, and the average half-life across all groups was 17.2 h.

At the same time, a 3-month oral toxicity study in dogs was initiated to support subsequent longer duration clinical dosing. The MK-4882 low-, mid-, and high-dose levels in this study were 10, 50, and 1,000 mg/kg/day. The high-dose group received 1,000 mg/kg/day for 2 weeks, with doses lowered sequentially to 500 mg/kg/day and then 200 mg/kg/day. The MK-4882 exposure in the low-dose group was 14 μM h which was seven times higher than the clinical efficacy target of 2 μM h. Exposure for the high-dose group was not proportional to the low-dose group (AUC = 71 μM h) due to limited absorption. Noteworthy in this study was the finding that one of six dogs in the high-dose group showed white-matter brain degeneration at necropsy which did not repeat in several follow-up studies. As a consequence of this toxicological finding, along with the viral breakthrough evidenced in the preclinical proof-of concept efficacy studies and the fact that more promising compounds were starting to emerge from the laboratories, MK-4882 were placed on hold in February of 2011.

5 Second-Generation Analogues of MK-4882

The viral breakthrough evidenced in the preclinical proof-of-concept efficacy studies for MK-4882 necessitated the design of newer NS5A inhibitors with improved safety and virologic profiles against the various genotypes and resistance mutations. SAR of the benzofuran core structure suggested that the introduction of a cyclic constraint could result in more potent inhibitors. The initial strategy involved linking the C3 benzofuran carbon to the C2' phenyl carbon to afford tetracyclic core structures **27** and **28** (cyclization mode a; Fig. 3).

Evaluation of these compounds in the replicon assay showed that these modifications resulted in a loss in potency versus the wild-type forms of GT1a and 1b as well as the key mutants L31V and Y93H. An alternative mode of cyclization (mode b) was examined and was made possible by converting the benzofuran core to an indole scaffold which allowed cyclization from the indole nitrogen to the phenyl C2' carbon through either an ethylene bridge (**29**) or an oxygen-containing bridge (**30–34**). These modifications afforded inhibitors that possessed a tetracyclic indole scaffold which proved to be equipotent to MK-4882 versus GT1a and GT1b wild-type replicon systems. Further evaluation showed that the GT1a Y93H potency was weakened with the carbon analogue **29** (EC_{50} = 170 nM) (Table 7). This activity was improved by incorporating an oxygen atom in the two-atom bridge of compound **30** (Y=O; GT1a Y93H EC_{50} = 5 nM). Noteworthy is the fact that the aminal linkage was extremely stable to hydrolytic cleavage even under forcing conditions. Despite having an improved virologic profile, the unique tetracyclic indole-containing compound **30** proved to be cytotoxic in the low-micromolar range (CC_{50} ~1 μM).

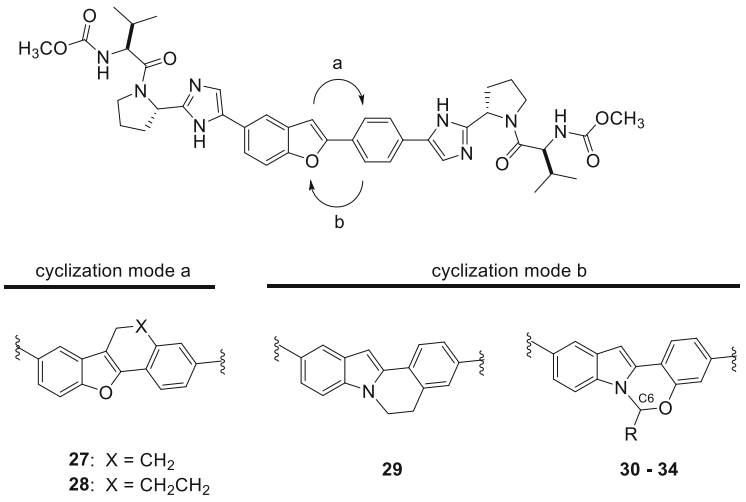


Fig. 3 Design strategy for second-generation NS5A inhibitors

Table 7 SAR for tetracyclic inhibitors

Cmpd	R	Genotype, EC_{50} (nM)					CC_{50} (uM)
		1b	2a	1a	1aL31V	1aY93H	
MK-4882	–	0.001	0.04	0.01	6	24	9
29	–	0.02	0.9	0.002	nd	170	1
30	H	0.001	0.16	0.002	2	5	1
31	CH_3	0.003	0.007	0.003	7	8	nd
32	CH_2CH_3	0.002	0.001	0.002	1	4	nd
33 (EBR)	S-Ph	0.003	0.003	0.004	0.5	2	>25
34	R-Ph	0.005	0.010	0.002	nd	60	>25

SAR analysis showed that the addition of an alkyl or aryl substituent at C6 could abrogate the cytotoxicity while maintaining a favorable potency profile. Initial studies focused on substituting the tetracyclic indole core with small alkyl groups (Table 7) [8]. The addition of a methyl group (**31**) showed good potency against genotypes 1a, 1b, 2a, and 3a, while the activity was weak on genotypes 2b (EC_{50} = 23 nM), 4a (EC_{50} = 0.03 nM), and 1a L31V (EC_{50} = 7 nM). An ethyl analogue (**32**) offered a potency profile similar to the methyl analogue with the exception of 10× better potency on GT2b (EC_{50} = 2 nM). Increasing the size of the alkyl group to *n*-propyl, *n*-butyl, or *n*-hexyl group did not result in improved potency and gave similar results as the ethyl analogue. The addition of fluorine atoms to the alkyl chain also had little effect on the virologic profile, while the introduction of either a terminal cyano group or an ester functional group reduced the potency against the GT1a Y93H mutant. The corresponding carboxylic acid analogue lost activity across all genotypes.

In addition to linear alkyl groups, a series of branched alkyl groups were incorporated into the tetracyclic scaffold at the C6 aminal carbon. The introduction of an isopropyl group resulted in a tenfold loss in GT2a and GT1a Y93H activity versus the corresponding ethyl analogue, while a cyclopropyl group improved the virologic profile in the replicon assay against genotypes 2b, 3a, and 1a Y93H. The potency profiles of cycloalkyl analogues of increasing ring size were also evaluated but proved to be >10× weaker against genotype 2b and several genotype 1a mutants.

Attention next turned to the incorporation of a variety of aryl and heteroaryl substituents for SAR evaluation. We began these SAR studies with the unsubstituted phenyl group and showed that the resulting mixture of diastereomers had a good potency profile. Chiral SFC separation of the two diastereomers afforded compound (**33**) and its diastereomer **34**. While the virologic profiles of the two diastereomers showed equivalent potency values in the wild-type replicons, compound **33** proved to be 25-fold more potent versus the GT1a Y93H mutant.

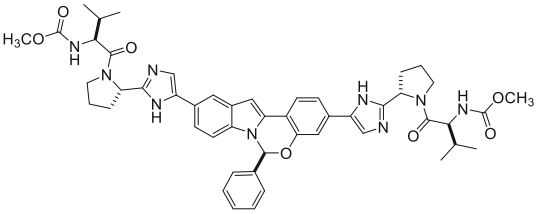
A series of six-membered heteroaromatic analogues were also synthesized and evaluated, but each of these compounds failed to show a better profile than the parental phenyl compound (**33**) [9]. Additionally, substitution of the C6 phenyl ring

generally resulted in poorer overall virologic profiles when compared to compound **34**. Notable exceptions were found with the *p*-*c*-propylphenyl and *p*-biphenyl analogues as well as the 3-alkoxyphenyl analogues which showed improved potency versus each of the genotypes and mutants assayed. Despite the improvements in potency, the pharmacokinetics of each of these analogues proved to be inferior to the unsubstituted phenyl compound; thus they were not advanced.

After extensive profiling, dimethyl *N,N′*-([(6*S*)-6-phenylindolo[1,2-c][1,3]benzoxazine3,10-diyl]bis{1*H*-imidazole-5,2-diyl-(2*S*)-pyrrolidine-2,1-diyl[(2*S*)-3-methyl-1-oxobutane-1,2-diyl]})dicarbamate (**33**), also known as L-002469825, MK-8742, Elbasvir, and EBR supplanted MK-4882 as Merck's lead clinical compound (Table 8). EBR was specifically described in US Provisional Patent Application No. 61/247,318, filed September 30, 2009, and PCT Application No. PCT/US2010/028653, filed March 25, 2010. The PCT application published on September 29, 2010, as International Publication No. WO 10/111,483. Both applications name inventors from Merck & Co., Inc. and WuXi AppTec Co., Ltd.

EBR was found to be highly potent against most HCV genotypes tested with EC_{50} values in the low-picomolar range and modest potency shifts in the presence of 40% NHS. EBR maintained significant potency against most of the NS5A mutations in the screening panel and showed (on average) an order of magnitude improvement relative to MK-4882. EBR potencies against the key L31V and Y93H mutants in the GT1b background were 0.01 and 0.05 nM, respectively, while in the genotype 1a background, they were 0.5 and 2 nM. EBR also demonstrated a favorable genotypic

Table 8 EBR in vitro potency profile GT1–4 and key GT1 mutants



Genotype	EC_{50} (nM)	Genotype	EC_{50} (nM)
1a WT	0.004	**1b WT**	0.003
1a Q30H	0.03	**1b L28V**	0.004
1a Q30R	0.5	**1b R30Q**	0.009
1a L31F	0.08	**1b L31F**	0.05
1a L31V	0.5	**1b L31V**	0.01
1a Y93C	0.2	**1b Y93C**	0.005
1a Y93H	2	**1b Y93H**	0.05
1a Y93N	2	**2a (JFH) WT**	0.003
2a WT	0.003	**2b (JFH)**[a]	3
3a (con1)[a]	0.02	**4a(con1)**[a]	0.003

[a]Chimeric replicons with indicated NS5A patient sequences cloned into con1 or JFH background

virologic profile (Table 8). The decreased potency in the GT2b cell line is attributed to the presence of a methionine residue at position 31 of NS5A versus a leucine present in GT2a [10].

6 Pharmacokinetics and Metabolism Studies

The pharmacokinetics of EBR was studied in Wistar Han rats, beagle dogs, and cynomolgus monkey (Table 9). The i.v. clearance was moderate and constituted ~14–29% of hepatic blood flow in all three species. The V_{dss} was moderate, and the effective half-life was also moderate (2.5–5.9 h). The terminal half-life was longer (4–16 h) than the effective $T_{1/2}$, suggesting rate-limited return from tissue compartments. The oral bioavailability was low to moderate (9–35%) for all three species. The low to moderate bioavailability is likely due to limited absorption which is consistent with low passive permeability in MDCKII cells of 47 nM/s. Preclinical modeling of EBR suggested a high potential for low-dose once-daily dosing in the clinic.

EBR showed high plasma protein binding in all species (>99.9%). The compound was well distributed to the target organ (liver/plasma ratio in rats ~200×), while its uptake by the brain was low (brain/plasma ratio = 0.26). There was no indication of untoward accumulation or auto-induction upon multiple dosing in rats, with good exposure multiples obtained in dose limiting toxicity studies in the rat as well as in dog pharmacology study. In rats, the compound-derived radioactivity was excreted in urine, bile, and feces after IV administration with a significant percent of dose excreted in feces likely by secretion or efflux from the GI tract wall. Approximately 13% of the dose was excreted unchanged in urine, bile, and feces. Human PK and dose projection was carried out based on both allometry and IV/IVC which showed the effective half-life range of 10–14 h consistent with once-daily dose regimen with a dose range of ~50 mg, encompassing both loading and maintenance dose ranges. The compound was metabolized by hepatocytes of all species including humans to oxidative metabolites (M + 16 and M + 32) which were also the same metabolites observed in rats and dogs in vivo, with no glutathione or acyl glucuronide metabolites observed. There was no human-specific metabolite. EBR was found to be neither an inhibitor nor a potent inducer of major human CYPs; therefore potential DDI as a perpetrator with other CYP substrates was predicted to be low.

Table 9 Preclinical pharmacokinetics of elbasvir

Species	Cl (mL/min/kg)	$T_{1/2}$ (h)	PO C_{max} (uM)	PO AUC (uM*h)	%F
Rat[a]	24 ± 8.0	4.2 ± 1.0	0.36 ± 0.3	2.3 ± 1.0	~9
Dog[b]	8.4 ± 2	7.7 ± 2.0	0.29 ± 0.02	1.7 ± 0.3	~35
Monkey[b]	5.2 ± 0.3	16 ± 4.0	0.1 ± 0.04	1.2 ± 0.4	~17

[a]5 mg/kg IV (3%DMA in 40% HPβCD; 30 mg/kg PO (0.4% HPMC in water))
[b]1 mg/kg IV (20% HPβCD; 2 mg/kg PO (10%T80/90% PEG400))

EBR was a substrate for human CYP3A4 only. Given that drug was eliminated unchanged in both rats and dogs suggesting other elimination pathways in addition to CYP3A4 metabolism, the potential for DDI as a victim for CYP3A4 inhibitors or inducers was also predicted to be low. EBR was shown to be a substrate for human OATP1B3.

7 Preclinical Safety Assessment Studies

EBR had no effect on hERG current up to the maximum tested concentration of 30 μM (400× to 1,300× the projected human C_{max} = 20–70 nM). When corrected for plasma protein binding in humans (~99%), this value was approximately 600,000×–5,600,000× the projected human C_{max} (Fu ~0.23–0.7 nM). Similarly, EBR had no effect on I_{Ks} and I_{Na} and minimal effect on I_{CaL} currents at 30 μM. EBR had no effect on heart rate or arterial blood in conscious rats at doses ≤40 mg/kg, and at doses up to 50 mg/kg, there were no test-article-related findings, thus establishing a no-observed-effect level (NOEL) at ≥50 mg/kg.

Potential hydrolysis products (both carboxylic acid and amine) from the parent structure were visually inspected for literature-based structural alerts for genotoxicity and found to be negative. EBR was tested for mutagenicity in a five-strain exploratory microbial mutagenesis (Ames) assay with and without S-9 up to 5,000 μg/plate and was found to be negative. As an antiviral compound, EBR was also tested for the induction of micronuclei in vitro in Chinese hamster ovary cells at 3 h after dosing with and without S9 and at 24 h after dosing without S9. The compound was negative up to 5 μM, and the top dose scored was limited by the precipitation of the test article. In addition, EBR was tested for micronucleus induction in rat bone marrow from a 4-day oral biomarker gene expression study at 1 day after the last treatment at 30 and 300 mg/kg/day for 4 days and found to be negative.

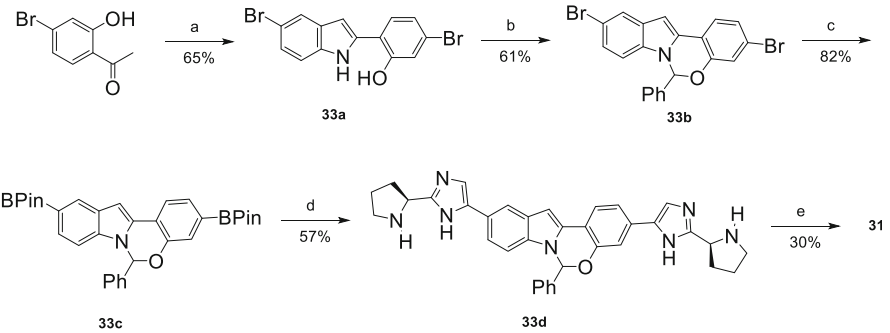
EBR was evaluated in an exploratory oral pharmacology study in dogs and an exploratory 7-day oral tolerability study in male rats at doses up to 750 mg/kg/day which corresponded to projected exposure multiples of 30–70× [AUC] and 40–120× [C_{max}]. There were no test-article-related findings; and the NOEL (and NOAEL) for this study was ≥750 mg/kg/day. EBR was also administered orally to Beagle dogs at doses up to 750 mg/kg. Assessments consisted of mortality, physical signs, and serum biochemistry evaluations which showed no adverse events nor any changes in serum biochemistry parameters.

In longer-term safety studies, no target organs of toxicity were identified, following oral EBR administration of up to 1,000 mg/kg/day, in toxicology studies in mice, rats, and dogs for up to 1, 6, and 9 months.

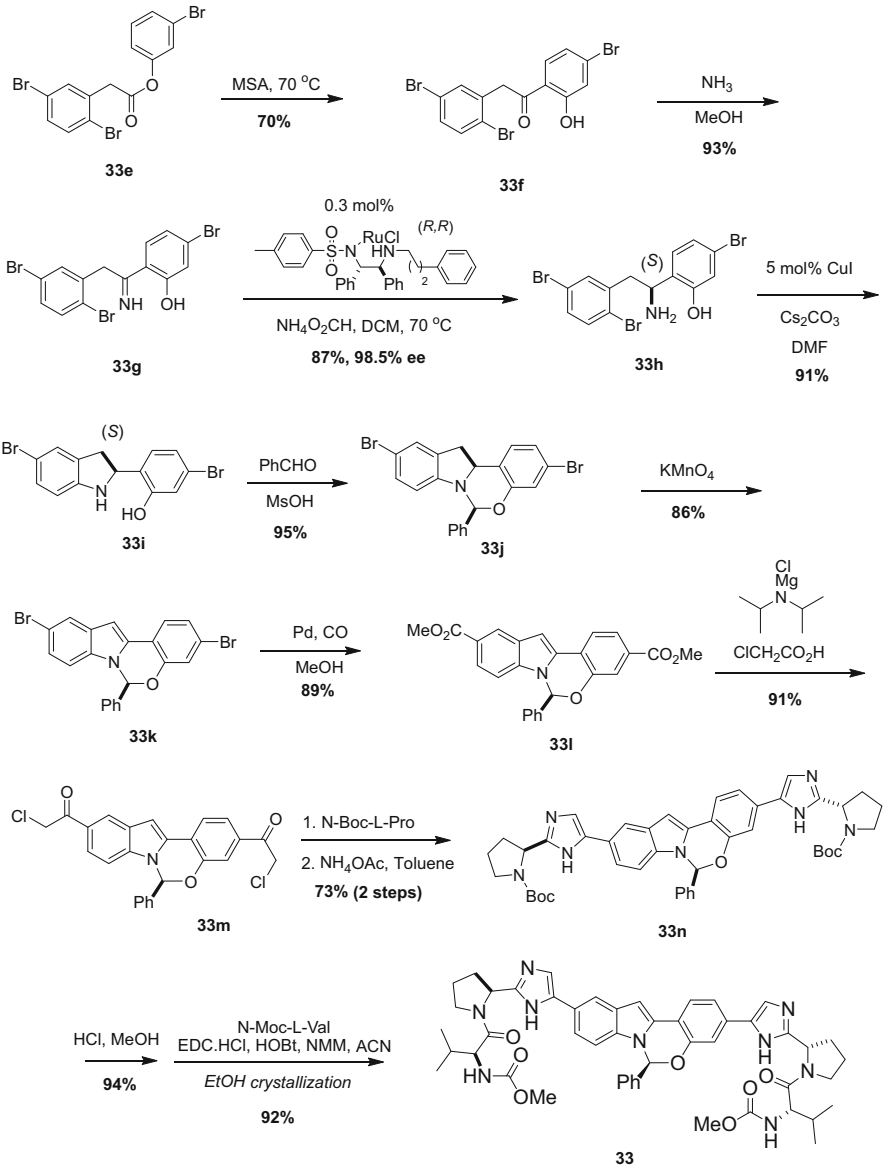
8 Synthesis

The first-generation synthesis of EBR proceeded in eight linear steps (twelve total steps) from commercially available materials with a 3% non-optimized yield from the longest linear sequence (Scheme 2). Thus, the C2' phenylindole intermediate **33a** was prepared by starting from 5-bromoacetophenone and *p*-bromophenylhydrazine using well-established two-step Fisher indole conditions. The indole NH group was cyclized onto the C2' phenolic group using standard alkylating conditions to give the racemic tetracyclic scaffold **33b** in good overall yield. The dibromide intermediate was subsequently converted into the corresponding pinacolboronate ester **33c** using standard procedures. Boronate ester **33c** was then coupled with two equivalents of the heterocyclic bromide **22b** in the presence of a catalytic amount of $Pd(dppf)Cl_2$ followed by workup, and deprotection of the Boc groups provided the desired compound **33d** in 57% yield. Amide coupling with *N*-methoxycarbonyl-L-valine afforded compound **33** as a mixture of diastereomers which were easily separated by SFC chromatography. EBR was the second diastereomer to elute and was determined to be the *S,S,S,S,S* diastereomer by single-compound X-ray analysis.

An enantioselective synthesis of the tetracyclic scaffold (**33k**) was developed by the Merck Process Chemistry team and featured a highly enantioselective asymmetric hydrogenation of an imine (**33g** → **33h**) and a directed stereochemical relay strategy that leveraged a dynamic diastereoselective condensation to produce the challenging hemiaminal stereocenter [11, 12]. The improved synthesis of EBR required only ten linear steps for completion in the longest linear sequence and proceeded in 30% overall yield without the need for chromatography (Scheme 3).



Scheme 2 Medicinal chemistry route for the synthesis of Elbasvir. (**a**) i. *p*-bromophenylhydrazine, AcOH, EtOH, reflux, ii. PPA, 110°C. (**b**) α,α-dibromotoluene, K_2CO_3, DMF, 100°C. (**c**) Bis (pinacolato)diboron, KOAc, $Pd(dppf)Cl_2$, dioxane, reflux. (**d**) i. 22b, Na_2CO_3, $Pd(dppf)Cl_2$, THF, water, reflux; ii. HCl, MeOH. (**e**) *N*-Moc-L-valine, BOP, DIPEA, DMF and then chiral SFC



Scheme 3 Process chemistry route for the synthesis of elbasvir

9 Early Phase Clinical Trials

EBR was approved as a development candidate by Merck on December 9, 2010. In 2011, EBR was evaluated in healthy young male volunteers to assess the initial safety and plasma pharmacokinetics of single rising oral doses from 5 to 300 mg in healthy young male subjects. Proof of pharmacology targets were based on achieving exposures with a high likelihood of attaining proof of concept in a phase Ib study as judged by benchmarks set by BMS-790052. Using BMS-790052 PK and viral load data following 14 days of QD administration, the PK/PD relationship was assessed to determine the steady-state and Day 1 C_{24hr} targets that were likely to result in a 3 $\log_{10}$ viral load decline from baseline. The PK targets were adjusted for both potency against the GT1a virus and protein-binding differences between BMS-790052 and EBR using the EC_{90} values determined in 40% normal human serum. On this basis, the steady-state PK target for EBR was set at $C_{24hr,ss} \geq 3$ nM.

A summary of the mean plasma concentration-time profiles in healthy male subjects is presented in Fig. 4. Results from the study showed that the compound was rapidly absorbed, with a median T_{max} of 3.5–4.0 h for the 5–300 mg doses, and a

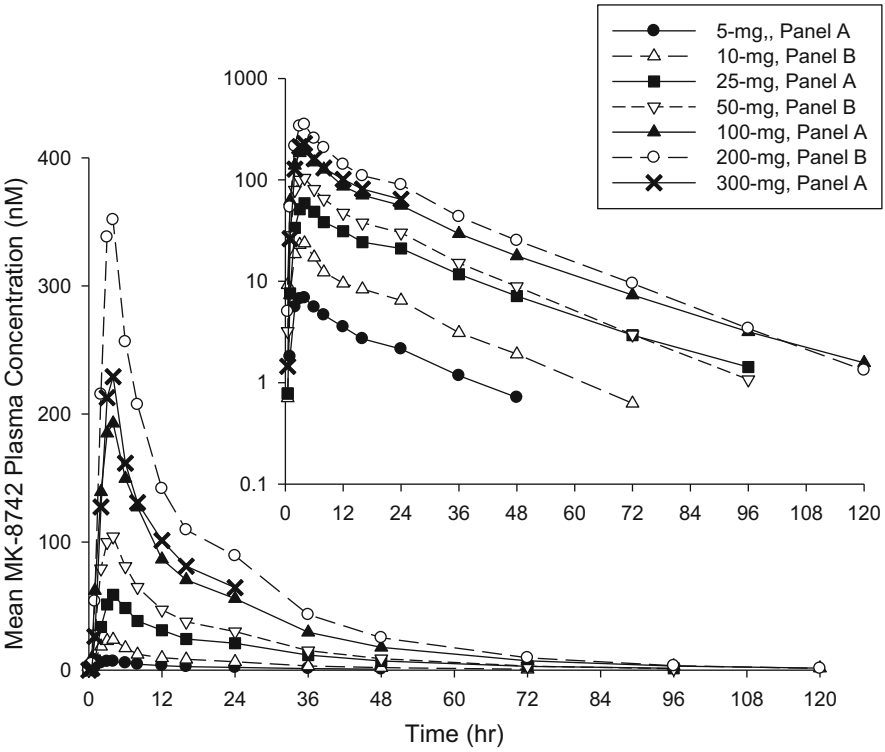


Fig. 4 Arithmetic mean plasma concentration profiles for EBR (EBR) following single oral 5–300 mg doses of EBR ($N = 6$/panel, linear scale, inset = semi-log) (LLOQ = 0.283 nM)

mean time delay $\leq$0.5 h was observed in most subjects at all doses. Drug concentrations appeared to decline after T_{max} in a biphasic manner, with the second phase initiating at about 12 h post-dose across doses. The mean terminal half-life ($T_{1/2}$) was ~14.5–19.1 h across the dose range studied and was consistent with the human PK predicted half-life of 14 h based on allometric scaling of preclinical data.

In 2013 results from a phase 1b randomized double-blind, placebo-controlled, multiple-dose study in HCV GT1-infected patients (PN002, "A Multiple Dose Study to Evaluate the Safety, Pharmacokinetics and Pharmacodynamics of EBR in Hepatitis C-Infected Males") demonstrated efficacy after once-daily dosing at a range of doses between 5 and 100 mg. Following once-daily dosing of 50 mg of EBR QD $\times$ 5 days, the mean maximum reduction in HCV viral load in GT1b patients was -5.1 (0.30) $\log_{10}$ IU/mL. The pattern of initial viral load decline was similar to that of the 10 mg panel. Following cessation of 50 mg dosing on day 5, the viral load remained suppressed at day 13 with a mean reduction of -4.75 $\log_{10}$ IU/mL followed by a rebound to -1.7 $\log_{10}$ IU/mL of VL decline by 3 weeks post-dose and a return to approximately baseline by month 2 after dosing (Fig. 5).

In the phase 2 dose-ranging study in combination with the protease inhibitor MK-5172 (Grazoprevir, GZR), 50 mg QD of EBR provided efficacy similar to that obtained with 20 mg QD EBR in a 12-week therapy with 100 mg QD GZR and

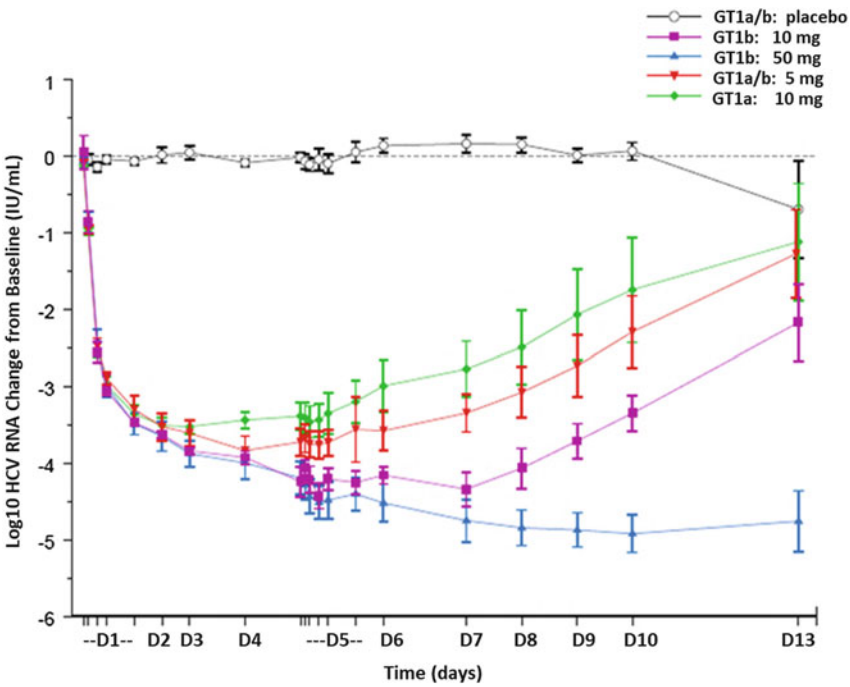


Fig. 5 Profiles of the change from baseline in $\log_{10}$ HCV RNA for GT1 HCV-infected male patients after receiving multiple once-daily doses of elbasvir/placebo for 5 days. *HCV RNA BLOQ is 25 IU/mL and that the BLOQ samples were imputed to 0.5 $\times$ 25 IU/mL

ribavirin, with SVR12 rates of 100% (22/22) and >95% (23/24) observed at 20 and 50 mg, respectively. While SVR12 response rates were similar between 20 mg QD and 50 mg QD EBR, the higher dose was selected for further evaluation in combination with 100 mg QD GZR.

10 Summary

On January 28, 2016, the US Food and Drug Administration approved Zepatier™ (elbasvir and grazoprevir) for the treatment of adult patients with chronic hepatitis C virus GT1 or GT4 infection. The discovery of elbasvir was the result of an intensive research effort that combined components from both internal and literature compounds that first led to a series of benzofuran-based NS5A structures which exhibited good potency versus genotype 1b. A detailed medicinal chemistry effort powered by a team of chemists from WuXi AppTec was then undertaken and included the exploration of various amide bond isosteres. This work ultimately led to the incorporation of two imidazole subunits and afforded compounds with sub-nanomolar EC_{50} values against genotypes 1a and 2a. Further optimization of this series using an expanded panel of HCV genotypes and clinically relevant NS5A-resistant mutant strains led to the discovery of the early lead compound MK-4882 which showed efficacy in a nonhuman primate model at a moderate dose. Viral breakthrough with the genotype 1a-infected chimpanzee, however, focused attention on developing analogues that were more potent against resistant variants while maintaining or improving the broad genotype profile. Strategic incorporation of a cyclic constraint and further lead optimization efforts led to the discovery of EBR.

Compliance with Ethical Standards

Conflict of Interest The author declares that he has no conflict of interest.

Ethical Approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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HCV NS5A as an Antiviral Therapeutic Target: From Validation to the Discovery and Development of Ombitasvir and Pibrentasvir as Components of IFN-Sparing HCV Curative Treatments

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Abstract While IFN-based hepatitis C virus (HCV) treatment regimens required long treatment duration, they only achieved a limited cure rate in HCV-infected patients and were accompanied by significant therapy-based side effects. The first curative IFN-sparing therapies revolutionized HCV treatment by utilizing a cocktail of mechanistically orthogonal direct-acting antiviral (DAA) agents to achieve much higher cure rates in a shorter period of time and with fewer side effects. One of the drug targets that these therapies usually engaged was the HCV NS5A protein. This chapter reviews the Abbott/AbbVie HCV NS5A program, which discovered

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inhibitors of this protein using an in vitro phenotypic screen, validated the mechanism in vivo, and ultimately discovered two FDA-approved NS5A inhibitors ombitasvir (OMB) and pibrentasvir (PIB). OMB, a first-generation NS5A inhibitor, is a component of two FDA-approved IFN-sparing DAA therapies (Viekira Pak™ and Technivie™) with approval to treat genotypes 1 and 4, respectively. PIB, a next-generation NS5A inhibitor included in AbbVie's next-generation therapy Mavyret™ (or Maviret™), prevents replication of HCV genotypes 1–6 and exhibits an improved resistance profile relative to other FDA-approved first-generation NS5A inhibitors.

Keywords Abbott, AbbVie, ABT-267, ABT-530, DAA, HCV, Hepatitis C, IFN-sparing, Maviret, Mavyret, NS5A, Ombitasvir, Pibrentasvir, Technivie, Viekira Pak

1 Introduction

Abbott Laboratories was one of the first companies to commercialize a blood screening test for hepatitis C virus (HCV) in 1990 and has maintained a continuous flow of improvements and new platform technologies through the present day. This early and sustained commitment to HCV by our colleagues in Abbott's Diagnostic Division, combined with the growing awareness of the significant medical need for well-tolerated and effective treatments for HCV infections, piqued the interest of the antiviral drug discovery team within the Pharmaceutical Products Division. (Note: The Pharmaceutical Products Division was spun off from Abbott as a separate company in 2013 and is now known as AbbVie). Although the antiviral drug discovery team was heavily involved in HIV research in the 1990s, work that would lead to the discovery of the marketed HIV protease inhibitors ritonavir and lopinavir, a small exploratory effort was initiated in the HCV space. Several biochemical screens were conducted to identify inhibitors of the NS3/4A protease and the NS5B RNA-dependent RNA polymerase. Whereas the protease inhibitor screen yielded very little that was ultimately useful, among the polymerase inhibitor screening hits was a fragment with weak binding affinity [1]. Elaboration of this fragment resulted in the discovery of dasabuvir, a nonnucleoside RNA polymerase inhibitor that is a component of the marketed drug for HCV genotype 1 infections known as Viekira Pak™ [2]. Beyond biochemical screens, the availability of subgenomic HCV replicons afforded the opportunity to conduct a cell-based phenotypic screen to identify inhibitors of HCV replication. The following sections in this chapter provide some detail with regard to how a compound identified in the replicon inhibition screen ultimately gave rise to ombitasvir and pibrentasvir, NS5A inhibitors that are components of the marketed HCV drug treatments Viekira Pak™ and Mavyret™, respectively.

2 Discovery of AbbVie's First-Generation HCV NS5A Inhibitors

2.1 Screening Hit and the Establishment of NS5A Inhibitors as a Viable HCV Drug Discovery Approach

A phenotypic screen was initiated in our laboratories to identify compounds that inhibited replication of the HCV genotype 1b subgenomic replicon. Naphthyridine **1** (see Fig. 1) emerged as an interesting hit from this screen, inhibiting replication of

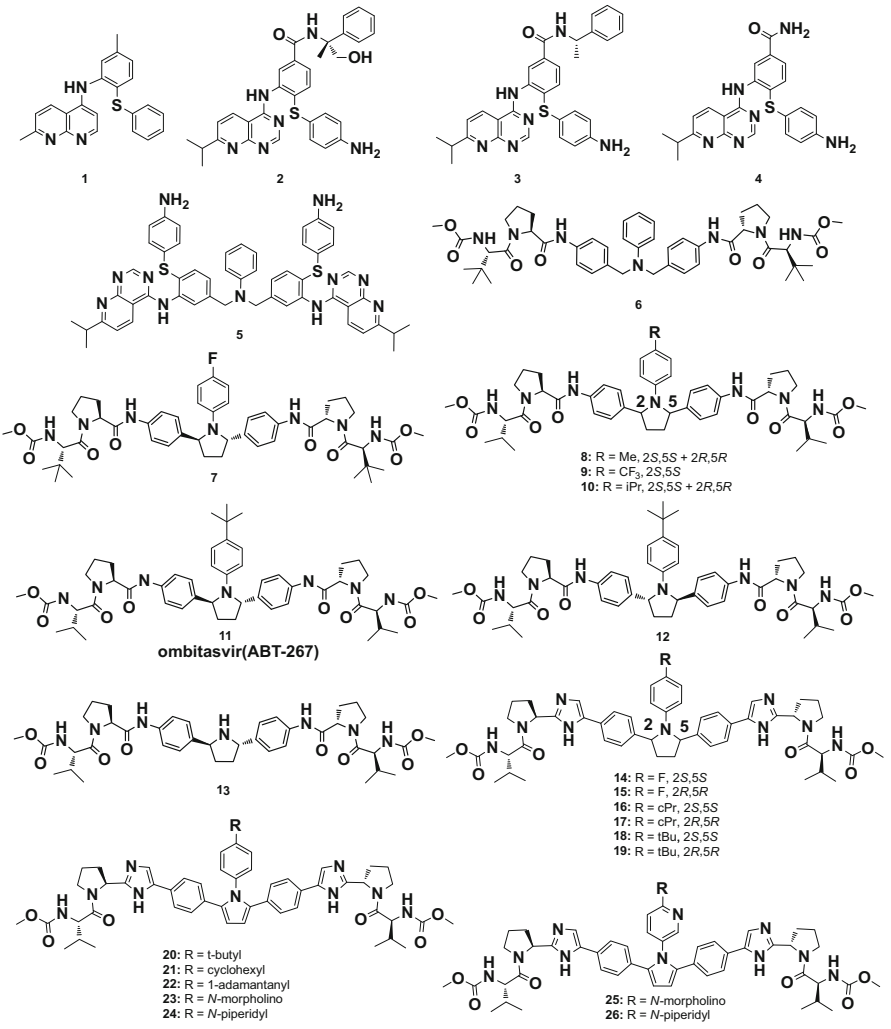


Fig. 1 Structures of NS5A inhibitors

the genotype 1b replicon with an $EC_{50} = 20$ nM, whereas cellular toxicity only occurred at concentrations that were >400-fold higher. The mechanism of action for **1** was initially elusive as it showed no inhibition in HCV NS3 protease, NS3 helicase, NS5B polymerase, HCV IRES, or EMCV IRES assays. However, treating genotype 1b replicon cells with **1** under conditions where replication of the HCV subgenome is essential for cell viability led to the selection of subgenomic variants with mutations in the NS5A gene, corresponding to amino acid substitutions at position 31 (L31F or V) or 93 (Y93C or H). Replicon cells containing the NS5A L31V or Y93C variants conferred 45–150-fold resistance toward **1**, whereas no resistance was conferred upon HCV protease or polymerase inhibitors, strongly suggesting that the mechanism of action for **1** was mediated, in some way, through the NS5A protein. Additional in vitro studies showed that the combination of **1** with HCV polymerase inhibitors could inhibit HCV subgenomic 1b replication much more effectively than when either compound was used alone, establishing **1** and its putative NS5A mechanism of action as a new class of HCV inhibitors that are synergistic when combined with the more mainstream polymerase (or protease) inhibitors.

Although these in vitro results were compelling, there was considerable uncertainty with regard to whether they would translate into robust antiviral efficacy in the in vivo setting. This uncertainty arose from the presence of adaptive mutations in the NS5A gene of the subgenomic replicon that are not present in fully genomic, infectious virus, thereby raising the possibility that the replicon inhibitory effects by NS5A inhibitors such as **1** might be an artifact of the subgenomic replicon system. Therefore, an in vivo proof-of-concept study in the HCV genotype 1b infected chimpanzee model was conducted in collaboration with the Southwest Foundation for Biomedical Research. Pyrido-pyrimidine compound **2**, with a genotype 1b replicon $EC_{50} = 80$ nM measured in the presence of 40% human plasma, elicited a 1.65 $\log_{10}$ decline in viral load in this model when IV dosed at 2.5 mg/kg q8h × 5 [6]. This clear viral load decline, coupled with the in vivo selection of NS5A L28M and Y93H resistant variants (which were also selected in in vitro experiments), validated this chemical series and replicon NS5A inhibitors in general, as a viable approach for achieving viral load declines in the in vivo setting.

2.2 *Identification of the E-Ring Pharmacophore and the Move to C-2 Symmetrical Compounds*

One of the challenges for the program was achieving potent inhibition in genotype 1a replicons. A breakthrough occurred with compound **3** which demonstrated inhibition of genotype 1a and 1b replicons with EC_{50} values in the 20–45 nM range. Notably, the close analog **4** exhibited no activity in the genotype 1a replicon at 50 μM, suggesting that the benzylic "E-ring" contributed at least 1,000-fold toward the activity of **3** in the genotype 1a replicon. Given that uniform activity in

genotypes 1a and 1b was a goal for the program, this important E-ring pharmacophore was retained throughout the duration of the project.

In a quest for identifying strategies that might lead to further potency improvements, the team was aware of the published X-ray crystal structure for the domain I form of HCV NS5A which established that this part of the HCV NS5A protein exists as a C-2 symmetrical dimer [3]. Exploiting this C-2 symmetry in some way had appeal to the team, based on its previous success in creating C-2 symmetrical inhibitors of HIV protease, likewise a C-2 symmetrical protein dimer [4]. This background, combined with emerging reports that others were having success in deriving highly potent NS5A inhibitors using a C-2 symmetric strategy stimulated an exploratory effort to create C-2 symmetrical dimeric molecules from our pyrido-pyrimidine series [5]. Compound **5** is an example from this series whose EC_{50} values of 4.9 and 0.05 nM in the genotype 1a and 1b replicons, respectively, represented at least a 9–400-fold improvement over the "monomeric" pyrido-pyrimidines such as **3** and **4**, thereby validating the C-2 symmetric approach for improving potency.

2.3 N-*Phenylpyrrolidine Series Discovery*

However, drug discovery challenges remained for the C-2 symmetric pyrido-pyrimidine series, especially with regard to achieving microsomal stability, oral bioavailability, and uniform genotype 1a/1b potency. Several strategies were investigated to address these concerns, but the most successful one involved keeping the central core with the E-ring and incorporating more drug-like ends. Compound **6** exhibited replicon potencies (EC_{50} = 7.2 and 0.1 nM toward genotype 1a and 1b replicons, respectively; see Table 1) that were comparable to **5** but had the added benefit of being at least 28% orally bioavailable as evaluated in an 8-h rat pharmacokinetic study. Attempts to improve potency and microsomal stability via conformational constraint resulted in the discovery of the *N*-phenylpyrrolidine series. 2,5-disubstituted *N*-phenyl pyrrolidine inhibitor **7** is an early compound from this series and demonstrated 23% oral bioavailability and an IV half-life in rat that was too long to be accurately measured in a 24 h pharmacokinetics study (Table 2). Compound **7** also exhibited replicon EC_{50} values that were 16–27-fold more potent than the acyclic comparator **6**. The large potency improvement for the pyrrolidine inhibitor is likely due to stabilization of an active conformation provided by the rigid heterocyclic core. The team was sufficiently encouraged by the long plasma half-life and sub-nanomolar replicon potencies to mount an extensive SAR campaign on the *N*-phenylpyrrolidine series.

Table 1 In vitro activity against HCV GT1a and GT1b in the replicon assay and ADME data

	Replicon potency (nM)[a]				ADME		
	0% human plasma		40% human plasma		Solubility[b]	% Remaining[c]	
Compd	GT1a	GT1b	GT1a	GT1b	pH 7.2	HLM	RLM
6	7.2	0.100	69	0.685	2.6	29	57
7	0.263	0.006	15.5	0.528	4.4	52	56
8	0.720	0.040	4.36	0.204	9.9	42	44
9	0.057	0.007	0.650	0.072	3.4	58	68
10	0.033	0.008	0.706	0.086	1.9	65	67
11	0.014	0.005	0.186	0.056	2.7	60	73
12	0.138	0.088	0.986	0.090	1.8	n/a[d]	65
14	0.009	0.008	0.358	0.255	1.1	37	45
15	0.029	0.009	0.714	0.297	1.2	49	48
16	0.012	0.007	0.310	0.130	4.0	36	63
17	0.005	0.008	0.094	0.094	1.3	34	68
18	0.004	0.003	0.129	0.082	1.4	73	68
19	0.003	0.004	0.061	0.078	2.9	77	70
20	0.018	0.013	0.504	0.562	<0.7	67	79
21	0.018	0.010	0.284	0.148	1.4	66	75
22	0.011	0.007	0.293	0.251	10.5	93	100
23	0.045	0.010	0.667	0.290	1.1	54	65
24	<0.1	0.010	0.249	0.159	3.7	48	82
25	0.047	0.020	0.876	0.526	2.0	29	41
26	0.008	0.008	0.178	0.144	1.6	53	61

[a]GT1a-H77 and GT1b-Con1 replicon inhibitory effects were determined as described previously
[b]Kinetic solubility (μM) determined by chemiluminescent nitrogen detection (CLND) in 10 mM phosphate buffer (pH 7.2)
[c]Percentage of parent compound remaining after 30-min incubation
[d]Not available

2.4 Phenyl-Amino Pyrrolidine Series

The objective for the team was to identify a compound for clinical development that possessed sub-nanomolar potency in both genotype 1a and 1b replicon assays, as well as cross-species pharmacokinetics that would be consistent with once-daily dosing in humans. Replicon activity was determined with and without the addition of 40% human plasma in order to assess the effect that binding to plasma proteins could have on potency of the inhibitors, in vivo. Further potency improvement was obtained with alkyl substituents in the para-position of the *N*-phenyl group, such that increasing the size of the 4-alkyl substituent provided better replicon activity, particularly in genotype 1a. Thus, 4-methyl-substituted analog **8** was 12-fold less active than 4-trifluoromethyl analog **9** and 20-fold less active than 4-isopropyl analog **10** in the 1a replicon assay (Table 1). Potency differences were more modest in the 1b replicon. Importantly, both **9** and **10** provided sub-nanomolar activity against both 1a and 1b replicons in the presence of 40% human plasma.

Table 2 Pharmacokinetic parameters for selected compounds[a]

Compd	Species	IV[b]			Oral[b]				
		$t_{1/2}$	Vss	Cl	$t_{1/2}$	T_{max}	C_{max}	AUC	F
7	Rat	NC[c]	NC[c]	<0.39	NC[c]	3.0	0.11	1.85	23
9	Rat	6.0	8.9	1.2	12.1	2.2	0.03	0.50	16
	Dog	6.0	1.3	0.16	5.8	4.7	0.07	0.85	5.5
11	Rat	9.9	5.9	0.65	15.9	3.7	0.01	0.29	6.2
	Mouse	11.0	1.7	0.11	11.1	6.0	0.38	7.65	29
	Dog	7.9	1.8	0.18	7.3	3.3	0.64	8.00	57
	Monkey	4.4	1.5	0.38	5.0	3.3	0.29	2.40	35
12	Rat	5.5	2.2	0.43	8.6	4.7	0.02	0.22	3.1
14	Rat	4.2	3.2	0.72	6.7	2.0	0.04	0.43	9.5
15	Rat	3.6	1.2	0.25	3.9	2.2	0.19	1.52	12
16[d]	Rat	5.8	3.4	0.54	7.3	3.7	0.09	1.20	19
18	Rat	6.6	2.3	0.33	11.6	2.0	0.03	0.36	3.7
19	Rat	6.1	1.5	0.34	7.2	1.5	0.02	0.17	2.0
	Mouse	NC[c]	NC[c]	0.09	NC[c]	10.0	0.14	2.23	3.9
	Dog	8.8	0.9	0.07	8.9	3.3	0.32	4.32	12
	Monkey	6.2	1.2	0.16	7.6	4.3	0.26	3.35	20
20	Rat	6.0	2.3	0.41	7.8	2.8	0.07	0.82	11
21	Rat	6.9	1.9	0.32	5.9	4.5	0.02	0.30	3.2
22	Rat	12.7	1.7	0.10	4.3	3.5	0.05	0.43	1.5
23	Rat	3.5	2.4	0.57	6.9	7.0	0.08	1.06	20
26	Rat	4.2	1.2	0.27	3.9	10.0	0.29	3.85	35
	Mouse	NC[d]	NC[d]	<0.025	NC[d]	11.0	0.85	14.4	13
	Dog	5.0	0.7	0.11	3.7	3.3	1.08	13.2	57
	Monkey	2.7	0.8	0.24	2.7	4.0	0.14	0.81	8.0

[a]Units: $t_{1/2}$ (h); Cl (L/h/kg); C_{max} (μg/mL); $AUC_{0-24\ h}$ (μg h/mL); F (%)
[b]Doses: 3 mg/kg IV and oral for rat and mouse, 1 mg/kg IV and 2.5 mg/kg oral for dog and monkey
[c]*NC* not calculated
[d]Dosed as a mixture of A-1246114.0 and A-1246108.0

The 4-tert-butylphenyl analog **11** provided 1a and 1b replicon EC_{50} values of 0.014 nM and 0.005 nM, respectively. The 40% human plasma attenuating effect on potency was just over tenfold for both genotypes. The threefold 1a/1b potency difference for **11** is in stark contrast to the >72-fold difference seen with **6**, which clearly illustrates the benefit of the pyrrolidine core for this inhibitor series. The pyrrolidine stereochemistry in **11** is important to potency, as this 2*S*,5*S*-trans isomer is tenfold more active in the 1a replicon assay when compared to the 2*R*,5*R*-trans isomer **12**. The activity difference in the 1b replicon assay was on the order of twofold, once again favoring the *S*,*S*-trans isomer **11**. While proper orientation of the substituents at positions 2 and 5 on the pyrrolidine ring is important for inhibitory effect as evidenced by the activity differences for **11** and **12**, the *t*-butylphenyl substituent on the pyrrolidine nitrogen plays an equally critical role for potency of these inhibitors. An analog that lacks this substituent (**13**) was 10,000-fold less

active than **11** in the 1a replicon assay ($EC_{50} = 2$ μM in both 1a and 1b replicon in the presence of 40% human plasma).

Pharmacokinetic profiling of promising compound **11** was conducted across species (Table 2). The pharmacokinetic profile in rat, monkey, and dog was characterized by low plasma clearance values in rat (0.46 L/h kg) and monkey (0.38 L/h kg), with even lower values in dog (0.18 L/h kg) and mouse (0.11 L/h kg). The compound was characterized by moderate to high volumes of distribution in all species (Vss), with values of 1.5–1.8 L/kg for mouse, dog, and monkey but higher values for rat (4.8 L/kg). The apparent elimination half-life ranged from 4.4 h in monkey to 11.4 h in rat. Oral bioavailability values ranged from 24.8% in rat to 57.3% in dog. The bioavailability in dog for this sparingly soluble compound was markedly affected by formulation, with slow absorption (T_{max} 12.6 h) and low bioavailability (10%) obtained from an aqueous suspension; more rapid absorption was noted with a lipid-based solution formulation (T_{max} 1.8 h), with a fourfold increase in bioavailability (*F* 41%). Compound **11** concentrations in the liver exceeded levels in the plasma by 10–12-fold at the 24-h timepoint in mice administered single doses at 1, 3, 10, or 30 mpk.

2.5 *Phenyl Imidazole Pyrrolidine Series*

In an effort to explore the effect of changes to the bis-phenyl amide linker groups in analogs such as **11**, we made some chemical modifications. One of the changes investigated was replacement of the bis-phenyl amide units with slightly longer and more acidic bis-phenyl imidazole linker units. Six of these analogs are shown in Fig. 1 (**14**–**19**). Within this set, compounds with different stereochemical configurations around the central pyrrolidine core (2*S*,5*S* and 2*R*,5*R*) in conjunction with three different "E-ring" para-phenyl substituents (fluoro, cyclopropyl, and tert-butyl) are represented. In general, the stereochemistry around the pyrrolidine core (2*S*,5*S* or 2*R*,5*R*) did not lead to significant replicon potency differences in the matched analogs (**14** and **15**, **16** and **17**, **18** and **19**). The most potent bis-phenyl imidazole analog made was **19**, which displays GT1a replicon potency in 40% human plasma of 0.061 nM and the corresponding GT1b replicon potency of 0.078 nM (Table 1). This analog displays roughly threefold better potency in GT1a and nearly equivalent GT1b potency when compared to the best bis-phenyl amide analog, **11**. It should also be noted that these two analogs surprisingly differ in their stereochemistry around the central pyrrolidine ring (2*R*,5*R* for **19** and 2*S*,5*S* for **11**). Analog **19** had similar solubility and ADME parameters when compared with **11**. The pharmacokinetic parameters of some of the bis-phenyl imidazole analogs are shown in Table 2. In general, the bis-phenyl imidazole series compounds showed lower oral bioavailability than the bis-phenyl amide analogs, with the most potent analog **19** displaying a good IV half-life and clearance in rat but with low plasma AUC and bioavailability values upon oral administration. Clearance in mouse and dog were also low with long half-life in mouse that could not be calculated from a 24 h study,

although oral bioavailability was also low in these species. In monkey, **19** also demonstrated a long IV plasma half-life with low clearance; however, the oral AUC and bioavailability values were found to be higher than in the other species.

2.6 Phenyl Imidazole Pyrrole Series

While the introduction of the pyrrolidine group resulted in improved potency and pharmacokinetics compared to the acyclic analogs, additional SAR was conducted with the goal of improving potency and PK. One approach was to design new cores that might be more synthetically accessible than the pyrrolidine core to enable more diverse E-ring SAR exploration. One such modification was to replace the pyrrolidine ring with a pyrrole, a transformation which removed two stereocenters. Initially, the pyrrole equivalent to **11** was synthesized (not pictured) and found to be about 100–300-fold less potent in both genotypes 1a and 1b. While several additional modifications to the E-ring and substitution on the pyrrole ring were attempted, we were unable to break into the sub-nanomolar potencies enjoyed by the pyrrolidine series. Incorporating the pyrrole core with the bis-phenyl imidazole series generated **20** (Fig. 1). Biochemical evaluation of this molecule found it to be the first in the pyrrole series to possess sub-nanomolar potency in the presence of 40% HP (1a/1b; 0.50 nM/0.56 nM). In rat PK studies, it demonstrated oral bioavailability comparable to **11** (see Tables 1 and 2).

Further SAR on the E-ring pocket of the pyrrole series demonstrated that similarly sized and larger substituents were tolerated without significant decreases in potency, such as cyclohexyl (**21**), adamantyl (**22**), morpholino (**23**), and piperidinyl (**24**). We found that while there was no significant change in potency, in most cases oral bioavailability was negatively impacted. One exception was *N*-morpholino compound **23** which demonstrated 20% oral bioavailability in rat. Previous experience on earlier, internal projects had shown that 2-aminopyridines sometimes had positive effects in rat oral bioavailability [7]. Thus, we synthesized and evaluated the 2-aminopyridine analogs, **25** and **26**. The more potent piperidinyl analog (**26**) showed comparable GT1a potency to **11** and improved oral bioavailability in rat PK ($F = 35\%$). The compound demonstrated a long half-life and low clearance in mouse. In dog, the compound demonstrated low clearance and high oral bioavailability (57%), although it demonstrated a short half-life and lower oral bioavailability in monkey.

2.7 Advanced Characterization and Selection of Ombitasvir (ABT-267)

Several compounds were selected for advanced characterization for potential selection as a clinical candidate. From a virological perspective, high potency against

HCV GT1a and GT1b was viewed as a necessary requirement. Based on their high potencies against GT1a and GT1b in the presence of 40% HP, **11**, **19**, and **26** underwent additional characterization. As shown in Table 3, potencies across the other genotypes were also evaluated where compound **11** showed a potential advantage over the other two compounds. Compound **11** demonstrated relatively uniform potency across the genotypes 1 through 5, with lower potency observed at GT6a. This is in contrast to compound **19** which showed lower potency at GT2a and GT3a and compound **26** which showed lower potency at GT2a, GT2b, GT3a, GT5a, and GT6a. Activity against commonly selected GT1-resistant variants in the replicon assay against NS5A inhibitors is shown in Table 4. In general, high levels of resistance were observed for several variants for all three compounds. HCV GT1a variants at M28, Q30, and Y93 conferred high resistance against compound **11**. Overall, these same variants conferred a lower level of resistance against compound **19** when compared with analog **11**. Phenyl imidazole pyrrole analog **26** demonstrated a comparable profile to **11**, although the HCV GT1b Y93H variant demonstrated higher resistance against **26** relative to **11**.

Compounds **11**, **19**, and **26** exhibited good in vitro microsomal stabilities in human and rat liver microsomes (Table 1), and metabolic stability in hepatocytes suggests low clearance across species (data not shown). The compounds showed low solubility in their amorphous forms at pH 7.2 (Table 1), while higher solubility was observed in fed and fasted simulated intestinal fluids (FeSSIF and FaSSIF). No inhibition of CYP1A2, 2C9, 2C19, 2D6, and 3A4 (IC50 $>$ 30 μM) and no significant CYP3A4 or CYP1A2 mRNA induction in human hepatocytes was observed with

Table 3 Potency across genotypes in the stable replicon assay

	Replicon EC_{50} pM (fold)[a]						
Compd	GT1a	GT2a	GT2b	GT3a	GT4a	GT5a	GT6a
11	14	12 (0.9)	4.3 (0.3)	19 (1.4)	1.7 (0.1)	3.2 (0.2)	366 (30)
19	3	159 (53)	21 (7)	578 (192)	1.9 (0.6)	n.t.[b]	n.t.[b]
26	8	62 (7.7)	1,033 (129)	4,818 (602)	3.6 (0.4)	1,820 (228)	1,536 (192)

[a]Fold loss in activity is relative to GT1a-H77
[b]*n.t.* not tested

Table 4 HCV Genotype 1a transient replicon EC_{50}s (fold resistance) for variants selected against GT1a and GT1b in vitro

	GT1a EC_{50} nM (fold)[a]						GT1b EC_{50} nM (fold)[a]	
Compd	WT	M28T	M28V	Q30R	Y93C	Y93H	WT	Y93H
11	0.0027	24.5 (9,065)	0.159 (58)	2.18 (800)	4.6 (1,675)	113 (41,383)	0.0008	0.06 (77)
19	0.0004	0.063 (146)	0.003 (6)	0.026 (61)	0.044 (102)	0.322 (742)	0.001	0.015 (15)
26	0.0023	0.473 (208)	0.006 (3)	1.27 (559)	0.674 (297)	5.8 (2,555)	0.002	1.07 (535)

[a]Fold loss in activity relative to wt

any of the three advanced analogs. Human, rat, dog, and monkey plasma protein binding was determined to be high (>99%). Observed PAMPA permeability was low for the compounds, while results from the Caco-2 model suggested that **26** has low-to-moderate permeability and were unclear for **11** and **19** due to low recovery and non-specific binding. While **11** and **19** were generally stable compounds, **26** was unstable to UV light (320–395 nm) in pH 7.4 solution. Although this sensitivity could be circumvented by proper light protection, radiolabeled tissue distribution studies in rats indicated that **26** distributed to skin (pigmented/nonpigmented) and eyes and that photo-safety testing should be conducted.

Compounds **11**, **19**, and **26** were well tolerated at the maximum oral exposures in 14-day mouse toxicology studies. In addition, they were predicted to have human half-lives consistent with QD dosing (≥12 h). Ultimately, **11** was selected as the first clinical candidate, being renamed as ABT-267, and ombitasvir in later clinical testing [8, 9]. The pharmacokinetics, safety, and tolerability were evaluated in a phase I study in healthy volunteers following single doses of 5–350 mg and multiple doses of 5–200 mg, where the half-life ranged from 18 to 26 h and 25 to 34 h, respectively [10, 11]. ABT-267 was safe and well tolerated across all dose groups. The antiviral activity of ABT-267 was initially evaluated during 3-day monotherapy in HCV GT1-infected treatment-naïve subjects at doses ranging from 5 to 200 mg [12]. On day 3, dose-normalized Cmax and AUC values were similar across doses. ABT-267 demonstrated C_{max} values ranging from 5.7 to 442 ng/mL and a half-life ranging from 25 to 32 h across the dose groups. As shown in Fig. 2, ABT-267 decreased HCV RNA up to 3.10 $\log_{10}$ IU/mL during 3-day monotherapy with a nearly 3 log reduction observed in all dose groups. The drug candidate was generally

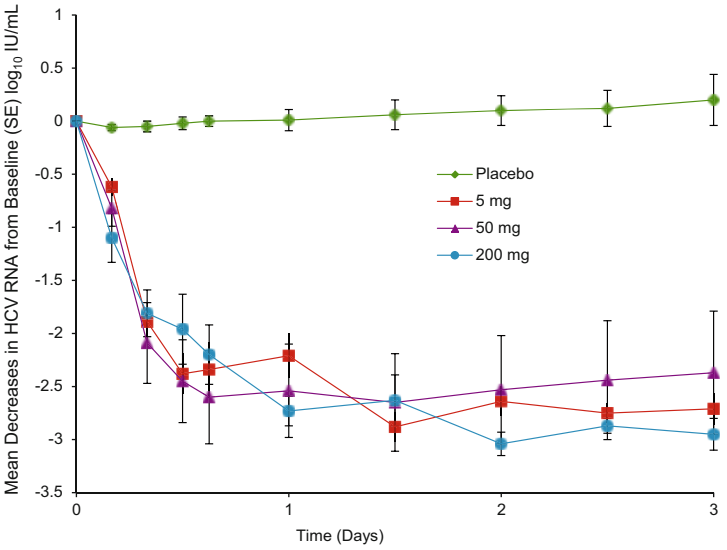


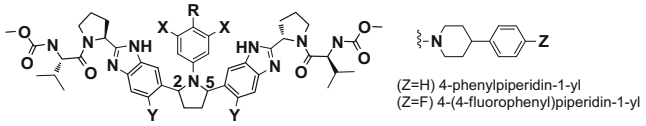
Fig. 2 Mean decreases in HCV RNA from baseline during 3-day monotherapy with ABT-267 (ombitasvir) in HCV GT1-infected treatment-naïve subjects

well tolerated at all doses, and there were no serious or severe adverse events, no clinically significant laboratory abnormalities, and no subjects discontinued. Most adverse events were mild and were not dose related. These findings supported continued development of ABT-267 as a once-daily NS5A inhibitor, and subsequent clinical trials were conducted in combination with NS5B polymerase inhibitor dasabuvir and NS3/4A protease inhibitor paritaprevir/ritonavir with or without ribavirin. Efficacy and safety data from phase III clinical trials supported the regulatory filing and marketing approval of ombitasvir (ABT-267), as part of Viekira Pak™ for the treatment of GT1 HCV in December of 2014.

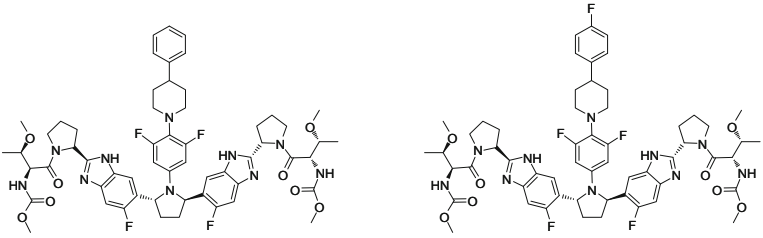
3 Discovery of a Next-Generation NS5A Inhibitor [13]

While the medicinal chemistry strategy for the discovery of a next-generation NS5A inhibitor was to retain the potent antiviral properties of ombitasvir, additional improvements to genotype coverage and the resistance profile were mandatory. Furthermore, development of a compound with an improved resistance profile could potentially translate into requiring fewer DAAs in the curative combination, as well as possibly shorten the 12-week treatment duration, which, with very limited exceptions, was the most common treatment time course in first-generation peg-IFN/RBV-sparing DAA-based therapies. As shown above, AbbVie's first-generation NS5A inhibitor, ombitasvir, exhibited potent EC_{50}s (ranging 0.82–19.3 pM) against HCV genotypes 1–5 and an EC_{50} of 366 pM against genotype 6a in the replicon assays. In vitro resistance selection experiments in genotype 1–6 replicons selected variants which demonstrated reduced susceptibility to the actions of ombitasvir, by factors often greater than 1,000-fold [8]. Variants of NS5A amino acid positions 28, 30, and 93 were most commonly detected in patients experiencing virologic failure with the first-generation NS5A inhibitors ombitasvir, daclatasvir, and ledipasvir. We therefore examined the resistance profiles of the newly synthesized compounds against representative amino acid substitutions, M28T, Q30E, Q30R, Y93C, Y93H, and Y93N in genotype 1a and Y93H and Y93N in genotype 1b replicons.

An alternative inhibitor scaffold (Fig. 3 and Table 5) investigated the impact of the "linker" moiety, as well as the absolute stereochemistry at carbons 2 and 5. While the phenyl amide linker pair, present in ombitasvir and **12** (see Fig. 1), presented the desired broad genotype coverage with the chirality at carbons 2 and 5 being *S*,*S* (vide supra), the 2*S*,5*S*-benzimidazole analog **27** was weakened in genotype 1a in the presence of 40% human plasma, despite being an isosteric replacement for the linker found in ombitasvir (see Fig. 3). Surprisingly, the 2*R*,5*R* isomer (**28**) provided reasonably potent activity against most of the genotypes tested. Coupled with the reduced potency fold loss of first-generation resistant variants relative to WT genotypes 1a and 1b (see Table 7), when compared to ombitasvir, the properties of **28** indicated that this compound could serve as a promising lead for next-generation HCV NS5A inhibitor discovery.



compd	pyrrolidine stereochem.	X	Y	R
27	2*S*,5*S*	H	H	t-butyl
28	2*R*,5*R*	H	H	t-butyl
29	2*R*,5*R*	H	H	F
30	2*R*,5*R*	H	H	cyclohexyl
31	2*R*,5*R*	H	H	*N*-morpholinyl
32	2*R*,5*R*	H	H	4-phenylpiperidin-1-yl
33	2*R*,5*R*	F	H	4-phenylpiperidin-1-yl
34	2*R*,5*R*	F	H	4-(4-fluorophenyl)piperidin-1-yl
35	2*R*,5*R*	H	F	t-butyl
36	2*R*,5*R*	F	F	4-phenylpiperidin-1-yl
37	2*R*,5*R*	F	F	4-(4-fluorophenyl)piperidin-1-yl



38 **PIB (Pibrentasvir, ABT-530**

Fig. 3 Structures of bis-benzimidazole NS5A inhibitors

Our first objective was to establish the structural changes required of **28**'s progeny to achieve high potencies across genotypes 1–6 and then leverage those discoveries toward compounds with significantly improved resistance profiles to maintain effectiveness against first-generation NS5A inhibitor-resistant variants. Extensive modifications of the central *N*-phenylpyrrolidine core unveiled a number of interesting observations (see Table 5). The relatively weak activity of fluorinated derivative **29** in comparison to *t*-butyl analog **28** and cyclohexyl-substituted compound **30** demonstrated the importance of substitution at the para-phenyl position. Replacement of the cyclohexyl substituent to yield the more polar morpholino derivative **31** revealed that increased hydrophilicity was not well tolerated. Although replacement of the morpholino group with the more lipophilic 4-phenyl piperidine **32** did not improve the antiviral activity across genotypes, attenuating the basicity of the piperidine ring through the introduction of fluorine at positions X and Y (**33**) did achieve that objective across genotypes 1–6. Having reached the important goal of

Table 5 Antiviral activity (EC_{50}, pM) of benzimidazole linker NS5A inhibitors in HCV stable replicons

	Inhibition of HCV stable replicons containing NS5A from genotypes 1–6 EC_{50} (pM)									
			40% H. plasma							
Compd	1a	1b	1a	1b	2a	2b	3a	4a	5a	6a
27	71	13	1,980	354	NT	NT	NT	NT	NT	NT
28	9	13	148	268	152	10	6	6	NT	NT
29	38	25	490	427	28	21	31	14	NT	14
30	4	9	107	178	10	7	8	9	NT	NT
31	379	382	902	2,070	598	960	1,200	316	NT	NT
32	324	325	1,510	1,510	468	452	481	243	NT	402
33	2	6	76	183	8	6	6	4	3	8
34	3	8	78	126	20	13	13	9	NT	21
36	2	6	58	135	8	3	4	4	2	9
37	1	3	70	172	3	2	1	1	1	3

Table 6 Pharmacokinetic parameters for selected benzimidazole-linked NS5A inhibitors[a]

		IV			Oral				
Compd	Species	$t_{1/2}$	Vss	Cl	$t_{1/2}$	T_{max}	C_{max}	AUC_{last}	F
28	Mouse[b]	2.0	0.6	0.38	2.6	1.7	0.27	1.36	11
33	Mouse	12.9	0.34	0.02	[c]NC	16	0.44	6.83	[c]NC
34	Mouse	[d]NT	[d]NT	[d]NT	[c]NC	13	0.27	4.59	[c]NC
35	Mouse	[d]NT	[d]NT	[d]NT	4.39	7.0	0.63	5.51	[c]NC
36	Mouse	[d]NT	[d]NT	[d]NT	[c]NC	15	0.99	14.0	[c]NC
37	Mouse	[d]NT	[d]NT	[d]NT	[c]NC	24	1.85	26.1	[c]NC
38	Mouse	[c]NC	0.1[e]	<0.004	[c]NC	7.0	0.96	13.8	[c]NC
	Rat	6.5	0.1[e]	0.10	6.4	3.7	0.12	1.2	5.1
	Dog	4.0	0.1[e]	0.13	3.4	4.0	0.41	4.7	24.2
	Monkey	5.4	0.1[e]	0.11	7.5	2.7	0.19	1.6	8.1
PIB	Mouse	2	0.09[e]	<0.003	[c]NC	11	1.27	20.0	[c]NC
	Rat	6.2	[d]NT	0.07	7.0	5.3	0.28	3.6	9.9
	Dog	7.1	0.1[e]	0.097	8.3	3.67	0.63	6.67	29.8
	Monkey	8.3	0.07[e]	0.15	5.69	4.0	0.29	2.25	14.1

[a]Units: $t_{1/2}$ (h); Cl (L/h/kg); C_{max} (μg/mL); $AUC_{0–24\ h}$ (μg h/mL); F (%). Routine doses: 3 mg/kg IV and oral for rat mouse, 1 mg/kg IV and 2.5 mg/kg oral for dog and monkey
[b]5 mg/kg IV and oral mouse
[c]*NC* not calculated
[d]*NT* not tested
[e]V_C (L/kg)

pan-genotype activity in replicon assays, we turned our attention to a problem that was identified with the original lead bis-benzimidazole inhibitor, **28**. Pharmacokinetic properties (Table 6) for this compound were poor across preclinical species in comparison to the bis-anilide inhibitor ombitasvir, particularly in rodent, where it

was found that very low plasma levels were obtained with oral dosing. Unexpectedly, it was found that larger and more lipophilic E-rings provided higher circulating plasma levels upon oral exposure, e.g., compounds **33** and **34**. An exploratory effort modifying the benzimidazole linker identified symmetrically substituted 5-fluorobenzimidazole analog **35** as an analog with plasma exposures that were several fold higher than that achieved with **28** upon oral administration of a similar dose in mouse [14]. Introducing the fluorine substituent into the benzimidazole groups of pan-genotype inhibitors **33** and **34** gave **36** and **37**, respectively, which provided plasma levels with oral dosing in mouse that were improved several fold in both cases. A notable feature of these compounds is the long T_{max} and significant plasma concentrations of drug at 24 h, which prevent half-life calculations but result in significant enhancement of AUC_{last}. Replicon inhibition across genotypes for **37** ranged from 1 to 3 picomolar, a potency range far better than had been achieved in any inhibitor series before, including the bis-benzimidazole series prior to fluorination of the heterocyclic groups.

Further characterization of many of these analogs also showed that progress was being made against HCV 1a and 1b NS5A first-generation inhibitor-resistant variants. Compounds were evaluated in a transient replicon assay, and fold losses in EC_{50} potencies relative to 1a and 1b NS5A "wild-type" replicons are shown in Table 7. This table illustrates the SAR of a variety of substituent patterns that, while found to show promise across wild-type genotypes 1–6, resulted in significant variation in their ability to suppress the replication of clinically relevant ombitasvir-resistant variants. Examination of the data reveals that the greatest resistance emerged with the Q30E, Y93H, and Y93N variants in genotype 1a. Y93 variants of genotype 1b, where tested, were susceptible to all analogs shown in Table 7. There appears to be a general trend that increased size at position "R" (Fig. 3) correlates with an improved ability to suppress replication with lower multiples of the wild-type EC_{50}. One particular exception to this trend is found for

Table 7 SAR of NS5A inhibitors – fold resistance of HCV genotype 1a/1b NS5A transient replicon variants vs. wild type

	HCV genotype 1a/1b NS5A variants vs. wild type (fold resistance)							
	1a[a]						1b	
Cmpd	M28T	Q30E	Q30R	Y93C	Y93H	Y93N	Y93H	Y93N
28	4	61	14	7	216	510	2	NT
30	>17	>90	>20	>30	>577	NT	1	NT
31	2	6	7	5	87	NT	1	NT
32	2	4	3	2	4	5	1	1
33	3	69	7	6	72	145	1	1
34	2	20	3	3	20	25	2	NT
36	1	12	2	6	49	50	1	1
38	1	2	1	1	6	5	1	0.3
PIB	2.1	2.4	1.7	1.7	6.7	6.7	0.6	0.6

[a]Observed as resistant variants in ombitasvir single-agent clinical studies

30, where the wild-type EC_{50} was more potent than the lowest concentration tested. Although the fold resistance pattern for **32** is superior to any other compound in Table 7, it should be noted that Table 5 shows the pan-genotype replicon potencies for this compound to be much weaker than **33**, **34**, and **36**. Indeed these three compounds come close to fulfilling the virology profile requirements for a next-generation analog and deliver a profound improvement in performance against resistant variants when compared to the first-generation NS5A inhibitor ombitasvir or **28**, while demonstrating good PK in mouse (Table 6).

The beneficial effects observed by introduction of fluorine atoms at several key positions in our developing chemical matter were sufficiently noteworthy to the team so that we favored late-stage research activities to include these structural modifications. As the majority of the early and intermediate medicinal chemistry activities focused on the central and linker regions of the lead structures in both the first-generation and next-generation efforts, it was observed that substitution of the Moc-Val capping groups also affected the virological properties. In particular, efforts in the first-generation medicinal chemistry studies showed that oxygen-containing amino acids could beneficially affect the resistance profile of the resulting analogs (data not shown). The intriguing possibility of importing those observations into the next-generation medicinal chemistry effort resulted in the synthesis of the Moc-methyl-threonine-capped compounds shown in Table 8. Analogs **38** and PIB (pibrentasvir, ABT-530) demonstrated potent inhibition of the HCV replicon across genotypes 1–6. However, just as gratifying were the very low fold losses measured against the first-generation NS5A inhibitor-resistant variants, thus achieving two critical project objectives (Table 7) [15]. Upon oral administration in mouse, the plasma exposures of both compounds compare favorably to prior compounds in Table 6. Interestingly, both compounds show much lower exposures in rat than in mouse, with plasma exposure in dog showing better performance than either monkey or rat. While the virology of both compounds are similar, PIB's PK properties do seem to show a slight advantage, and it was therefore elevated to clinical status.

Pibrentasvir was further characterized to determine how frequently resistant colonies would emerge from replicons containing NS5A genes from genotypes 1a, 1b, 2a, 2b, 3a, 4a, 5a, and 6a at both 10× and 100× of the WT EC_{50}s (Table 9) [15]. After determining the resistant NS5A gene sequences that conferred resistance, mean EC_{50}s of PIB against these variants (and therefore fold resistance vs. WT in transient replicons containing these single or double point mutations) were measured, as well as replication efficiency to estimate the mutant's "fitness." No surviving colonies were detected when HCV 1b, 2b, 4a, 5a, and 6a NS5A-containing replicons were incubated in the presence of 10× or 100× of PIB's EC_{50}. Upon incubation with PIB at 10× EC_{50}, genotypes 1a, 2a, and 3a showed colony survival of 0.0065%, 0.00015%, and 0.0003%, respectively. At 100× EC_{50}, only genotype 1a NS5A containing replicons survived the incubation with a frequency of 0.0002%. At 10× EC_{50}, replicons of genotype 1a and 3a display the Y93H resistant variant as either the most frequently prevalent or only surviving colony detected, and genotype 2a displays only two colonies both of which possess amino acid changes at two locations (F28S/M21I or P29S/K30G). At 100× EC_{50}, only genotype 1a NS5A

Table 8 Antiviral activity (EC_{50}, pM) of Moc-*O*-methyl-Thr-capped NS5A inhibitors in HCV stable replicons

	Inhibition of HCV stable replicons containing NS5A from genotypes 1–6 EC_{50} (pM)									
			40% H. plasma							
Compd	1a	1b	1a	1b	2a	2b	3a	4a	5a	6a
38	1	3	43	138	3	3	3	3	2	4
PIB	1.8 ± 0.86	4.3 ± 1.7	64 ± 14	200 ± 54	2.3 ± 0.65	1.9 ± 0.59	2.1 ± 0.66	1.9 ± 0.61	1.4 ± 0.36	2.8 ± 0.67

Table 9 Selection of NS5A amino acid substitutions by PIB in replicon cell lines with NS5A from HCV genotypes 1–6 and resistance of these substitutions to pibrentasvir

Genotype[a]	Colony survival (%)[b]		NS5A amino acid substitutions	Prevalence in replicon selection[c]		Mean EC_{50} ± SD (pM)	Fold change in EC_{50}[d]	Replication efficiency[d] (%)
	10× EC_{50}	100× EC_{50}		10× EC_{50}	100× EC_{50}			
1a[e]	0.0065	0.0002	Q30D[f]	0/20	1/4[g]	68 ± 37	94	50
			Q30 deletion	0/20	1/4[g]	2,555 ± 268	3,549	0.5
			Y93D	0/20	1/4[g]	NV	NV	<0.5
			Y93H	18/20	0/4[g]	4.8 ± 1.5	6.7	18
			Y93N	1/20	0/4[g]	5.1 ± 2.1	7.1	25
			H58D + Y93H	0/20	1/4[g]	1,612 ± 272	2,238	13
1b	0	ND	NA	NA	NA	NA	NA	–
2a[e]	0.00015	0	F28S + M31I	2/3 g	NA	14,303 ± 2,722	14,448	–
			P29S + K30G	1/3 g	NA	2.3 ± 0.36	2.3	–
2b	0	0	NA	NA	NA	NA	NA	–
3a[e]	0.0003	0	Y93H	3/3[h]	NA	1.5 ± 0.19	2.3	–
4a	0	0	NA	NA	NA	NA	NA	–
5a	0	0	NA	NA	NA	NA	NA	–
6a	0	0	NA	NA	NA	NA	NA	–

NA not applicable, *ND* not done, *NV* not available, as the EC_{50} value could not be determined due to low replication efficiency of the replicon containing the amino acid substitution

[a]Genotype of NS5A in replicon cell lines

[b](Number of surviving colonies/number of input replicon cells) × 100

[c]Number of times an amino acid substitution was found out of the total number of colonies analyzed

[d]Relative to the respective wild-type replicon

[e]EC_{50} values for wild-type replicons in transient transfection assays: genotype 1a = 0.72 pM, genotype 2a = 0.99 pM, and genotype 3a = 0.65 pM

[f]Substitution with double nucleotide changes

[g]Denominator indicates total number of colonies that survived selection out of 2×10^6 input cells

[h]Denominator indicates total number of colonies that survived selection out of 1×10^6 input cells

replicons show survival, with only four colonies detected starting from 2×10^6 input cells. The genotype 1a NS5A mutants with the highest fold resistance are the Q30 deletion and H58D + Y93H variants with a fold change in EC_{50} of 3,549-fold and 2,238-fold vs. WT, respectively. However, the replication efficiency of replicons containing these changes is significantly lower at 0.5% and 13% of WT. The Q30D mutation shows the highest replication efficiency at 50%, conferring a 94-fold change in EC_{50} relative to WT.

4 Clinical Studies

PIB in combination with glecaprevir (GLE, ABT-493) has been the subject of multiple clinical studies, having successfully completed phase I–III studies in the USA and abroad. The combination GLE/PIB (trade name Mavyret in the USA and Maviret in Europe) was approved in the USA and Europe in August 2017. A partial summary of some of these clinical studies is shown below.

SURVEYOR-1 (genotypes 1, 4, 5, and 6) and SURVEYOR-2 (genotypes 2 and 3) were phase II, open-label, multicenter dose-ranging trials in non-cirrhotic patients with chronic HCV genotype 1–6 infection who were either previously untreated or only treated with pegylated interferon plus ribavirin (Table 10) [16]. Doses of GLE and PIB were varied, with or without ribavirin (RBV, total daily dose 1,000 mg for patients $<$75 kg or 1,200 mg for patients $\geq$75 kg) for 8 or 12 weeks. Primary efficacy endpoints were the percentage of patients that achieved a sustained virologic response 12 weeks after completing treatment (SVR12).

Across all studies 319/449 (70%) patients experienced adverse events, with the majority reported as mild in severity. The most common ($\geq$10%) adverse events in RBV-free treatment were fatigue, headache, and nausea, with a greater frequency of these events occurring in RBV-treated patients. Three patients discontinued treatment prematurely due to adverse events. Serious adverse events were reported in seven patients, none of which were considered to be related to the study drugs. Table 10 shows that the most difficult to treat patients were infected with genotype 3, with the higher dose combination of 300 mg GLE and 120 mg PIB demonstrating superior efficacy than the lower dose combinations. Based on this observation, combined with the desire to maintain a consistent dosing paradigm across patients in genotypes 1–6, this particular dose combination was chosen for further studies. For treatment-naïve or PEG-IFN/RBV-treated HCV genotype 1, 2, 4–6 patients, SVR12 was achieved at between 96 and 100% of the patient groups tested, with no significant differences detected between 8- and 12-week treatment durations in genotype 2 patients. In genotype 3, for the treatment arm containing both treatment-naïve and PEG-IFN/RBV-treated individuals, 12 weeks of treatment at 300 mg GLE and 120 mg PIB resulted in 28/30 (93%) patients achieving SVR. Two separate treatment arms receiving the same doses had the treatment-naïve patients achieving 28/29 (97%) SVR_{12} after 8 weeks of treatment and the PEG-IFN/RBV-treated individuals achieving 22/24 (92%) SVR_{12} after 12 weeks of treatment.

Table 10 SURVEYOR-1 and SURVEYOR-2 virologic response during and after treatment and reasons for non-response

Genotype	Prior Tx history	Dose GLE + PIB	Tx duration (weeks)	Sustained virologic response, n/N (%)		Reasons for non-response, n (%)			
						Virologic failure		Non-virologic failure	
				PTW4	PTW12[a]	Breakthrough	Relapse	Missing SVR12 data	Early Tx discontinuation
1	TN or PR	200 + 120	12	40/40 (100)	40/40 (100)	0	0	0	0
	TN or PR	200 + 40	12	38/39 (97)	38/39 (97)	0	1 (3)	0	0
	TN or PR	300 + 120	8	34/34 (100)	33/34 (97)	0	0	0	1 (3)
2	TN or PR	300 + 120	12	24/25 (96)	24/25 (96)	0	0	0	1 (4)
	TN or PR	200 + 120	12	24/24 (100)	24/24 (100)	0	0	0	0
	TN or PR	200 + 120 + RBV[b]	12	25/25 (100)	25/25 (100)	0	0	0	0
	TN or PR	300 + 120	8	53/54 (98)	53/54 (98)	0	0	0	1 (2)
3	TN or PR	300 + 120	12	28/30 (93)	28/30 (93)	0	1 (3)	1 (3)	0
	TN or PR	200 + 120	12	28/30 (93)	28/30 (93)	0	2 (7)	0	0
	TN or PR	200 + 120 + RBV[b]	12	29/31 (94)	29/31 (94)	1 (3)	0	0	1 (3)[c]
	TN or PR	200 + 40	12	28/30 (93)	25/30 (83)	1 (3)	2 (7)	1 (3)	1 (3)
	TN	300 + 120	8	28/29 (97)	28/29 (97)	0	0	1 (3)	0
	PR	300 + 120	12	23/24 (96)	22/24 (92)	1 (4)	1 (4)	0	0
4, 5, 6	TN or PR	300 + 120[d]	12	34/34 (100)	34/34 (100)	0	0	0	0

Tx treatment, *PTW* post treatment week, SVR_{12} sustained virologic response at post treatment week 12, *TN* treatment naïve, *PR* peg-IFN/RBV-experienced, *RBV* ribavirin

[a]Primary endpoint

[b]RBV total daily dose 1,000 mg for patients <75 kg or 1,200 mg for patients ≥75 kg

[c]Patient discontinued treatment at week 10 and was found to be reinfected with HCV genotype 1a during post treatment follow-up

[d]Includes two patients who received GLE 200 mg + PIB 120 mg for 12 weeks

On- or post treatment virologic failure occurred in 10/449 (2%) of the treated patients. One genotype 1a patient who received the lowest GLE/PIB dose combination experienced relapse post treatment at week 4. The remaining nine patients with virologic failure were infected with genotype 3a with 6/9 receiving doses lower than the most effective GLE/PIB combination which was discovered during this dose-ranging study.

ENDURANCE-1 and ENDURANCE-3 were two phase III randomized, open-label, multicenter trials treating a total of 1,208 non-cirrhotic patients infected with either HCV genotype 1a/b or 3, which compared the outcomes of treating patients with 300 mg GLE and 120 mg PIB QD for either 8 or 12 weeks (Table 11) [17]. In the genotype 3 patient cohort, 1/3 of the patients enrolled were dosed with sofosbuvir-daclatasvir for 12 weeks in order to compare this patient outcome to the 8- and 12-week GLE/PIB HCV genotype 3 patient arms. Non-cirrhotic patients who presented positive for HCV genotype 1 infection could also be coinfected with HIV-1 and could either not have received treatment for HCV or have received an IFN-containing regimen with or without ribavirin or treatment with sofosbuvir with or without PEG-IFN. Genotype 3 patients needed to be treatment naïve. The safety profile of GLE/PIB in all patients was similar with the most common adverse effects ($\geq$10%) being headache and fatigue. Serious adverse events were reported in 1–2% of treated patients with none deemed to be related to the trial drugs. The results of these trials are summarized in Table 11. The results show that a high rate of HCV genotype 1- and 3-infected patients achieved SVR12 in both 8- and 12-week treatment times.

At baseline, the characteristics of patients were generally similar, but there were some notable differences. Among genotype 3-infected patients, prevalence of stage F3 fibrosis was higher in the 8-week GLE/PIB group (17%, as compared with 8–9%). The baseline HCV RNA levels in genotype 3 patients were 6 million IU/mL or higher in the 12-week GLE/PIB arm than in the SOF/DAC arm (28% vs. 12%). In this study, genotype 1 patients experienced the highest level of SVR12 upon treatment with GLE/PIB with the 8-week arm statistically demonstrating non-inferiority to the 12-week arm (348/351 patients and 351/352 patients, respectively). Genotype 3 patients treated for 12 weeks with GLE/PIB achieved SVR12 at a slightly lower rate in 222 out of 233 patients, whereas the 8-week arm achieved SVR12 rate of 149 out of 157 patients. Statistical analysis of these genotype 3 results showed non-inferiority of the 8-week regimen when compared to the 12-week treatment. Interestingly, the genotype 3 patients receiving 12 weeks of SOF/DAC achieved an SVR12 in 111 out of 115 patients. A statistical comparison showing superiority of 12 weeks of treatment of GLE/PIB was not attempted because of a statistical procedure which required both non-inferiority criteria to be met for the comparison between the 8-week and 12-week GLE/PIB treatment arms in order to proceed to testing the next ordered comparison.

As a result of these and other studies, the USA FDA approved Mavyret in August 2017 for the treatment of chronic hepatitis C viral infections of genotypes 1–6. Treatment duration is dependent on prior patient treatment experience, genotype, and whether or not the individual is non-cirrhotic or presents with compensated

Table 11 ENDURANCE-1 and ENDURANCE-3 treatment outcomes in the intention-to-treat population

Genotype	Tx	Tx duration (weeks)	Number of patients	SVR12	Reasons for failure, n (%)					
					Virologic failure		Non-virologic failure			
					Break-through	Relapse	Follow-up loss or missing SVR12 data	Early Tx discontinuation	Consent withdrawn	Nonadherence
1	GLE/PIB	12	352	351 (99.7)	0	0	1 (<1)	0	0	0
1	GLE/PIB	8	351	348 (99.1)	1 (<1)	0	1 (<1)	1 (<1)	0	0
3	GLE/PIB	12	233	222 (95)	1 (<1)	3 (1)[a]	4 (2)	1 (<1)	1 (<1)	1 (<1)
3	GLE/PIB	8	157	149 (95)	1 (1)	5 (3)	2 (1)	0	0	0
3	SOF/DAC	12	115	111 (97)	0	1 (1)	2 (2)	1 (1)	0	0

Tx treatment, *SVR12* sustained virologic response at post treatment week 12, *GLE/PIB* 300 mg glecaprevir/120 mg pibrentasvir QD, *SOF/DAC* 400 mg sofosbuvir/60 mg daclatasvir QD

[a]One patient had reinfection with HCV genotype 3, as determined by phylogenetic analysis

cirrhosis (Child-Pugh A). The shortest treatment period is 8 weeks for non-cirrhotic treatment-naïve individuals with HCV genotypes 1–6. Treatment of patients with no prior HCV treatment that present with compensated cirrhosis (Child-Pugh A) requires 12 weeks of therapy. Those that have received prior treatment are categorized according to genotype, treatment type, and cirrhotic state (no cirrhosis or compensated cirrhosis (Child-PughA)). Depending on category, treatment can be as short as 8 weeks or as long as 16 weeks.

From first-generation NS5A inhibitors, such as daclatasvir, ledipasvir, elbasvir, and ombitasvir, to the next-generation inhibitors such as velpatasvir and pibrentasvir, all have had a profound and powerful impact on the IFN-sparing treatment of HCV infection. These inhibitors, in combination with orthogonally mechanistic inhibitors, have allowed regimens that are better tolerated than IFN-based treatment and which have therefore unsurprisingly encouraged higher patient compliance. In combination, all this has resulted in higher cure rates, shorter treatment periods, improved convenience, fewer side effects, and ultimately lower costs for a cure. Moreover, it is expected that curing hepatitis C will not only reduce the burden on the healthcare system by reducing the rates of cirrhosis and hepatic carcinoma, but reduce the additional emotional and economic costs that HCV morbidity brings to patients' lives and their families.

Compliance with Ethical Standards

Funding All research described was funded by AbbVie.

Conflict of Interest All authors are employees of AbbVie and may own stock in the same.

Ethical Approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the clinical studies.

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Part II
HCV NS4B Inhibitors

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Evolution of HCV NS4B Inhibitors

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Abstract NS4B has remained for a long time an undisclosed target within the HCV drug discovery programs. However, impressive drug discovery efforts from 2005 to 2016 led to the identification of different chemical classes targeting NS4B as effective anti-HCV agents, and some of them act by impairing AH2-mediated membranous web formation or RNA-binding property. This book chapter aims to discuss research published on NS4B inhibitors focusing on hit identification and hit-to-lead optimization, also with respect to pharmacokinetic properties and structure-activity relationships raised for the different chemical classes taken into account. To date, the only clinical trial conducted with molecules targeting NS4B was focused on clemizole hydrochloride. However, even if NS4B ligands are not currently used in therapy, they can serve in the near future as new weapons to combat resistance to the current therapy.

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1 HCV NS4B Protein and Related Biochemical Assays to Discover New Binders

1.1 Structure and Function of NS4B

The NS4B protein characterization and functions have been already described earlier in this book and highlighted a key contribution of the protein in HCV replication and also cell transformation. However, before presenting the evolution of HCV NS4B inhibitors, a historical reconstruction of studies conducted on this protein deserves further analysis.

Studies on NS4B were first conducted at the end of the twentieth century when its subcellular localization was determined as a first step toward the understanding of its function [1, 2]. Indirect immunofluorescence and green fluorescent protein fusion experiments determined that NS4B is cytoplasmically localized in the perinuclear region where it adopts chicken wire-like and speckled patterns typical of a membrane-associated protein [1–3].

In 2001, Moradpour D. and co-workers published results on the subcellular localization of NS4B employing continuous human cell lines inducibly expressing NS4B, either individually or in the context of the entire HCV polyprotein [4]. In this study it was demonstrated that the majority of the protein was an integral endoplasmic reticulum membrane protein oriented toward the cytoplasm. Although NS4B was computationally predicted as an internal transmembrane protein with four or six domains, authors were unable to experimentally demonstrate the presence of transmembrane or lumenal fragments. These studies revealed that the protein had properties of a cytoplasmically oriented integral membrane protein, and it was observed that 40–70% of NS4B protein sedimented after in vitro transcription-translation in the presence of microsomal membranes.

Later, in 2003, Persson M. and co-workers described more in-depth the cellular location and topology of NS4B [5]. Their studies confirmed previous findings, but researchers also observed new membrane structures visible by immunofluorescence in cells expressing NS4B. The protein was found not only associated to endoplasmic reticulum (ER) but also able to promote rearrangement of intracellular membranes as a result of an intrinsic property. Furthermore, a computer analysis of the protein, conducted on gt 1a, predicted four transmembrane domains (TMDs) with both the C- and the N-terminal tails located in the cytoplasm. The topology organization was further refined through glycosylation studies; observing glycosylation at specific residues of the protein, researchers demonstrated that five TMDs were present instead of the four computationally predicted. Thus, the N-terminal tail of NS4B was thought to be translocated across the ER membrane by a posttranslational

mechanism. Although the dual topology of viral proteins is not unprecedented, for the first time, the work by Persson and colleagues demonstrated this behavior for HCV NS4B.

After the abovementioned key contributions, many scientists have been continuing to improve our understanding of NS4B, thus highlighting structural features and properties of this protein. In 2004, Glenn and co-workers reported on the identification of an N-terminal amphipathic helix (aa 6–29), now called AH1, responsible for NS4B membrane association, correct localization of the HCV replication complex proteins, and RNA elongation [6]. In 2009, two other helical regions (aa 42–66 at N-terminal and aa 229–253 at C-terminal) were successively identified and described by Moradpour and co-workers [7, 8]. The new alpha-helices (initially called AH2 and AH3, which was later called H2) were ascribed as important elements for NS4B membrane association and HCV replication complex formation. To note that the existence of AH1 segment was debated for some years until, in 2014, a key contribution confirmed the presence of an amphipathic α-helix between aa 4–32, with positively charged amino acid residues flanking this structural element and important in membranous web (MW) formation and RNA replication [9].

In summary, NS4B is a predominantly hydrophobic transmembrane protein possessing a multifunctional role within HCV replication. The protein has an N-terminal part (aa 1–69) and a central core with at least four predicted TMDs (aa 70–190) and a C-terminal part (aa 191–261) [4, 5, 8]. The NS4B N-terminus consists of two amphipathic α-helices, AH1 (aa 6–29) and AH2 (aa 42–66), with the last segment which is well conserved in all HCV genotypes (gts) and critical for HCV replication [6, 7, 9]. The N-terminal, oriented on the cytosolic face of ER, seems to cross the membrane yielding the fifth additional TMD also called TMX and promote AH1 translocation into the ER lumen [5]. The N-terminal translocation seems induced by NS4B dimerization/multimerization that promotes lipid vesicle aggregation and in turn seems to play an important role in MW formation for the recruitment of the HCV RNA replication complex [6–8, 10, 11]. Indeed, NS4B plays a structural role in HCV RNA replication complex formation due to the ability to reorganize the intracellular membranes into new membranous structures (e.g., MW) [6, 8, 11]. The NS4B central region harbors a nucleotide-binding motif (NBM) (Walker A motif) located between TM2 and TM3 domains (aa 129–135) with the typical "GXXXXGK" motif of a NTPase [5, 12]. Found in almost all HCV gts, the NBM Walker A permits binding and hydrolysis of GTP and ATP and the synthesis of ATP and AMP from two ADP molecules [13], and it is essential for HCV life cycle, as shown by mutagenesis studies [12]. Although the precise role of the NBM-mediated NTPase activity remains still unclear, it has been proposed that the NS4B GTPase activity plays an important role in cell transformation and tumor formation [14]. The analysis of the secondary structure of NS4B C-terminal reveals two α-helices, named H1 and H2 [8]. The first helix extends from residue 200 to 213 and is highly conserved among HCV gts, while the second is a less conserved twisted amphipathic α-helix composed of aa 229–253 as demonstrated by the 3D NMR structure (PDB code 2KDR) [11, 15, 16]. H2 (originally called AH3) mediates membrane association and it is also involved in the formation of a functional HCV

replication complex [8, 17, 18]. In addition, the C-terminal domain includes arginine-rich motifs at residues 192–193 and 247–248 able to bind the 3′ end of the HCV ss-(−)-RNA, an essential property for efficient in vitro viral replication [19]. NS4B C-terminal has also two palmitoylation sites at two terminal cysteine residues (aa 257 and 261) probably involved in NS4B oligomerization, but the role of C-terminal palmitoylation of NS4B in the HCV life cycle still remains unclear [20].

Attempts to express, purify, and realize membrane-associated NS4B constructs for subsequent studies of the 3D protein structure were conducted by Böckmann and co-workers, but unfortunately these studies did not lead to a definitive structure and thus deserve further investigations [21]. In 2017, Bartenschlager and collaborators reported on the characterization of conserved glycine-zipper motifs within NS4B TM helices 2 and 3 involved in NS4B self-interaction and that contribute significantly to HCV-induced membrane rearrangements, crucial for HCV replication [22]. To date, several functions are attributed to NS4B and can be summarized as follows: (a) recruitment of lipid raft from intracellular membranes, (b) MW formation through a remodeling of ER, (c) effects on HCV RNA translation, (d) modulation of NS5B RdRp activity, and (e) immunomodulation and malignant cell transformation that can in part explain the ability of HCV to facilitate the development of hepatocellular carcinoma [3, 23].

1.2 Screening Assays to Discover NS4B Ligands

The transmembrane nature of NS4B represents a challenge for protein expression and biochemical and structural characterization and has hampered the development of quick screening. Thus, the most prominent approach to discover NS4B ligands entailed the use of a phenotypic approach based on high-throughput screening (HTS) campaigns employing HCV replicons. The protein was successively identified as a target, carrying out genetic validation based on the identification of mutations in HCV genome sequence after compound exposure. All the compounds identified using this approach are discussed in the next section.

Nonetheless, some in vitro assays based on biophysical and/or biochemical HTS methods have been applied over the years and include (1) a microfluidic RNA-binding inhibition assay [19], (2) an AH2-mediated lipid vesicle aggregation inhibition assay [24], (3) a quenching fluorescence binding assay [25], and (4) a nontraditional approach based on encoded library technology (ELT) [26].

The microfluidic affinity assay has been developed by Glenn and co-workers with the aim to study the HCV RNA-binding properties of NS4B and, subsequently, to evaluate the capability of small molecules to inhibit the NS4B-RNA complex formation [19]. Interestingly, this assay was advantageously used in a HTS procedure which evaluated 1,280 compounds and led to the identification of 18 potential

hit compounds including clemizole hydrochloride (see Sect. 2.1). In this assay a flow layer containing fluorescently labeled HCV RNA is delivered through a chamber where a static layer formed by immobilized NS4B protein can bind the viral RNA. The increase of unbound RNA is related to the reduced affinity of NS4B for the RNA induced by the inhibitor. Its ability to interfere with the NS4B-RNA binding is expressed as IC_{50}, measured through a method based on mechanical trapping of molecular interactions [19].

Glenn and colleagues have also published a NS4B AH2-mediated lipid vesicle aggregation inhibition assay to evaluate the ability of small molecules to inhibit the NS4B-mediated MW formation, one of the key functions of the viral protein [24]. This assay consists of two consecutive experiments based on different biophysical methods: (1) the fluorescence microscopy and (2) the dynamic light scattering. In the first step, the aggregation of fluorescently labeled synthetic lipid vesicles upon addition of a synthetic AH2 peptide was monitored by fluorescence microscopy, and the intensity of fluorescence was compared in the absence and in the presence of the tested compound. Molecules able to reduce lipid vesicle formation passed to the second screen. Thus, dynamic light scattering measurements of lipid vesicle size were performed in the presence of a compound, and the inhibition activity was definitively confirmed. Finally, the best compounds were further analyzed in transient replication assays showing inhibition of HCV replication in a dose-dependent manner. This approach led to the identification of anguizole and an amiloride analogue as promising starting point for further developments (see Sects. 2.2 and 2.6, respectively). Detailed analysis of the aforementioned compounds revealed that AH2 function can be disrupted by either one of the two mechanisms: inhibition of NS4B AH2 oligomerization or inhibition of the ability of AH2 to associate with membranes.

The quenching fluorescence binding assay is based on measuring fluorescence variations of a recombinant NS4B upon ligand binding, allowing also for the determination of K_D. This assay was exploited by Chunduru and collaborators who identified and patented different anti-HCV compounds targeting NS4B (see Sect. 2.6) [25].

Thompson and co-workers at GlaxoSmithKline have advantageously exploited ELT to screen an unprecedented large collection of small molecules as N4SB binders (see Sect. 2.6) [26]. Several combinatorial libraries were built by conjugating drug-like building blocks with short coding double-strand DNA tags as markers of each chemical library. Split/mix methods were applied to achieve DNA-tagged libraries with wide chemical diversity that were screened by affinity selection on the immobilized NS4B target protein. Bound molecules were first separated from non-bound molecules and then removed by heat elution. After translation of the amplified DNA tagging sequences into reporter protein, chemical libraries containing the NS4B protein binders were indirectly identified. The confirmation of ligands was carried out through the resynthesis of molecules, without the DNA tag, belonging to the identified chemical libraries. Finally, the K_D of each compound was separately determined using a radiolabeled known NS4B ligand in a displacement assay.

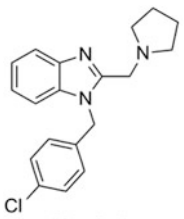
2 HCV NS4B Inhibitors

Until a few years ago, little data was available in the literature about anti-HCV agents targeting NS4B. The last decade has highlighted that NS4B also represents an appealing drug target, thus making this protein one of the last studied targets in HCV drug discovery. Indeed, many hit compounds have been identified by different screening procedures, and hit-to-lead optimization campaigns have been reported. Despite the fact that no drugs acting as NS4B inhibitors have been approved, promising preclinical candidates belonging to different chemical classes have been identified and extensively reviewed [27, 28].

2.1 Clemizole

The old H_1 antihistaminic drug clemizole hydrochloride [29] was one of the first compounds targeting HCV NS4B, identified in 2008 by Glenn and co-workers at Stanford University [19]. In the microfluidic RNA-binding assay, clemizole was shown to be a potent NS4B RNA-binding inhibitor but was a weak inhibitor of HCV replication (Fig. 1) [19, 30]. The discrepancy between biochemical potency and antiviral activity was attributed to low membrane permeability. In addition, NS4B was validated as target of clemizole since two important aa mutations in HCV gt 1b were generated: W55R in AH2 region and R214Q in the cytoplasmic C-terminal segment [19, 30]. Later, clemizole in combination with the first generation of HCV protease inhibitor (i.e., boceprevir or telaprevir) demonstrated a promising synergistic and gt-independent antiviral activity [30].

Successive structure-activity relationship (SAR) campaigns, carried out around clemizole, were not successful, and a number of new analogues possessed undesirable hERG activity [31–34].



Clemizole
IC_{50} (NS4B) = 24 nM
EC_{50} (gt 2a) = 8 μM
EC_{50} (gt 1b) = 23 μM

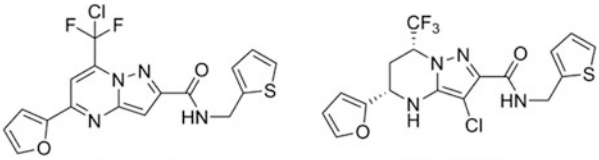
Fig. 1 Structure and activities of clemizole

Thanks to its well-known safety profile, clemizole is the only NS4B inhibitor evaluated into Phase 1B clinical trials in treatment-naïve HCV chronically infected patients (gt 1 and gt 2), but no results have been reported [35].

2.2 *Anguizole and Structurally Related Compounds*

The pyrazolo[1,5-*a*]pyrimidine anguizole (Fig. 2) was discovered in 2005 by Chunduru and colleagues at ViroPharma through the quenching fluorescence binding assay and demonstrated a good NS4B binder [25]. Anguizole was able to reduce HCV protein expression, as determined by an ELISA-based HCV replication assay, without significant toxicity [25, 36]. Later on, Glenn and his team demonstrated that anguizole hampered the interaction of the NS4B-AH2 with lipid vesicles and the lipid vesicle aggregation, thus suggesting a direct binding of the compound to the AH2 region [24, 37]. Furthermore, the molecule was active in a HCV replicon luciferase reporter assay resulting, at that time, in the first NS4B ligand endowed with sub-μM antiviral activity [24]; but the compound was inactive against HCV gt 2a (Fig. 2) [37]. In addition, resistant mutants on NS4B were identified after anguizole treatment of cells carrying the HCV gt 1b replicon, with the H94R mutation being the most common resistance mutation; the F98L and the V105M mutations were also observed in some HCV colonies [37]. Lee and colleagues had also shown that anguizole interfered with (1) NS4B dimerization/multimerization altering the protein subcellular localization and disrupting MW formation and (2) NS4B/NS5A interaction [38].

Successively, a partly saturated analogue of anguizole (compound **5**, called *AP80978*), having 5*S*,7*R* configuration, was reported by Rice and co-workers, at the Rockefeller University, as a sub-μM HCV replication inhibitor targeting NS4B (Fig. 2) [39].



Anguizole
K_D = 0.622 μM
EC_{50} = 0.63 μM[a]
EC_{50} (gt 1a) = 0.56 μM[b]
EC_{50} (gt 1b) = 0.31 μM[b]
CC_{75} > 100 μM[c]
CC_{50} > 50 μM[d]

5 (AP80978)
EC_{50} (gt 1a) = 0.62 μM
EC_{50} (gt 1b) = 0.63 μM
CC_{50} > 100 μM

Fig. 2 Structure and activities of anguizole and compound **5**. [a] ELISA-based HCV replication assay; [b] HCV replicon luciferase reporter assay. [c] Crystal violet staining-based assay. [d] Cell proliferation reagent WST-1-based assay

Compound **5** was active against gts 1a and 1b but was inactive against gt 2a, and its potency, gt specificity, and resistance profile were very similar to those of anguizole [39].

One of the first examples of a successful hit-to-lead optimization campaign, which led to the identification of a preclinical candidate, was carried out at GlaxoSmithKline on molecules structurally related to anguizole [40–44]. As reported by Shotwell and co-workers in 2012, the project started from the identification of hit imidazo[1,2-*a*]pyridine **6** (Fig. 3), through a cell-based HTS using a HCV gt 1b replicon luciferase reporter assay. It was demonstrated that compound **6** specifically bound to NS4B and induced the production of NS4B-resistant mutants [40]. Interestingly, key mutations were H94N, F98L, and V105M, the same observed for anguizole. Initial modifications on hit **6** entailed the replacement of the C-3 bromine with a chlorine and of the C-5 1*H*-4-pyrazolyl with a 3-furyl, in analogy to anguizole, leading to an equipotent analogue. Optimization of the amide side chain started by replacing the chiral substituted pyrrolidine with a piperidine to yield achiral compounds, while different aromatic and aliphatic heterocyclic rings were explored in place of the 2-thiophenyl ring, which was identified as a main metabolic site by in vitro/in vivo studies.

Among the new compounds, derivative **7** having an oxazolidinone moiety at the amide side chain retained high affinity for NS4B and showed very potent antiviral activity in the low nM range (Fig. 3) [40]. Despite its interesting activity, derivative **7** was still far from desirable pharmacokinetic (PK) properties, being characterized by quick in vivo clearance due to metabolism of oxazolidinone ring [40]. Iterative cycles of optimization focusing on modification of the amide side chain allowed the identification of the piperazinone nucleus as suitable replacement for the piperidinyl oxazolidinone, with *N*-cyclohexyl derivative **8** being a low nM NS4B binder and a good inhibitor of HCV replication (Fig. 3). The presence of the hydrophobic pendant ring was a key feature for obtaining a strong NS4B binding. A 4-hydroxyl substituent was added at the cyclohexyl ring (i.e., **9**, Fig. 3) in an attempt to increase the solubility and to reduce oxidative metabolism on the cyclohexyl ring, observed in compound **8**. Interestingly, compound **9** retained comparable NS4B affinity and HCV replicon activity, with the *anti*-configuration preferred over the corresponding *syn* arrangement. The final round of optimization focused on the C-5 position of the imidazo[1,2-*a*]pyridine nucleus with the aim to replace the metabolically labile furyl ring (data not shown) with different alkyl and/or cycloalkyl groups [40–44]. C-5-Cyclopropyl derivatives, exemplified by compound **10** (known as *GSK8853*), retained the same tight binding to NS4B and comparable anti-HCV activity with respect to parent **9** (Fig. 3) [40–44]. Compound **10** was also characterized by an improved metabolic stability, a better in vivo clearance, and more favorable oral bioavailability in rats and dogs when compared to the direct analogue **9** [40]. Resistance passaging in HCV replicons with compound **10** generated mutants carrying single-point mutations within the NS4B sequence (H94R, F98L, V105M) that were moderately (nearly 30-fold) to highly (nearly 350-fold) resistant.

Due to the good balance between anti-HCV activity and PK properties, compound **10** became the lead within the imidazo[1,2-*a*]pyridine series, and thus its

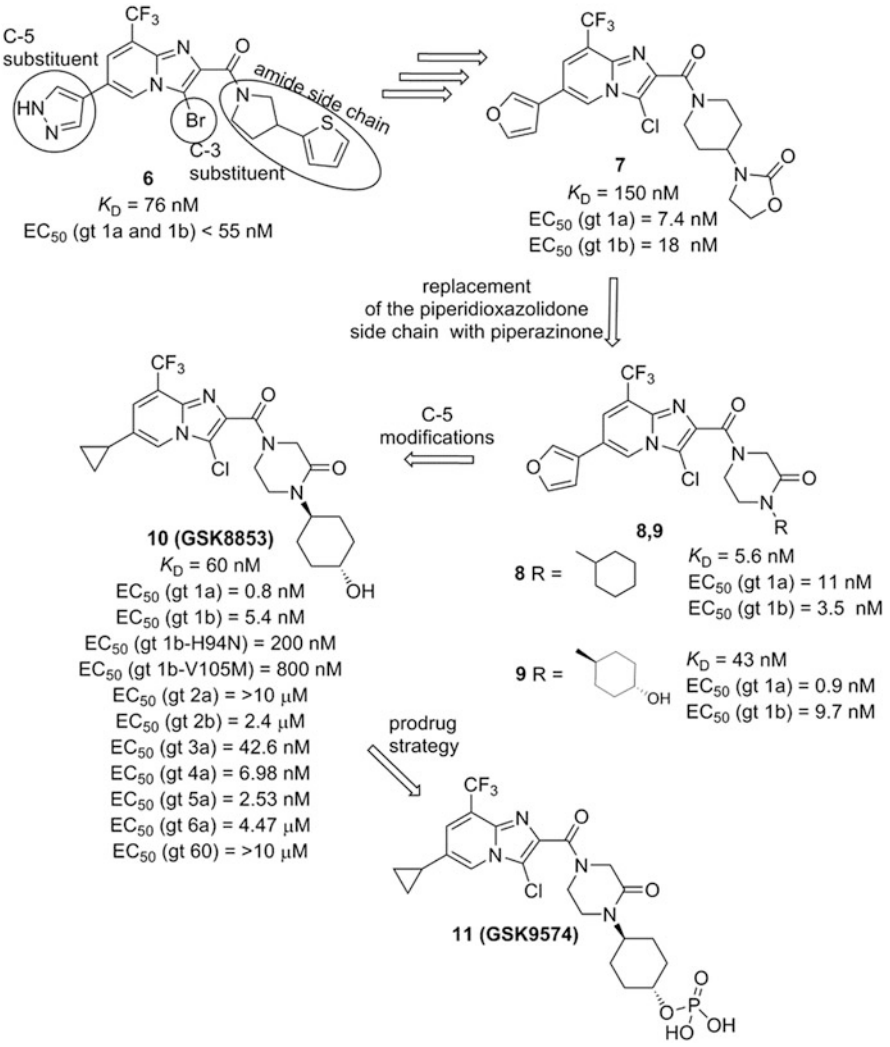


Fig. 3 Structures and activities of representative imidazo[1,2-*a*]pyridines summarizing the chemical optimization process and highlighting the main modification starting from hit **6** to lead **10** and its phosphate prodrug **11**, the first preclinical candidate among the NS4B inhibitors

activity was assessed across a wide panel of different gts showing EC_{50} in the nM range against gts 3a, 4a, and 5a, while it was only a weak inhibitor of gts 2b and 6a. However, compound **10** was inactive against gts 2a and 6o (Fig. 3) [45].

In 2013, Peat A. J. and co-workers reported the in vivo antiviral activity and safety of lead compound **10** [44, 45], but despite improved PK properties, the candidate molecule did not reach an appropriate plasma concentration in rats for preclinical safety studies, due to reprecipitation phenomena. The use of

corresponding phosphate **11** (known as *GSK9574*, in Fig. 3) instead matched the requirements for the in vivo studies, achieving an EC_{90} of 29 nM in PXB mice infected by HCV gt 1a leading to a viral load reduction of 4 log units in a 7-day study [44, 45]. This result provided the first in vivo proof-of-concept that an optimized NS4B inhibitor could be developed into an anti-HCV drug. However, during the 7-day safety study, an adverse cardiovascular event was observed for **10**, and its further development was abandoned [44, 45].

The limitations associated with compound **10**, such as low solubility, decreased activity against NS4B mutants (H94N, F98L, V105M), and the cardiovascular toxicity, prompted researchers at GlaxoSmithKline to engage a strategy based on a isosteric replacement of the imidazo[1,2-*a*]pyridine core with the pyrazolo[1,5-*a*] pyridine, as exemplified by derivatives **12–15** (Fig. 4) [44, 46]. Overall, the pyrazolopyridines possessed (1) acceptable physicochemical properties (measured LogD = 4.1 for **12**), (2) improved NS4B binding affinity, and (3) antiviral activity against wild-type and stable H94N NS4B mutant replicons. To note, the presence of a [3.1.0]bicyclohexane at the piperazinone nitrogen provided the sub-nM anti-HCV

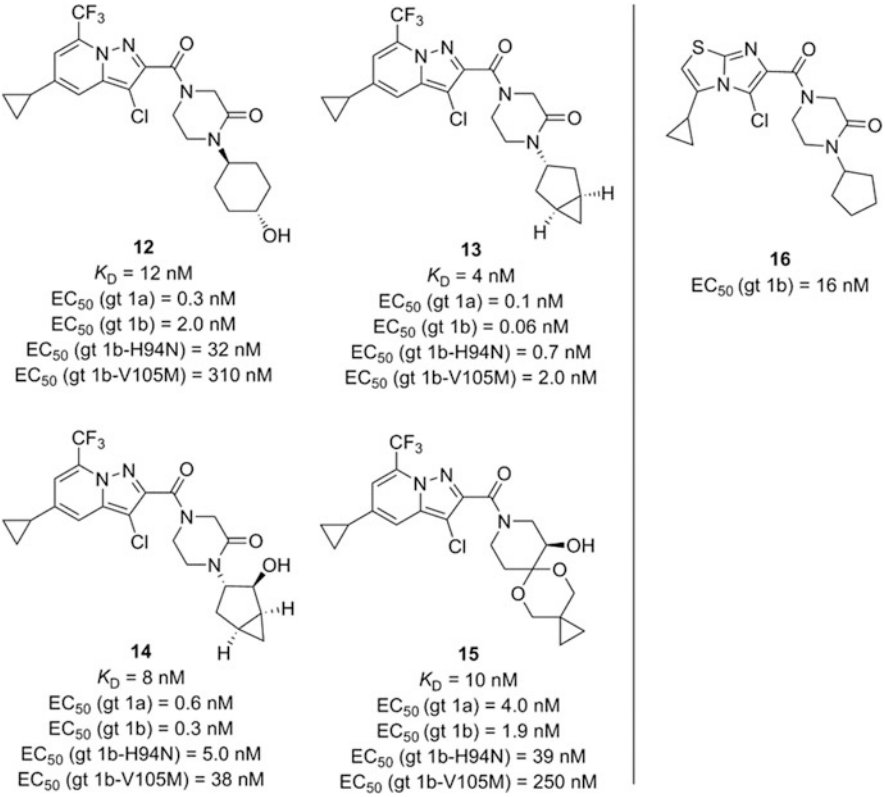


Fig. 4 Structures and activities of representative pyrazolopyridines **12–15** (left) and imidazo[2,1-*b*] thiazole **16** (right)

activity of derivative **13**, highly potent also against the H94N- and V105M-resistant replicons. The dextrorotatory hydroxyl derivative (*S*,*S*,*S*,*S*) **14** was designed in an attempt to reduce the lipophilicity (measured LogDs = 4.6 vs. 6.4, for **14** and **13,** respectively) and simultaneously to improve metabolic stability (in rat clearance of **14** and **13** were 25 and 70 mL min^{-1} kg^{-1}, respectively). Indeed, as shown for imidazopyridines, the insertion of a hydroxyl group reduced metabolism of **14**, compared to its parent analogue **13** without affecting anti-HCV activity. Interestingly, the [3.1.0]bicyclohexanol isomers of **14** bound to NS4B with a similar nM affinity but showed different anti-HCV activity especially against H94N- and V105M-resistant replicon, thus indicating the *S,S,S,S* configuration was the most favored.

Despite an increase in lipophilicity, compound **14** (LogD = 4.6) showed an increased solubility in biorelevant medium in comparison to imidazopyridine lead **10** (LogD = 3.1) [44]. Furthermore, the increased hydrophobicity was commeasured to a higher binding affinity for the target protein. In vitro studies showed also that derivative **14** did not inhibit CYP450 isoforms, and in vivo PK investigation indicated a low-to-moderate clearance across different species, high oral bioavailability, and high plasma concentration [44]. Based on the impressive anti-HCV activity and its very promising in vivo PK profile, compound **14** proceeded into 7-day preclinical safety studies in mice without the need of a prodrug [44], but no results have been reported.

Yu and collaborators, at the Sichuan University, pursued a scaffold hopping approach replacing the imidazo[1,2-*a*]pyridine with the imidazo[2,1-*b*]thiazole nucleus as exemplified by derivative **16** (Fig. 4) [47]. The validation of NS4B as the target was demonstrated by evaluation of compound **16** in an array of known resistant replicons of NS4B as well as of NS3/4A, NS5A, and NS5B. The compound retained the same order of activity in all the replicons with the exception of H94R, F98C, and V105M NS4B mutants. Interesting results were obtained when hit compound **16** was evaluated in association with different DAAs in HCV gt 1b replicon observing synergistic effect with simeprevir, daclatasvir, and sofosbuvir, and an additive effect was demonstrated with clemizole [47].

2.3 6-(Indol-2-yl)pyridine-3-sulfonamides and Related Compounds

Chen and his research team, at PTC Therapeutics, reported a novel anti-HCV chemotype based on the indole core and exemplified by weak HCV gt 1b inhibitor **17** identified through a cell-based HTS (Fig. 5) [48]. At that time, no studies to elucidate the molecular target of the molecule were reported. Chemical optimization based on (1) the replacement of the unsuitable 3-nitro with a cyano group, (2) the shifting of the *para*-methoxyphenyl to the indole C-2 position, and (3) the ethylation of the nitrogen led to more potent derivative **18** (Fig. 5) [48].

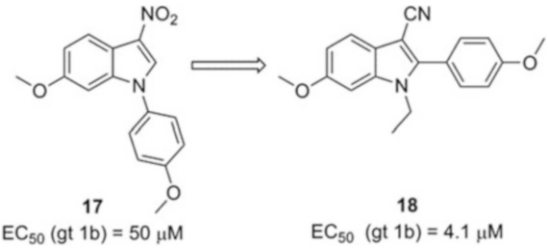


Fig. 5 HTS-derived indole **17** and initial optimization to inhibitor **18**

Then, systematic investigation of different substituents at *N*-1, C-5, and C-6 and at the C-2 phenyl ring provided sub-μM HCV replication inhibitors with SI > 130 as exemplified by representative derivatives **19–24** (Fig. 6) [48]. They are characterized by the presence of an alkylated sulfonamide at the C-2 phenyl ring, in place of the *para*-methoxy group, small linear or cyclic alkyls at the indole nitrogen, and mainly lipophilic fluorinated substituents at C-6 position. Additional SAR information indicated that small alkyls at the sulfonamide moiety were preferred over larger substituents, while polar groups at the C-6 position of the indole core or removal of the substituents were not tolerated. Furthermore, C-5 as well as C-5/C-6 di-substitution gave less active compounds, with the active C-5 fluorine derivative **24** representing an interesting exception (Fig. 6). Also the reverse sulfonamide **25** retained the same potency in the sub-μM range (Fig. 6) [48]. Again, NS4B was still not recognized as the target for this chemical class albeit compound **22**, used as chemical probe, was shown to be inactive against the most-exploited HCV proteins (i.e., NS5B polymerase and NS3/4A protease) [48].

The hit-to-lead optimization process of indoles proceeded in a joint program between PTC Therapeutics and Merck starting from derivative **25** characterized by low solubility and metabolic liability [49]. Furthermore, in vivo production of inactive *N*-sulfonamido dealkylated metabolite was observed when the compound was orally administered in rats. At first, the C-2 phenyl ring was replaced by nitrogen-containing heteroaryl rings, and the 6-(indol-2-yl)pyridine-3-sulfonamide **26** showed the most potent replicon activity coupled with improved in vitro metabolic stability, as demonstrated by human liver microsomal (HLM) clearance evaluation (Fig. 7). Then, more in-depth SAR exploration indicated that the combination of 6-difluoromethoxy/1-cyclobutyl as in compound **27** or 6-cyclopropyl/1-cyclobutyl as in compound **28** granted a good balance between potency and metabolic stability (Fig. 7). The metabolic improvement of these compounds was achieved introducing lipophilic electron-withdrawing groups on the alkyl chain of the sulfonamide moiety to suppress oxidative aminosulfonyl *N*-dealkylation.

Accordingly, compound **29** was characterized by a very good metabolic stability and a potent low nM anti-HCV activity and used as chemical probe to demonstrate the mechanism of action for the whole compound class (Fig. 7) [49]. In fact, NS4B was determined to be the molecular target for the 6-(indol-2-yl)pyridine-3-

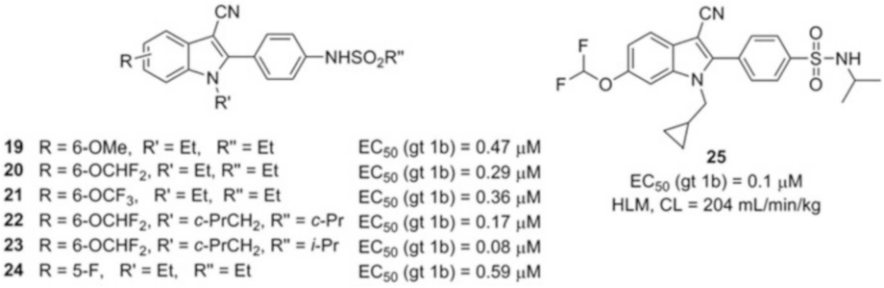


Fig. 6 Structures and activities of representative indoles **19**–**25** summarizing the chemical optimization process

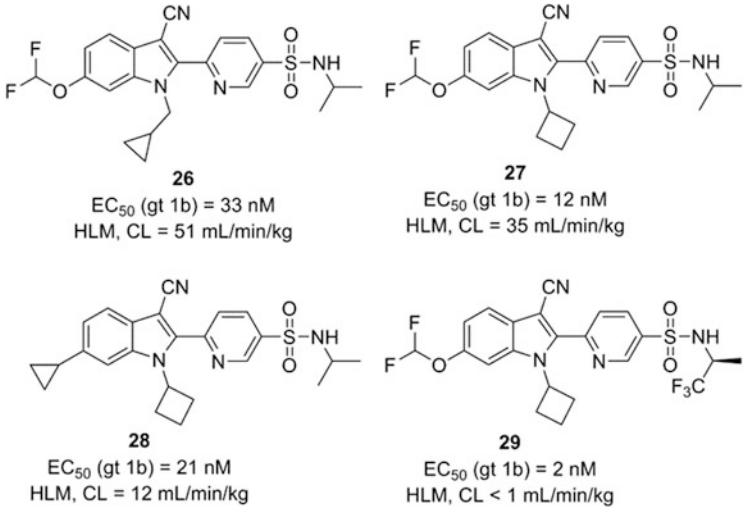


Fig. 7 Structures and activities of representative 6-(indol-2-yl)pyridine-3-sulfonamides

sulfonamide class, employing the replicon assay in which mutations in the NS4B sequence were induced after treatment with compound **29**. The most frequent mutation was a F98L substitution that produced a significant loss in activity (>70-fold). Interestingly, this mutation is localized in the TM1 domain of NS4B, the same region involved in generation of escape mutants identified for anguizole and related compounds.

Successive medicinal chemistry efforts pointed toward a further improvement of the physicochemical/PK properties of derivative **29** [50]. Chemical modifications focused on the benzene ring of the indole core and the *N*-1 substituent. It was observed that the contemporary presence of a fluorine at C-5 and of small lipophilic alkyls at C-6 reduced oxidative metabolism on the indole benzene ring and that the introduction of aryl groups at the *N*-1 position improved oral bioavailability in rats.

The synthesis of analogues containing different combinations of these chemical modifications was pursued leading to a new set of compounds having the 5-F/6-alkyl di-substitution and the pyrimidinyl moiety as *N*-1 aryl substituent, as exemplified by derivatives **30**, **31**, and **32** (*PTC725*) (Fig. 8), and showing high anti-HCV potency, good PK properties, and great metabolic stability.

6-Ethyl derivative **32** emerged as the most promising lead due to its excellent potency and favorable balance in PK properties [50]. Compound **32** was characterized by comparable low nM potency against gt 1a and 1b and a high degree of selectivity; however, it showed significantly less activity against HCV gt 2a. Selection of resistant gt 1b replicons revealed substitutions in NS4B sequence, especially H94R, F98L, and V105M. Retrospectively, the low potency against gt 2a can be explained by the presence of L98 naturally expressed in the wild-type gt 2a. Worthy of note, the lead **32** showed a low nM activity also against HCV gt 3a [51]. Interestingly, combination of compound **32** with boceprevir or VX-222 (non-nucleoside NS5B inhibitor) resulted in an additive or a synergistic effect, respectively, against HCV gt 1b replicon [50]. After oral administration, lead compound **32** showed good PK properties in rats and dogs, but a poor bioavailability was observed in monkeys [50]. Due to its excellent safety profile, it has been advanced into preclinical development, but no further data has been reported.

In another report, a related series of azaindoles has been reported (Fig. 9) [52]. In comparison with the 6-(indol-2-yl)pyridine-3-sulfonamide series, a slight decrease in activity was observed for the 4- or 5-azaindoles (e.g., **33** and **34**, respectively),

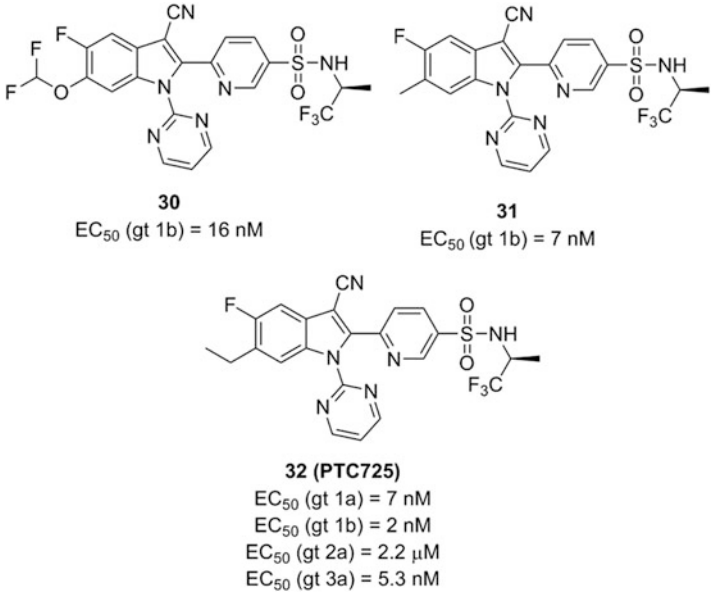


Fig. 8 Structures and activities of representative 6-(indol-2-yl)pyridine-3-sulfonamides summarizing the chemical optimization process leading to preclinical candidate **32** (PCT725)

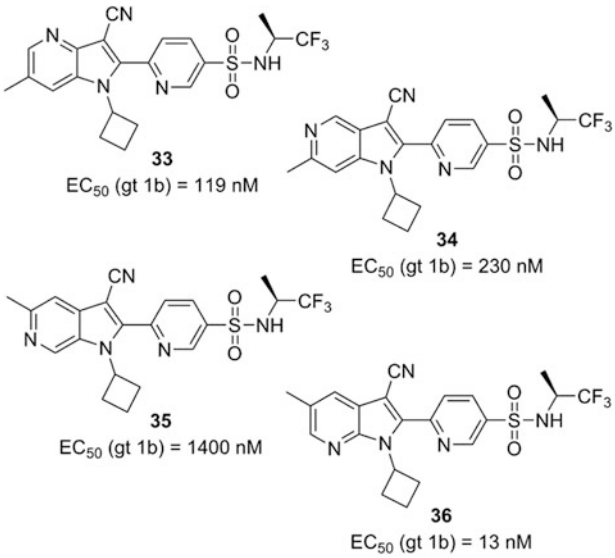


Fig. 9 Structures and activities of 6-(azaindol-2-yl)pyridine-3-sulfonamides

while the 6-azaindole subseries (e.g., **35**) showed a drop in potency. Conversely, potent derivatives, exemplified by compound **36**, were obtained by placing the nitrogen atom at position 7.

A further evolution around the 6-(indol-2-yl)pyridine-3-sulfonamides led to carboxamide analogues (**37–40**) endowed with broader genotypic anti-HCV activity (Fig. 10) [53]. In particular, compounds **38–40** derived from **37**, which was identified as a byproduct during the optimization of lead **32** wherein the indole 3-cyano group was hydrolyzed to a carboxyamide functionality. Biological testing highlighted a reduced but still interesting activity for compound **37**, and then several derivatives were synthesized and tested against HCV gts 1a, 1b, 2a, and 3a. The SAR around the new indole nucleus was thus reevaluated, taking into account that the cyano group may be advantageously replaced by an amide function. However, for the design of new derivatives, the 5-F in combination with a small alkyl or a fluorinated substituent at the C-6 position of the indole was retained in order to reduce the oxidative glutathione conjugation, as learned from the previous SAR studies. Unlike the 3-cyano derivatives, in 3-carboxamides, a cycloalkyl N-1 substituent was favored instead of an aryl group, and substituted phenyl was preferred over a pyridine at the C-2 indole position. As a result, 2-(4-sulfonamidophenyl)-indole 3-carboxamides **38–40** showed the best balance of activity across the gts used, with EC_{50} values ranging from sub-nM to low nM, including gt 2a, and thus serving as a new promising starting point for the identification of NS4B inhibitors with broad anti-HCV gt activity (Fig. 10).

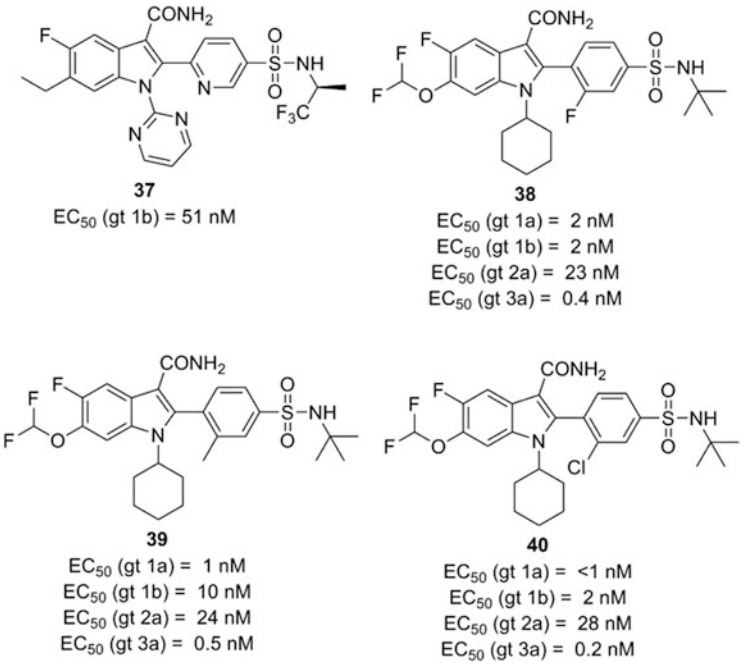


Fig. 10 Structures and activities of representative 2-(4-sulfonamidophenyl)-indole 3-carboxamides

2.4 Piperazinone Derivatives

Kakarla and co-workers, at Pharmasset, reported a new anti-HCV chemotype targeting NS4B, based on the piperazinone scaffold [54]. Exploiting a cell-based HTS (HCV gt 1b replicon luciferase reporter assay) of their in-house library, piperazinone **41** was identified as a promising and selective anti-HCV agent (Fig. 11). Generation of NS4B mutant replicons (residues 90 and 98 in protein sequence) revealed its mode of action. The *S,S* configuration at C-3 and C-6 piperazinone stereocenters and the *trans-R,R* configuration of the (2-phenylcyclopropyl)carbonyl side chain of the starting hit were critical since other modifications were not tolerated.

Preliminary SAR investigation around hit **41** indicated that the endocyclic unsubstituted amide of the piperazinone scaffold was a key pharmacophoric element, and therefore, N-alkylation or carbonyl reduction was detrimental [54–56]. Simplification of the side chain was attempted replacing the chiral cyclopropyl bridge with olefinic or aromatic/heteroaromatic linkers. In particular, a *trans* double bond (**42**) and an isoxazole ring (**43**) were good replacements for the cyclopropyl bridge (Fig. 11); indeed, a *cis* version of **42** or other aryl or heteroaryl groups instead

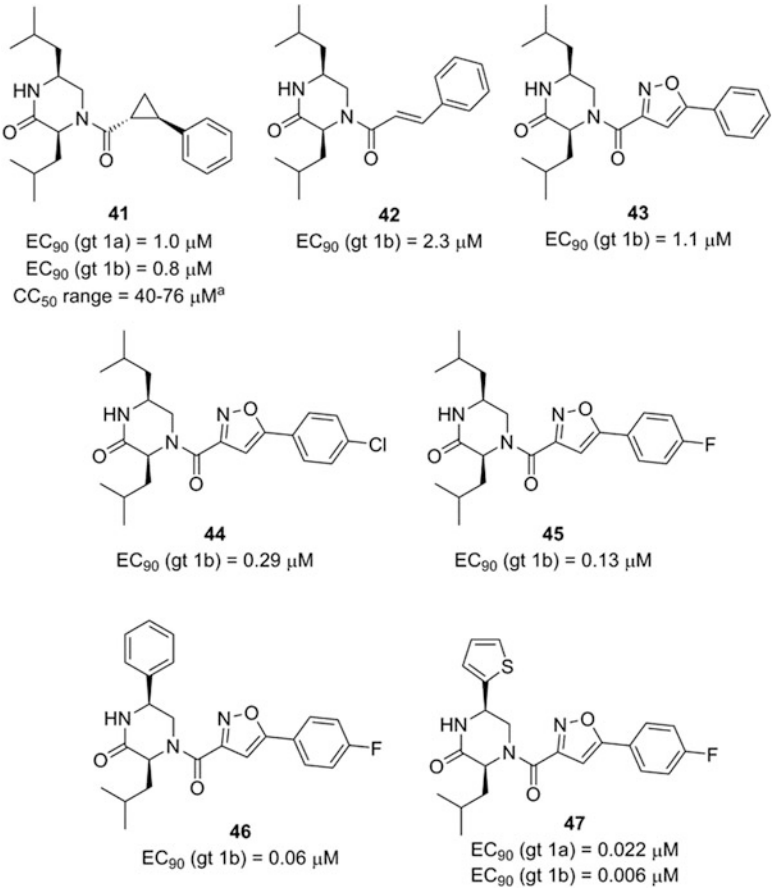


Fig. 11 Structures and activities of representative piperazinones from the HTS-derived hit **41** to compound **47**, the most potent within the series. [a]Range values from four different cell lines (Huh7, HepG2, BxPC3, and CEM)

of the isoxazole abolished the anti-HCV activity. Starting from piperazinones cinnamide **42** and isoxazolylamide **43**, two sets of analogues carrying different substituents at the phenyl side chain ring were prepared. The best results were observed with the isoxazolylamide subseries, as exemplified by the potent *p*-chloro, the *p*-fluorophenyl-substituted derivatives **44** and **45** endowed with sub-μM activity (Fig. 11).

SAR exploration continued by replacing the isobutyl group at the C-6 position of the piperazinone core with either different hydrophobic moieties (acyclic, branched, cyclic, and saturated/unsaturated alkyls) or more polar alkyl and aryl substituents, having H-bond forming properties [54–56]. In general, acyclic and cyclic alkyls were tolerated, while the addition of polar functionalities caused a decrease in antiviral potency. On the contrary, the insertion of unsaturated systems increased

potency as showed by the phenyl analogue **46** and other C-6 heteroaryl analogues, exemplified by compound **47** having a 2-thiophene as C-6 substituent (Fig. 11). Indeed, this compound showed the highest potency in the replicon assay. In addition, derivative **47** showed good activity against HCV gt 1a but was inactive against HCV gt 2. Due to their lack of broad genotype (gt) coverage, the anti-HCV piperazinones targeting NS4B were abandoned by Pharmasset and did not progress into further preclinical development [54].

2.5 2-Oxadiazoloquinoline Derivatives

A series of NS4B inhibitors based on the 2-oxadiazoloquinoline scaffold has been reported by Phillips and collaborators at Gilead Sciences [57]. Noteworthy, the hit-to-lead development of the 2-oxadiazoloquinolines led to derivatives characterized by potent pan-genotypic anti-HCV activity. The initial hit **48** was already endowed with excellent and selective activity against HCV gt 1b, but it was inactive against HCV gt 2a and characterized by high lipophilicity (Fig. 12). Thereby, the optimization strategy adopted aimed at reducing lipophilicity and obtaining anti-HCV gt 2a activity. Modification focused on the replacement of the two phenyl groups at each end of the molecule with more polar and less planar substituents. Thus, the phenyl ring at the aminoxadiazole moiety was replaced by several cycloalkyl ethers with the methylene-1,3-dioxolane moiety emerging as the best one. On the other hand, alkyl ethers (e.g., trifluoroethyloxy) at C-6 position of quinoline core proved to be effective replacements of the C-6-phenyl group. Moreover the C-8-trifluoromethyl on the quinoline nucleus was replaced by a *tert*-butyl group (Fig. 12). As a consequence of these medicinal chemistry efforts, derivative **49** was obtained and compared to **48**, and it showed (1) reduced lipophilicity (LogD = 3.9), (2) significant improvement in anti-HCV gt 2a activity, and (3) a fourfold increase of the potency in gt 1b replicon. As a consequence, compound **49** was submitted to a NS4B binding assay (scintillation proximity assay using a recombinant HCV NS4B gt 1b) and showed a K_D of 31 nM (Fig. 12).

The classical bioisosteric replacement of the oxygen with a NH group in the C-6 substituent further enhanced the anti-HCV activity, as exemplified by compound **50** (Fig. 12). Starting from this latter compound, the methylene-1,3-dioxolane group was replaced by different hydroxy cycloalkyl rings, furnishing potent compounds against both HCV gt 1b and 2a. Indeed, compound **51** and **52** having a hydroxycyclobutyl and hydroxycyclohexyl, respectively, resulted in an impressive increase in the anti-HCV activity against both gt 1b and gt 2a (Fig. 12). Being the most potent derivative, compound **52** was also evaluated in a panel of HCV replicons to assess the activity across a broad range of gts as well as against 1b-resistant mutants displaying high potency against all the replicons included in the study. Finally, PK studies in rats indicated lead **52** as a promising preclinical drug candidate in terms of half-life and oral bioavailability [57]. Overall, among the

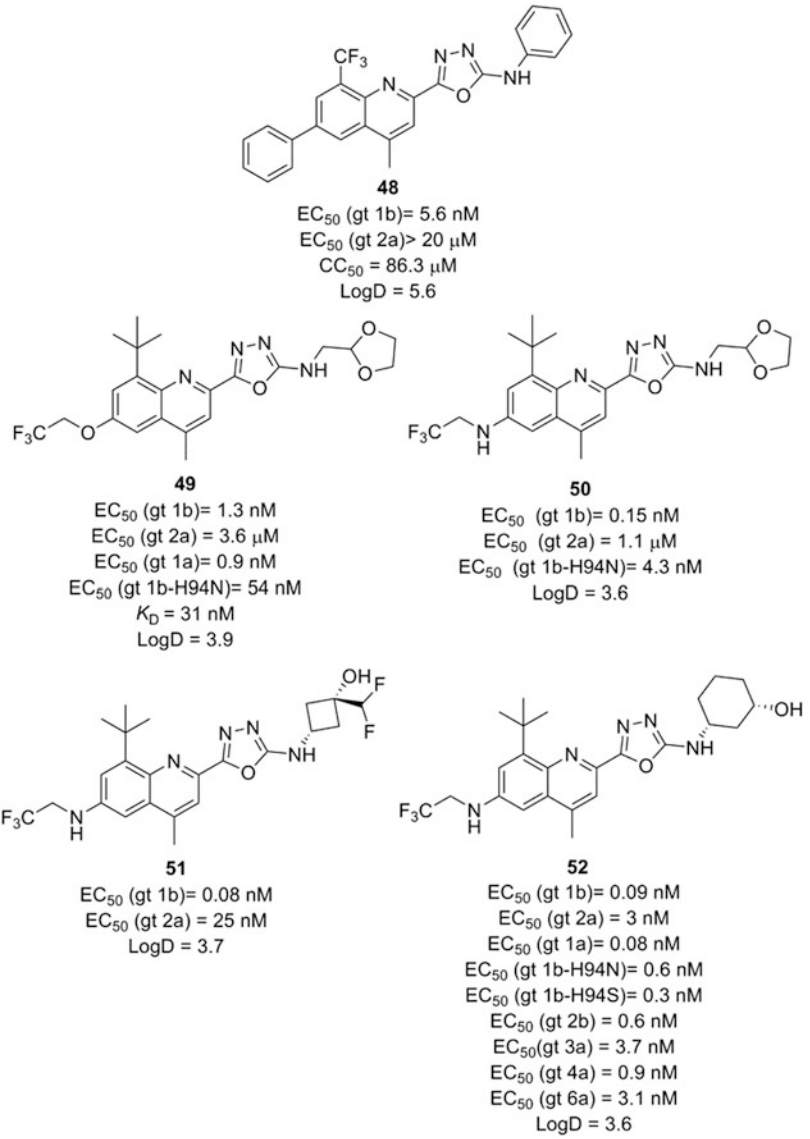


Fig. 12 Structures and activities of representative 2-oxadiazoloquinolines summarizing the chemical optimization process from the initial hit **48** to pan-genotypic anti-HCV lead **52**

NS4B binders, **52** can be considered to be the most potent and have the broadest anti-HCV activity reported so far.

2.6 Other NS4B Binders

Other NS4B ligands have been reported in literature without any information regarding a systematic chemical optimization.

For example, the quenching fluorescence binding assay was exploited by Chunduru and colleagues to identify not only anguizole but also compounds **53**–**59** (Fig. 13) [25]. Albeit these compounds bound NS4B with low μM affinity, only for the triazinoindole derivative **58** were mutations in NS4B sequence (K52R, G120V, A210S) generated [25].

Through the AH2-mediated lipid vesicle aggregation inhibition assay, Gleen and co-workers identified a series of amiloride analogues, exemplified by compound **60**, which were able to inhibit HCV replication in both gt 1b and gt 2a without showing significant cytotoxicity (Fig. 14) [24, 58].

An ELT screening approach using immobilized NS4B was pursued by Thompson and collaborators at GlaxoSmithKline [26]. For this study, 28 libraries containing one million to eight billion compounds were screened, and two families of NS4B binders were identified. The first was dominated by the bipiperidyl-triazine scaffold and the other by the spiro-diazaundecane pyrimidine core. Authors focused on the

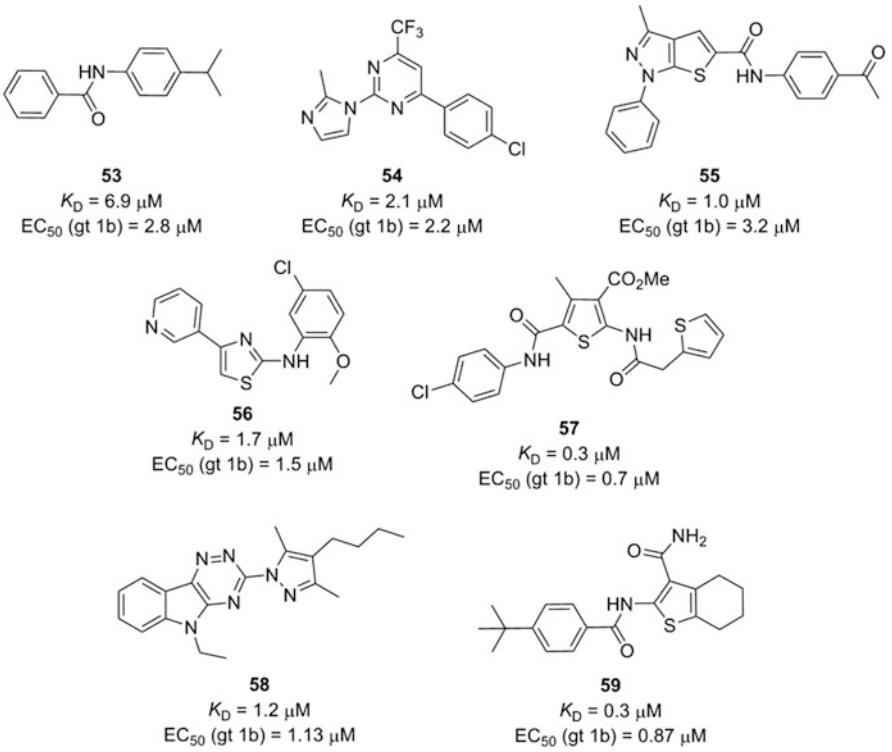


Fig. 13 Structures and activities of compounds **53**–**59**

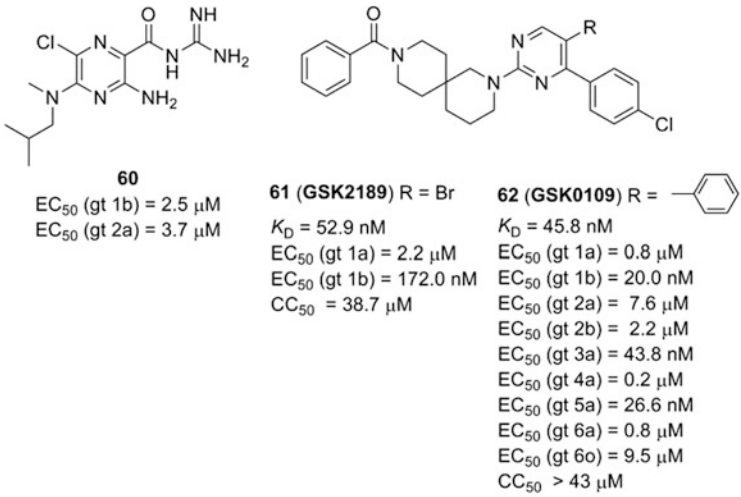


Fig. 14 Structures and activities of amiloride **60** and of ELT-derived compounds **61** and **62**

latter compound class identifying compound **61** (*GSK2189*, Fig. 14) resulting the most potent ELT-derived hit able to bind NS4B with high affinity. Moreover, the hit compound exerted good potency against HCV gt 1b, while moderate to weak activity in gts 1a and 2a was observed, respectively.

Early optimization focused on obtaining a wide gt coverage led to derivative **62** (*GSK0109*, Fig. 14) endowed with a good biological profile against gts 1a and 2a. Interestingly, compound **62** was found to be a potent HCV replicon inhibitor of gts 3a and 5a, a good inhibitor of gts 4a and 6a, and a modest inhibitor of gts 2b and 6o. Mutational studies highlighted that the activity of compound **62** was sensitive to F98L alteration, while it was still active in replicons carrying H94R and V105M mutations.

3 Final Overview and Future Directions

Over the years, the NS4B function and structure have been investigated, and new anti-HCV agents targeting this viral protein have been reported. However, the NS4B membranous nature hampered its purification and crystallization; thus the lack of structural data on NS4B did not allow structure-based drug design programs.

Nonetheless, some biochemical assays have been developed to identify NS4B ligands able to block either the RNA-binding activity (microfluidic affinity assay) or

the MW formation (AH2-mediated lipid vesicle aggregation inhibition assay). Also the innovative ELT approach has been exploited toward identification of NS4B binders. However, most of the promising compounds described so far were derived from hits identified through HCV replicon-based HTS, followed by genetic validation of NS4B as target. The information available about mutation sites are depicted in Fig. 15 [27].

The currently available direct-acting antiviral combinations for chronic HCV treatment are definitely breakthroughs in the field of life sciences. Indeed, these new therapies are able to cure the infection (SVR > 90%), but sooner or later, these therapeutic regimens will have to deal with the development of drug resistance, thus making necessary the use of new DAAs. To note that, the new drugs are less effective against HCV gt 3, while some NS4B binders (i.e., **32**, **37**, and especially **52**) proved to be efficacious also against this gt. In this context, the development of drugs targeting NS4B is certainly of great interest.

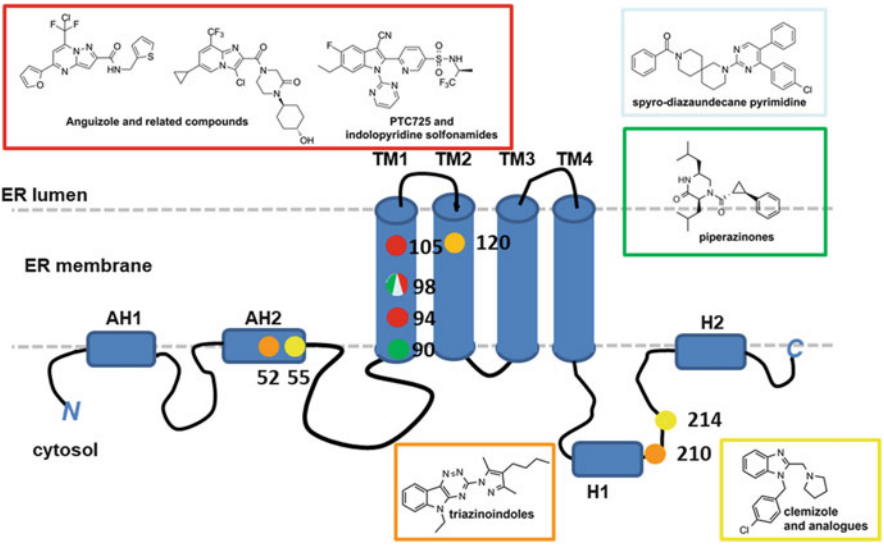


Fig. 15 Schematic representation of NS4B structure showing the mutation sites that confer drug resistance to representative ligands. Amino acid changes associated with resistance are depicted in color-coded circles according to the different ligand classes: (1) H94R, F98L, and V105M mutations at the TM1 segment confer resistance to anguizole and related compounds as well as to other structurally unrelated chemical families (i.e., indolopyridine sulfonamides and piperazinones); (2) the single mutation F98L was observed for the spiro-diazaundecane pyrimidines; (3) W55R mutation within the AH2 amphipathic α-helix and R214Q within the cytoplasmic C-terminal segment of NS4B confer resistance to clemizole and analogues; (4) the K52R replacement at the AH2 region, G120V in the TM2 segment, and A210S within the *C*-terminal segment are responsible of the resistance for triazinoindole derivatives (reused with permission and free of charge from Ref. [27])

Compliance with Ethical Standards

Conflict of Interest Giuseppe Manfroni declares that he has no conflict of interest. Rolando Cannalire declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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Part III
Clinical Trials and Combination Therapy

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The Evolution of Clinical Trials for Hepatitis C

Viviana Figueroa Diaz, Mary Olson, and Ira M. Jacobson

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Abstract The development of well-tolerated treatments that attain nearly universal cure of hepatitis C virus (HCV) infection, less than 30 years after the long-sought discovery of the causative agent, ranks as a landmark achievement of modern medicine. In the broadest sense, the international effort to address this global public health problem can be divided into an era of nonspecifically targeted therapy centering on interferon, a relatively brief "hybrid period" combining interferon and ribavirin with direct-acting antiviral agents (DAAs), and the latest era of DAA combination regimens. One of the most notable features of this story is the quantum leap in efficacy for DAA therapy to extraordinarily high levels instead of the years-long incremental steps that might have been anticipated. Similarly gratifying is the

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foundation on which the concept of curability, unique to HCV thus far in human virology, has been solidified based on the combination of our understanding of the molecular biology of the virus and the rarity, dating back to the interferon era, of virologic relapse after attainment of sustained virologic response. Although, at least until recently, the number of therapeutic agents was very limited, the combination of viral and host diversity ensured the development of a rich literature reflecting hundreds of treatment studies which dominated the scientific programs of the international liver meetings for many years. Viewed panoramically through a retrospective lens, the field developed in a logical sequence by first making the most out of the limited tools which were available and later by building on the remarkable elucidation of HCV biology by the scientific community and the paradigm of combination therapy for viral infection established in the HIV field to get us where we are today.

Keywords Direct-acting antiviral agents (DAAs), Genotype, Hepatitis C, Interferon, Ribavirin, Virologic cure

1 Introduction

The ability to cure hepatitis C virus (HCV) infection in nearly all recipients of currently available direct-acting antiviral (DAA) treatments, attained less than 30 years after the landmark publications heralding the discovery of this elusive virus in 1989, ranks as a major triumph of modern medicine [1–3]. The ingenuity of the scientific community, the resources dedicated by the pharmaceutical industry, the energetic involvement of the medical community, and the motivation of untold thousands of patients to participate in clinical trials were instrumental elements in the effort to address this enormous international public health problem. The consistent observation that the virus seldom reappears after it has been undetectable by molecular assays such as polymerase chain reaction (PCR) for a few months after completion of treatment, combined with our understanding of the life cycle of a virus that does not have a phase involving genomic archiving, has vindicated the distinctive status of HCV as a virus about which we can uniquely use the word “cure” to describe the outcome of successful therapy.

A retrospective assessment of the evolution of clinical trials for hepatitis C results in a division of the process into two major phases. The first was the interferon era, which had its onset over 30 years ago, while an intense search for the mysterious causative agent of what had become known as “non-A, non-B hepatitis” was still ongoing. Including the latter 1980s, when interferon was undergoing clinical trials, the “interferon phase” lasted over a quarter century and featured the addition of ribavirin as an adjunct to interferon as well as the development of pegylated interferon. Numerous clinical trials evaluated critical aspects of interferon-based therapy such as different interferon formulations, doses of both interferon and ribavirin, duration of therapy, response-guided therapy, and many specific populations. The new era of interferon-free DAA therapy was preceded by a “hybrid

model" in which pegylated interferon and ribavirin were combined with either of the two HCV protease inhibitors, telaprevir or boceprevir, for the first time in 2011 in the United States. Hailed as a great advance at the time, it is a reflection of the accelerated pace of the field that the use of these two drugs, along with any role for interferon, vanished in many countries within 2–3 years.

The goal of this chapter is to correlate drug development with scientific advances in understanding the biology of HCV, highlight the processes that led to the selection of the various agents used to treat hepatitis C patients over the years, influenced trial design, and culminated in the current highly effective regimens, resting on the fundamental principle of combinations of DAAs with great antiviral specificity and potency. In doing so, one cannot escape the parallel with earlier developments in antiretroviral therapy from which so much was learned. The evolution of treatment with interferon prior to DAA therapy is covered here to provide a comprehensive overview, but greater focus is on the direct-acting antivirals, initially with then without interferon. Further information about interferon therapy is available in [4]. The reader should bear in mind that while of necessity this review focuses on the clinical trials of the drugs that "made it to the finish line," many other agents, whether other formulations of interferon or ribavirin or members of the DAA classes (protease inhibitors, NS5A inhibitors, nucleotide polymerase inhibitors, non-nucleotide inhibitors, and other agents with alternative mechanisms of action, such as cyclophilin inhibitors and miR-122 inhibitors) failed because of efficacy or safety limitations, lack of partner drugs, or arrival on the scene too late to make the costs of further development worthwhile.

2 Early Days: Interferon

Approximately four decades ago, the scientific and medical communities began to focus on the potential therapeutic role of interferon in a variety of contexts because of its recognized combination of antiviral, immune modulatory, and antiproliferative properties [5–7]. Naturally derived interferon from sources such as fibroblasts and leukocytes excited such interest as a potential cancer treatment that readers of a feature article in *The New York Times* in 1981 could have been forgiven for taking away an impression that a miracle drug for cancer was on the horizon (http://www.nytimes.com/1981/04/26/magazine/putting-interferon-to-the-test.html). Early reports suggested potential benefit for hepatitis B [8–13]. The eventual role of nucleotides for viral hepatitis was also presaged by studies of adenine arabinoside for hepatitis B, alone or in combination with interferon [14]. The interest in interferon as an antiviral therapy made it logical to initiate studies in patients with a liver disease of viral etiology for which a causative agent had not yet been determined: non-A, non-B hepatitis. The major limitation of the early studies of what proved to be hepatitis C, which persisted through the initial approval of interferon in the early 1990s, was the need to rely upon serum aminotransferases as the endpoint of therapy because virologic testing was not yet available to serve as the far more appropriate endpoint of

therapy which it soon became with the advent of polymerase chain reaction (PCR) technology.

The international effort to study the therapeutic applications of interferon in human medicine was greatly facilitated by the development of recombinant interferon, which allowed for the availability of large quantities of purified preparations of interferon. The Liver Diseases Section at the National Institutes of Health, led by Dr. Jay Hoofnagle, pioneered the effort to study the effectiveness of recombinant human alpha interferon in patients with non-A, non-B hepatitis. In a case series of ten patients published in 1986, interferon given at an initial dose of 1 MU or 5 MU, at first once daily and then three times weekly for up to 12 months, resulted in rapid decreases of serum aminotransferase levels, often with normalization, in most patients for as long as treatment was continued, along with histologic improvement [15].

In the 2 years following the initial NIH publication, several similar studies were published using various interferons, including beta interferon, recapitulating the theme of interferon's ability to effect normalization of transaminase levels. Post-transfusion patients and others with classic risk factors quickly came to dominate most of the early study populations, still in the absence of available virologic markers [16, 17]. The capacity to achieve sustained biochemical response, a harbinger of the later concept of sustained virologic response (SVR), was demonstrated.

The field catapulted forward in 1989 with the publication of two landmark US studies. The first, representing an extension of the initial work at the NIH, was a double-blind, placebo-controlled trial of 41 patients who received 2 MU of recombinant human interferon alfa-2b three times weekly or placebo for 6 months. Nearly half the patients treated with interferon had normalization of aminotransferases on therapy, but only 10% had sustained biochemical response [18]. A simultaneously published multicenter US study randomized patients to 3 or 1 MU of recombinant interferon alfa-2b three times weekly for 6 months or to placebo. Response rates were higher in the 3 MU group, with 46% achieving normalization or near normalization of ALT by 6 months. Again, however, relapse was common [19].

A memorable feature of both trials establishing the efficacy of recombinant interferon alfa in non-A, non-B hepatitis is that the remarkable discovery of the hepatitis C virus was reported toward the completion phase of both trials by Michael Houghton and colleagues at the Chiron Corporation [1, 2]. Serologic testing of patient samples from both studies revealed that most patients had antibody to the newly discovered hepatitis C virus [3]. It is for this reason that the titles of both papers reporting the NIH and multicenter interferon were published in the *New England Journal of Medicine* indicated that they were studies on the treatment of chronic hepatitis C rather than the originally intended non-A, non-B hepatitis [18, 19]. These papers were among the first that featured the name for recombinant alpha interferon adopted in the INN (International Nonproprietary Name) classification, interferon alfa.

The studies on recombinant interferon alfa-2b led to its approval at a dose of 3 MU three times weekly for hepatitis C by the US Food and Drug Administration early in 1991 on the basis of the improvement in liver test parameters noted in the

clinical trials. Now obsolete for hepatitis C, interferon in pegylated form remains a frontline recommended therapy for hepatitis B, although the better tolerated oral nucleosides or nucleotides are currently far more commonly used for this disease.

A critical development at this time was the development of testing for HCV RNA by polymerase chain reaction assays. Early studies combining assessment of biochemical and virologic response demonstrated a predictable, though not invariable, correlation between the two, including normalization of ALT with viral suppression both during and after completion of therapy, and increases in ALT levels concomitant with virologic relapse after therapy with interferon are discontinued [20–26]. However, it became clear that ALT normalization on treatment, as well as sustained ALT response, occurred more frequently than the responses at comparable time points for HCV RNA, thus indicating that from a virologic viewpoint the capacity to eradicate infection was lower than estimated from the early studies using ALT as the primary endpoint [27]. In addition to the obvious mandate to redefine primary outcome of treatment virologically, an important consequence of the development of virologic testing was the capacity to vastly expand the identifiable population of infected patients, with a proliferation of studies now including "community acquired" hepatitis C [28].

The recognition that HCV consists of a population of viruses with substantial genomic variation followed the advent of virologic testing, and by 1991 the phrase HCV "genotypes" was appearing in the literature [29–34]. Simmons et al. laid the foundation for what became the standard classification of six major HCV genotypes based upon phylogenetic analysis of nucleotide sequences derived from part of the gene encoding a nonstructural protein (NS5, [35]). Subsequent studies showed that similar classifications could be derived by analysis of one of the envelope proteins as well as the highly conserved 5' untranslated region [36, 37]. Different genotypes were found to have up to 40% variability in genomic sequence with lesser degrees of heterogeneity characterizing different subtypes subsumed under individual genotypes, the most prevalent of which have been genotype 1a and 1b in the United States, Europe, Japan, and other areas [38, 39]. This classification was subsequently incorporated into the design of virtually all clinical trials of antiviral therapy for HCV and has persisted to the present era of direct-acting antiviral agents. It was not long before considerable variability in response to interferon therapy corresponding to HCV genotype was recognized, with genotype 1 being the least responsive and genotypes 2 and 3 considerably more so [40–42]. Genotype 4, which proved to be highly prevalent in the Middle East, especially Egypt, had an intermediate rate of response [43].

As clinical trials and observational studies on duration of therapy, variable doses, pretreatment viral load, predictors of response, rates of response in different populations, and side effects quickly proliferated [44–50], another alpha interferon, recombinant interferon alfa-2a, was developed. This molecule varies from alfa-2b by 1 amino acid in the 166 amino acid sequence of the protein, with efficacy and tolerability equivalent to that of interferon alfa-2b [51–56]. Interferon alfa-2a was approved for the treatment of hepatitis C in 1996, 5 years after the approval of interferon alfa-2b.

Yet another interferon alpha called consensus interferon marked the third and final commercially approved interferon to become available. Approved in 1997, consensus interferon was derived by placing the most common amino residue at each position of the alpha interferon molecule into a synthetic interferon molecule [57]. A phase 3 trial in treatment-naïve patients showed that 9 mcg three times weekly was superior to 3 mcg three times weekly for 24 weeks, leading to approval of the 9 mcg dose for treatment-naïve HCV-infected patients. Comparable rates of SVR were obtained in the same trial with interferon alfa-2b 3 MU three times weekly [58]. A second phase 3 trial in patients who had failed previous interferon therapy and received 15 mcg three times weekly yielded SVR over five times more frequently in relapsers than nonresponders treated for 24 weeks, and 48 weeks was superior to 24 weeks [59]. The longer duration of therapy became the approved dose for prior interferon failures. Consensus interferon received considerable attention and uptake in clinical practice for several years, but its use diminished, and eventually disappeared, with the advent of ribavirin in combination with interferons alfa-2a and alfa-2b and subsequently with the development of pegylated interferon alfa-2a and alfa-2b.

Concomitant with the advent of these alpha interferon molecules, the 1990s featured many advances in the understanding of virus-, host-, and treatment-related factors determining response beyond the differential rates of SVR across various HCV genotypes. The demonstrated capacity of a longer duration of therapy to attain higher rates of SVR, not by increasing rates of on-treatment response but by decreasing relapse, led to expansion of the approval of interferon alfa-2b to 18 to 24 months of treatment, although these prolonged durations of therapy were infrequently adopted in practice as opposed to 12 months [60–62]. It was also during this era that lower response rates were noted in African-American persons, even when corrected for the higher prevalence of genotype 1 in this population, as well as patients with hepatic cirrhosis, HIV coinfection, and other populations [63–65].

3 Interferon and Ribavirin Combination Therapy

The next leap forward in the evolution of HCV therapy was the introduction of ribavirin, a guanosine nucleoside analogue in search of a “therapeutic home” after it failed to fulfill its initial promise for HIV infection in the 1980s. One of the earliest clinical studies of ribavirin suggested efficacy in reducing ALT levels at a time when HCV RNA testing was still not available [66], with the observation on ALT normalization confirmed in a US study from the National Institutes of Health [67]. A subsequent multicenter study indicated that ribavirin monotherapy indeed resulted in normalization of ALT in up to half of HCV-infected patients but had very little antiviral efficacy [68].

Despite the lack of significant antiviral activity as monotherapy, ribavirin was combined with interferon alfa-2b in landmark phase 3 trials and significantly augmented the rates of SVR compared to those obtained with interferon alone. In

a US phase 3 trial, 912 treatment-naïve patients received interferon alfa-2b alone or in combination with ribavirin in a weight-based dose of 1,000–1,200 mg/day for 24 or 48 weeks. SVR was assessed at follow-up period of 24 weeks and was higher in patients who received combination therapy for 24 or 48 weeks (31–38%) than in those receiving monotherapy (6–13%). Patients with genotype 1 drove the difference between 24 and 48 weeks, with lower relapse rates in the 48-week group [69]. In an international phase 3 trial, interferon alfa-2b combined with ribavirin for 48 weeks resulted in SVR in 43% as compared with 35% treated with the combination regimen for 24 weeks and only 19% treated with interferon alfa-2b for 48 weeks. Again, patients with genotypes 2 and 3 fared better, as did patients with viral levels less than 2 million copies/ml, age 40 or less, minimal fibrosis, and female gender [70]. A third phase 3 trial in interferon monotherapy relapsers yielded SVR nearly ten times more frequently in patients given combination therapy rather than monotherapy for 6 months [71]. Other studies showed that nonresponders to interferon monotherapy had lower rates of SVR after combination therapy than prior relapsers [72]. It was with the advent of interferon and ribavirin that the already recognized difference in responsiveness to interferon-based therapy between genotypes 1 versus 2 and 3 was accentuated, and a difference in recommended treatment duration (48 versus 24 weeks) emerged.

The emergence of ribavirin as a useful adjunct to interferon generated much discussion, but no final resolution, of the question of what mechanism was responsible for the augmentation of response rates when a relatively ineffective antiviral drug in its own right was added to interferon. Potential explanations included IMPDH inhibition, immunomodulatory effects, direct inhibition of viral replication as a guanosine analogue, and "error catastrophe," based on the concept of incorporation of ribavirin into growing HCV RNA chains and the generation of an expanding population of defective virions [73–81].

4 Pegylated Interferon and Ribavirin

As interferon and ribavirin became established as the new standard of care, modifications of interferon in the form of pegylation were being studied. The addition of polyethylene glycol polymers of varying sizes to protein pharmaceutical agents had become established as a way to prolong the half-life of such products, minimize the peaks and valleys characterizing the pK profiles of standard interferon, decrease the dosing frequency to once weekly, and potentially improve the efficacy of therapy. Programs to pegylate interferon centered on the use of 12 kDa polyethylene residues for interferon alfa-2b and 40 kDa for interferon alfa-2a [82, 83]. In dose-ranging studies of peginterferon alfa-2b monotherapy, at three different doses, higher doses administered once weekly were more effective than a lower dose and also more effective than standard interferon 3 MU three times weekly [84].

Following phase 2 dose-ranging studies of peginterferon alfa-2b and ribavirin [85], the major phase 3 trial of peginterferon-2b in combination with ribavirin

centered on a dose of 1.5 μg/kg weekly as the starting dose. In 1,530 patients assigned to 1 of 3 arms, patients received interferon alfa-2b 3 MU three times weekly plus ribavirin 1,000–1,200 mg/day for 48 weeks, PEG IFN alfa-2b 1.5 μg/kg/week plus ribavirin 800 mg/day for 48 weeks, or PEG IFN alfa-2b 1.5 μg/kg/week for the first 4 weeks and then 0.5 μg/kg/week plus ribavirin 1,000–1,200 mg/day for 48 weeks. SVR occurred in 54%, 47%, and 47% of patients, respectively. In GT1 patients, the SVR rates were 42%, 34%, and 33%, while they were in the range of 80% patients with GT2 or GT3 [86].

Studies of peginterferon alfa-2a appeared contemporaneous with those on peginterferon alfa-2b. In one study, PEG IFN alfa-2a 180 μg was compared with interferon-2a 6 MU three times weekly for 12 weeks followed by 3 MU three times weekly for 36 weeks, with SVR rates of 39% and 19%, respectively [87]. In a second study of patients with bridging fibrosis or cirrhosis, interferon-2a at a dose of 3 MU for 48 weeks was compared with 90 μg or 180 μg of PEG IFN alfa-2a SVR24 rates were 8%, 15%, and 30%, respectively [88].

With the dose of pegylated alfa-2a 180 μg weekly now established, the major pivotal trial of combination therapy plus ribavirin compared 48 weeks of peginterferon alfa-2a 180 μg once weekly plus ribavirin 1,000–1,200 mg, peginterferon alfa-2a alone, or interferon alfa-2b 3 million units three times weekly plus daily ribavirin. SVR occurred in 56%, 29%, and 44%, respectively, with rates of 46%, 21%, and 36%, respectively, in genotype 1 [89]. A second phase 3 trial with four arms compared peginterferon alfa-2a 180 μg weekly for 24 or 48 weeks plus ribavirin at a low dose (800 mg/day) versus weight-based dose 1,000–1,200 mg/day. For patients with genotype 1, SVR rates were higher with 48 weeks, while neither duration of therapy nor ribavirin dose led to statistically different SVR rates for genotypes 2 or 3 [90].

The two pegylated interferons had similar adverse effect profiles and were approved in combination with ribavirin for 48 weeks for genotype 1 and 24 weeks for genotypes 2 and 3. Nearly all trials from this era combined genotypes 2 and 3, obscuring what later emerged as higher SVR rates for genotype 2 than genotype 3, but with genotype 3 still easier to eradicate than genotype 1, a situation that was to reverse itself early in the era of DAA therapy when the first DAA drugs were designed primarily to target genotype 1.

Successive FDA approvals of peginterferon alfa-2b and alfa-2a as monotherapies and of each in combination with ribavirin occurred between 2001 and 2003. There followed a period of intense competition in the marketplace, with proponents of one side or the other referring to such features as the simplicity of fixed- (PEG IFN alfa-2a) versus weight-based dosing (PEG IFN alfa-2b) of the two peginterferons, considerations of volume of distribution putatively favoring weight-based dosing, and purported variability in rates of sustained response with fixed dosing across different body weights.

Debate persisted for years and generated several comparative studies, culminating in the massive IDEAL study, a 3,000+ patient study in genotype 1 HCV infection comparing PEG IFN alfa-2b 1.0 μg/kg/week or PEG IFN alfa-2b 1.5 μg/kg/week, each with ribavirin 800–1,400 mg/day, versus PEG IFN alfa-2a 180 μg/week plus ribavirin 1,000–1,200 mg/day [91]. The trial yielded statistically equivalent rates of

SVR of 40%, 39%, and 38%, respectively, with PEG IFN alfa-2a attaining higher rates of on-treatment response but also higher rates of posttreatment relapse, resulting in the similar SVR rates. By the time this study was published, PEG IFN alfa-2a had for some time become the market leader, though both remained in widespread use and both were combined with the first two protease inhibitors, telaprevir (PEG IFN alfa-2a) and boceprevir (PEG IFN alfa-2b), along with ribavirin. However, most of the DAA inhibitors were subsequently studied in combination with peginterferon alfa-2a.

The years that followed the approval of each of the first two pegylated interferons in combination with ribavirin early in the new millennium can be characterized as an "era of refinement," during which their efficacy and safety were evaluated in diverse patient populations, including patients with normal ALT, HIV-/HCV-coinfected persons, African–Americans, liver transplant recipients, and patients with kidney failure, among others. Viral kinetic studies improved our ability to predict therapeutic outcomes, with the recognition that failure to attain at least a 2 log drop after 12 weeks of treatment predicted ultimate failure with such a high level of confidence that treatment could be discontinued at that point. Similarly, failure to clear HCV RNA by 24 weeks was highly predictive of failure, and a strategy of stopping therapy under those conditions at that time point was adopted, as was the 12-week "stopping rule" (ref). Trials suggested potential efficacy for prolonged duration of therapy to as long as 72 weeks in patients with genotype 1 with "slow response patterns" such as persistent viremia at week 4 or, more commonly, by a >2 log reduction at week 12 with attainment of HCV RNA undetectability at week 24 [92–96]. Conversely, other studies suggested that viral clearance by week 4 in patients with genotype 1 was conducive to shortened duration of therapy to 24 weeks in patients with low baseline viral levels [97–99]. Still other studies examined the possibility of shortening duration of therapy in patients with HCV genotype 2 or 3 to 12–16 weeks, with mixed results [100–103].

In a recapitulation of what happened when ribavirin was introduced, the development of peginterferon and ribavirin spawned many studies on retreatment of patients who had failed previous regimens, including both relapsers and nonresponders to standard interferon with or without ribavirin. The results were modest, with success in only a minority of patients who had failed IFN and RBV and were retreated with PEG IFN and RBV but, in the absence of other prevailing options, led to considerable real-world use. It became clear that prior relapsers had a higher chance of SVR than prior nonresponders to IFN and RBV, but even in prior relapsers, SVR was attained in only a minority of patients who had failed a combination of standard interferon and ribavirin [104–109].

In patients failing to attain SVR on interferon-based therapy, long-term maintenance therapy with interferon monotherapy was studied, building upon the histologic improvement noted on liver biopsy, extending even to virologic nonresponders, after courses of interferon in biopsy-containing studies [110]. The most important of these studies was the HALT-C trial, an NIH-funded study conducted in the United States, which compared 3.5 years of PEG IFN alfa-2a 90 μg/week ($n = 517$) to the same duration of no therapy ($n = 533$) in nonresponders to previous

nonresponders to PEG IFN and ribavirin. Although serum aminotransferases, the level of serum hepatitis C virus RNA, and histologic necroinflammatory scores all decreased significantly with PEG IFN alfa-2a, there was no difference in any of the primary clinical outcomes in death, liver decompensation, or hepatocellular carcinoma [111]. As a result of this and other trials, maintenance therapy never became a standard approach in clinical practice.

Trials were also designed to evaluate the optimal dosing of ribavirin, including what was at the time the largest HCV treatment study yet conducted, which showed that weight-based dosing in a range of 800–1,400 mg/day was superior to flat dosing in patients with genotype 1 receiving peginterferon alfa-2b and ribavirin [112]. The incremental efficacy of weight-based dosing of ribavirin was greatest in African–Americans, signaling that ribavirin's greatest impact may have been in patients with intrinsically poor response to interferon, with a doubling of SVR with weight-based dosing in this population from 10% to 21% [113]. Even with this increment in response, however, absolute response rates remained much lower in this population. With pegylated interferon alfa-2a, the dosing range of ribavirin 1,000–1,200 mg/day was applied from the time this regimen was introduced.

As an antiproliferative agent, interferon suppressed bone marrow production of all blood cell lines, but the capacity of ribavirin to cause hemolysis resulted in anemia being the most common hematologic problem associated with interferon and ribavirin combination therapy. Studies demonstrated that erythropoietin allowed for maintenance of higher ribavirin doses by reducing the need for, or degree of, ribavirin dose reduction engendered by anemia [114]. However, there were no randomized trials showing convincingly that such adjuvant therapy led to higher SVR rates. The use of erythropoietin remained common through the introduction of telaprevir and boceprevir in combination with interferon and ribavirin because of the incremental anemia induced by these protease inhibitors. However, significant concerns arose about thrombotic events with these agents, and the need for their use abated with the advent of DAA therapy [115].

African–Americans represented perhaps the quintessential population in which interferon-based therapy did not present a "level playing field" in terms of the opportunity for response. In one of the most notable trials evaluating this issue, Muir et al. found that PEG IFN and ribavirin therapy yielded markedly disparate SVR rates of 52% for non-African–Americans and 19% for African–Americans [116]. The explanation for the disparate response rates to interferon in HCV-infected African–American persons was in large part, though not wholly, elucidated in a brief landmark paper in 2009. In a genome-wide association study (GWAS), a single nucleotide polymorphism in the region of the IL-28B locus was pinpointed as a key differentiator of response to interferon, with the CC genotype associated with markedly superior response to CT or, even more so, TT. Persons of African descent, for undetermined reasons, had a higher prevalence of the T allele, accounting in large part for the reduced efficacy of interferon-based therapy [117–119]. In the last phase of the interferon era, IL28B (subsequently called interferon lambda 4 (IFNL4)) testing became commonplace among clinicians who used the predictive value of the test to help determine whether patients with relatively mild disease should

undergo treatment or have it withheld in favor of the hoped-for interferon-free era that had appeared on the horizon. Despite minor signals of a potential role of IL28B variants in influencing SVR rates with DAA therapy in a few studies [120], most studies showed no such signals, and few if any clinicians perform the test any longer.

HIV coinfection with HCV was consistently associated with a greater likelihood of progressive liver fibrosis and adverse liver-related outcomes [121]. As in monoinfected patients, studies in focusing on coinfected patients suggested higher response rates in HIV-/HCV-coinfected persons with PEG IFN plus ribavirin compared to standard IFN plus ribavirin [122–126]. Accordingly, peginterferon and ribavirin therapy was adopted as the standard approach to HCV in HIV-coinfected persons. However, only peginterferon alfa-2a and ribavirin were approved for this population by the US Food and Drug Administration.

One of the most challenging populations throughout the interferon era consisted of patients with renal failure. Patients on hemodialysis have a high prevalence of HCV infection, estimated at 9.3% in the United States [127]. For years, many kidney centers placed a high priority on curative HCV therapy before renal transplantation was offered, especially in patients with more advanced fibrosis, because of the perception that (a) interferon posed too high a risk of precipitating graft rejection after transplantation and (b) HCV-associated liver disease could progress more rapidly after transplantation [128]. PEG IFN monotherapy had reported success rates of up to 40%, with even higher rates reported when ribavirin was added, but many clinicians did not encounter such rates of success, and the severity of ribavirin-induced anemia in these patients was a major obstacle [129].

It was during the "era of refinement" with pegylated interferon and ribavirin as the centerpiece that the concept of SVR as tantamount to virologic cure firmly took hold, based upon the relative rarity, in the range of 1%, of virologic relapse after the standard SVR time point at that time of 24 weeks after discontinuation of therapy [130, 131]. This time point was subsequently modified to SVR12 with DAA therapy. In addition to the overwhelming weight of these empirical observations, collective confidence in the concept of curability of HCV infection arose from the maturation of our understanding of the HCV life cycle, which appears to involve no form of genomic archiving analogous to that which occurs with hepatitis B and HIV.

5 The Era of Direct-Acting Antiviral (DAA) Therapy

The limited efficacy of interferon-based therapy, especially in genotype 1 infection, and its poor tolerability profile further exacerbated by ribavirin led to a massive effort to develop specifically targeted antiviral agents. The deep-rooted conviction that the paradigm would eventually change was fueled by the successful development of antiretroviral therapy for HIV infection in the 1990s and by remarkable advances in HCV biology.

The elucidation of the organization of the HCV genome led to an understanding of the viral proteins – the NS3/4A serine protease, NS5A, and NSB HCV polymerase – that are critical for HCV replication and came to serve as the therapeutic targets against which their corresponding inhibitors have revolutionized the field. A critical juncture in the evolution of HCV therapy was the development and refinement of replicon systems which made it possible to subject putative antiviral agents to in vitro testing – an advance that was all the more historic because of the lack of animal models for HCV infection other than chimpanzees, at least until chimeric mouse models were developed much later [132]. The initial subgenomic in vitro replicon systems developed in the late 1990s [133], with subsequent refinements including adaptive mutations that increased their replicative efficiency [134–137], were of profound importance in later providing the opportunity to screen many putative antiviral agents for potency. They also became critical in the development of our understanding of the role of resistant variants in altering the sensitivity of the virus to the suppressive effects of these classes of agents.

The HCV NS3 protein contains the viral serine protease activity responsible for much of the polyprotein processing as well as an RNA helicase activity that is likely involved in genome replication. The NS4A protein serves as a cofactor for the activities of NS3 and is important in attaching NS3 to cellular membranes [138–140]. Critical to HCV RNA replication within the lipid-rich membranous web formed within the hepatocyte cytoplasm, the NS5A protein has also been suggested to be important for viral assembly [141, 142]. The NS5B protein serves as the viral RNA-dependent RNA polymerase. The NS5B RNA-dependent RNA polymerase can be inhibited by nucleos(t)ide or non-nucleoside inhibitors, the former by binding with the active site, which leads to chain termination of RNA synthesis, and the latter by allosteric effects.

The NS3/NS4A serine protease mediates proteolysis at the NS3/NS4A, NS4A/NS4B, NS4B/NS5A, and NS5A/NS5B junctions, suggesting a key role in HCV polyprotein processing and, therefore, viral replication [143–145]. The structure of the NS3/NS4A serine protease of HCV was determined by two different groups in the mid-1990s [144–146]. Given that the protease is critical to viral replication, and the profound importance that the development of HIV protease inhibitors played in advancing the field of HIV therapy, the identification and development of clinically useful HCV inhibitors became a goal of urgent priority.

The first HCV protease inhibitor studied in humans was BILN 2061 [147–149]. Studies of this agent in patients with HCV genotype 1 infection given 2 days of dosing demonstrated potent viral suppression with 2–3 log reductions of HCV RNA levels during exposure [150]. Viral rebound occurred soon after therapy was stopped. The results of these studies, representing a groundbreaking proof of concept, garnered enormous attention in an oral presentation at the 2002 meeting of the American Association for the Study of Liver Diseases [151]. Unfortunately, development of the drug was halted because of cardiotoxicity in monkeys, and it would be several years before further clinical data were reported with other protease inhibitors [152]. For the remainder of the first decade of the twenty-first century, while the “era of refinement” of peginterferon therapy moved steadily forward, the development of

protease inhibitors proceeded at an accelerating pace and ultimately became the first class of DAAs approved for clinical use in patients with hepatitis C.

The development of nucleotide polymerase inhibitors was an inevitable development in light of the success of this class of agents for human immunodeficiency virus (HIV) and hepatitis B virus (HBV) infections. The active site of the HCV polymerase is relatively highly conserved [153] compared to the sequences of the other viral proteins that have been therapeutically targeted, accounting for the better pangenotypic coverage, and the higher barrier to resistance, of even the early polymerase inhibitors than was the case for the first generation of protease and NS5A inhibitors. An early agent studied clinically in this class was NM283 (valopicitabine), which conferred <2 log reduction in HCV RNA and had gastrointestinal effects, never progressing to phase 3 [154]. Subsequent agents in this class had superior potency (≥ 2 log early reduction in HCV RNA), including IDX-184, R1479, R1626, and mericitabine (RG-7128), but there were significant adverse effects in certain cases. For a time, mericitabine, which was well tolerated, appeared poised for advanced development when it became the first polymerase inhibitor to be combined with a protease inhibitor (danoprevir, see below) in the landmark INFORM study, demonstrating profound if transient inhibition of viral replication over a dosing period of 28 days [155, 156]. However, mericitabine was supplanted by PSI-7977, which eventually became known as sofosbuvir (SOF), a central drug in the HCV therapeutic revolution owing to its 4 log potency, excellent safety, and very high barrier to resistance attributable to the low replicative fitness of the signature resistance-associated substitution (S282T) demonstrable in vitro [157]. A comprehensive early review of the development of nucleotides, featuring a rich discussion of the medicinal chemistry as well as the early clinical studies, is available from Dr. Michael Sofia, who played a key role in the development of sofosbuvir [158], earning a 2016 Lasker Award for his work.

Before the early 2000s, only limited characterization of the NS5A protein was available. Examination of NS5A using bioinformatics tools suggested the protein consisted of three domains and contained a zinc-binding motif within the N-terminal domain. Four essential cysteine residues within domain 1 collectively bind to a single structural zinc ion, and mutation of these residues results in the complete inhibition of RNA replication [159]. NS5A proved to be a nonenzymatic protein which plays a critical role in the viral life cycle, essential not only in facilitating HCV replication in the replicase complex but appearing also to play a role in viral assembly [160–162]. The initial report of clinical testing in HCV patients of the first-in-class NS5A inhibitor, daclatasvir (DCV), was greeted with fascination by a large audience congregating for hours around the relevant poster at the AASLD meeting in 2009. It was shown that a single 100 mg dose resulted in viral suppression for an entire week before the appearance of virologic rebound [163]. Years later, NS5A inhibitors have come to comprise a critical component of nearly all DAA regimens currently administered to hepatitis C patients because of their potency, tolerability, and relative lack of drug–drug interactions.

The final category of DAAs that has reached clinical practice are non-nucleotide polymerase inhibitors, which bind to sites on the NS5B polymerase away from the

active site and confer allosteric inhibition rather than chain termination as do nucleotide polymerase inhibitors. The former proved to be less potent than the more potent nucleotide polymerase inhibitors and have a lower barrier to resistance [164, 165]. A number of such drugs underwent trials, but only one, dasabuvir, entered the clinic in combination with paritaprevir and ombitasvir and is seldom used any longer (see below).

6 Interferon-Based DAA Regimens

Up to 60% of patients with hepatitis C virus (HCV) genotype 1 infection failed to have a sustained virologic response to therapy with peginterferon alfa plus ribavirin. The direct-acting antiviral (DAA) era of HCV therapy arrived in 2011 with the introduction of the NS3/4A protease inhibitors (PIs) telaprevir (TVR) and boceprevir (BOC) for HCV genotype 1 patients. The development program for these drugs lasted for several years and captivated a global audience as it became progressively more apparent that approval would be forthcoming based upon the incremental efficacy when either PI was added to peginterferon and ribavirin.

Early results with both PIs made it clear that in genotype 1 patients higher response rates resulted from combining either agent with peginterferon and ribavirin [166–172]. Both programs also moved the field forward by highlighting the role of resistance in virologic failure; delineating the resistant variants, largely common to both agent, which were the basis of this clinical problem; underscoring the variability in replicative fitness of resistant variants, a concept that later carried over into the other classes of antiviral agents; and determining the longevity of the resistant variants often found in patients who had suffered virologic failure [173–178]. An early understanding emerged of the variability in time to spontaneous clearance of resistant variants after conclusion of an unsuccessful course of treatment. It became apparent, for example, that with either TVR or BOC the resistant variants emerging after a failed course of therapy cleared more quickly in patients with genotype 1b than genotype 1a.

The phase 3 development programs for the two initial PIs were similar in important respects, but there were also significant differences. Both sets of phase 3 trial programs evaluated treatment-naïve patients and interferon-experienced patients in separate studies. Patients with cirrhosis were admixed with noncirrhotic patients, and subanalyses were performed that showed SVR rates to be significantly lower in cirrhotics, just as had been the case with peginterferon and ribavirin alone, but clearly superior to the results obtained with peginterferon and ribavirin alone. Both programs evaluated on-treatment viral kinetics carefully to establish “stopping rules” for futility, and both programs incorporated truncation of therapy to 24–28 weeks for treatment naïve patients with rapid virologic response. Throughout most of the TVR development program, all three drugs were started simultaneously. In contrast, the phase 3 BOC regimen was founded upon utilization of a 4-week “lead-in” of peginterferon and ribavirin followed by triple therapy. For both regimens, the

PI was given with peginterferon and ribavirin for 12 weeks followed by completion of therapy with peginterferon and ribavirin alone. Both development programs explored the utility of response-guided therapy, in which treatment duration was governed by attainment of virologic response at predefined time points.

ADVANCE was a phase 3 double-blind placebo-controlled trial in which 1,088 patients with HCV treatment-naïve GT1 patients were randomized to one of three groups: a group receiving TVR combined with peginterferon alfa-2a and ribavirin for 12 weeks (T12PR group), followed by peginterferon–ribavirin alone for 12 weeks if HCV RNA was undetectable at weeks 4 and 12 (termed an extended rapid virologic response, or eRVR) or for 36 weeks if HCV RNA was detectable at either time point; a group receiving telaprevir with peginterferon–ribavirin for 8 weeks and placebo with peginterferon–ribavirin for 4 weeks (T8PR group), followed by 12 or 36 weeks of peginterferon–ribavirin on the basis of the same HCV RNA response criteria; or a group receiving placebo with peginterferon–ribavirin for 12 weeks, followed by 36 weeks of peginterferon–ribavirin (PR group). Significantly more patients in the T12PR or T8PR group than in the PR group had a sustained virologic response (75% and 69%, respectively, versus 44%) [179]. Although 8 weeks of TVR came close to 12 weeks, this trial established that the optimal duration of TVR in combination with PR was 12 weeks, which became the standard when the regimen was approved. The ADVANCE trial also established a strong foundation for response-guided duration of therapy with peginterferon, ribavirin, and TVR.

The ILLUMINATE trial enrolled patients with chronic HCV GT 1 infection who had not previously received treatment. All patients received telaprevir, peginterferon alfa-2a weekly, and ribavirin for 12 weeks (T12PR12), followed by peginterferon–ribavirin. Patients who had an eRVR were randomly assigned after week 20 to receive the dual therapy for 4 more weeks (T12PR24) or 28 more weeks (T12PR48). Patients without an eRVR were assigned to T12PR48. Of 540 patients, 65% had an extended rapid virologic response. The overall rate of sustained virologic response was 72%. Among the 322 patients with an eRVR, 92% in the T12PR24 group and 88% in the T12PR48 group had a sustained virologic response [180]. This trial was instrumental in establishing a 24-week duration of total therapy as sufficient in patients meeting the criteria for rapid virologic response.

In the REALIZE study, 663 treatment (interferon)-experienced GT1 patients received 12 weeks of PR plus TVR followed by 36 weeks of PR alone, or a 4-week lead-in of PR followed by 12 weeks of triple therapy and 32 weeks of PR, or 48 weeks of PR therapy alone. SVR rates were 83% in prior relapsers, 59% in prior partial responders, and 29% in "null" responders, with no significant difference in overall rates of response from the patients treated with a lead-in phase but significantly superior to PR alone. The results of this trial indicated that a lead-in PR phase did not add significant efficacy to this regimen and that the addition of a potent protease inhibitor to PR could not overcome the disadvantage inherent in intrinsic nonresponsiveness to interferon, as defined by decremental gradients of response to earlier unsuccessful therapy [181].

Based on these pivotal trials, both treatment-naïve patients and relapsers, but not nonresponders, were considered eligible for response-guided therapy in practice. HCV RNA was determined at week 4 of therapy, and if it remained >1,000 IU/mL, the entire treatment regimen was discontinued. At week 12, TVR was discontinued, and an HCV RNA assay was performed, with continuation of PEG IFN and RBV alone. However, if the HCV RNA was >1,000 IU/mL at week 12 and/or the HCV RNA declined <2 log10, then the entire regimen was to be discontinued. The stopping rules were identical for treatment-naïve and treatment-experienced patients [182, 183].

The BOC phase 3 program consisted of two trials, one in treatment-naïve and one in treatment-experienced patients. SPRINT-2 evaluated BOC in combination with PR (peginterferon alfa-2b 1.5 mcg/kg/week with weight-based ribavirin 600–1,400 mg/day) in treatment-naïve patients with HCV GT1. Group 1 received PR for 48 weeks (PR48). Group 2 received PR for 4 weeks followed by PR with BOC 800 mg three times daily × 24 weeks. If the treatment week (TW) 8 HCV RNA was undetectable (early responder or EVR) and TW24 HCV RNA was undetectable, treatment was discontinued at TW28. If the TW8 or any subsequent treatment week HCV RNA was detectable but not detectable at TW24 (late responder), PR was continued for another 20 weeks for a total treatment duration of 48 weeks (BOC-response-guided therapy or RGT). Group 3 received PR for 4 weeks followed by BOC 800 mg three times daily plus PR for 44 weeks. Subjects with detectable virus at TW24 were discontinued. The overall SVR 24 rates for the 3 groups were 40%, 67%, and 68%, respectively. Subjects with an EVR had SVR rates of 86%, 89%, and 91%, respectively versus 31%, 37%, and 43%, respectively, if the subject did not have an EVR [184]. Other than the lead-in phase, these results were thematically similar to those in the treatment-naïve telaprevir studies with regard to the capacity to stop therapy earlier in the face of a rapid response and the higher SVR rates in patients with rapid responses than in those with slower responses even when the latter group received a longer duration of total therapy.

RESPOND-2 was the pivotal BOC trial in patients with genotype 1 who had previously failed PR. It compared PR for 48 weeks versus a 4 week lead-in of PR, followed by PR plus BOC for an additional 32 weeks or an additional 12 weeks of PR if HCV RNA was detectable at week 8 of treatment, versus a 4 week lead-in of PR plus 44 weeks of PR plus BOC. The overall SVR 24 rates were 21%, 59%, and 66%, respectively. Prior relapsers to PR had SVR24 rates of 29%, 69%, and 75%, respectively, while prior nonresponders to PR had SVR 24 rates of 7%, 40%, and 52%, respectively [185]. As a result of the way the phase 3 trials of BOC had been conducted, the approval for BOC included a 4-week lead-in with PR followed by BOC-RGT to determine the duration of therapy.

Post hoc analyses using data from the phase 3 trials were undertaken to determine whether protocol-specified stopping rules (detectable HCV RNA at week 24 for SPRINT-2 and at week 12 for RESPOND-2) could be refined and harmonized. They concluded that week 12 HCV RNA levels ≥100 IU/mL almost universally predicted a failure to achieve SVR in both treatment-naïve and treatment-experienced patients. In boceprevir recipients, the combination of two stopping rules – an HCV RNA level ≥100 IU/mL at week 12 and detectable HCV RNA at week 24 – maximized the

early discontinuation of futile therapy and minimized premature treatment discontinuation [186].

The introduction of TVR and BOC was hailed as a major advance in the treatment of genotype 1 HCV infection in 2011. Unfortunately, the enthusiasm for these medications was tempered by the added burden of adverse effects, including exacerbation of the anemia already engendered by peginterferon and ribavirin, and the adverse cutaneous effects of TVR, including the development of grade 3 rashes that could even include Stevens–Johnson syndrome. Neither PI was incorporated into pivotal trials in combination with other DAAs, and within 3 years the two initially approved protease inhibitors that had made medical history were obsolete.

While the trials of BOC and TVR were moving into the advanced phases of testing and then approval, another protease inhibitor, simeprevir (SIM), was also being developed and showed early promise of better tolerability and at least equivalent efficacy in genotype 1 HCV infection. In the phase 3 QUEST-1 trial, treatment-naïve HCV genotype 1 infection patients were randomly assigned in a 2:1 ratio to receive SIM or placebo plus peginterferon alfa-2a plus ribavirin for 12 weeks, followed by peginterferon alfa-2a plus ribavirin. Total treatment was 24 weeks if HCV RNA <25 IU/mL (undetectable or detectable) at week 4 and <25 IU/mL undetectable at week 12, otherwise 48 weeks, and 48 weeks in the placebo group. Treatment with SIM, peginterferon alfa-2a, and ribavirin was superior to placebo, peginterferon alfa-2a, and ribavirin, with SVR12 in 80% versus 50%, respectively [187].

In the phase 3 QUEST-2 trial, treatment-naïve patients with HCV genotype 1 infection were randomly assigned to receive SIM, peginterferon alfa-2a or alfa-2b, and ribavirin (SIM group) for 12 weeks, followed by peginterferon alfa-2a or alfa-2b plus ribavirin, versus placebo plus peginterferon alfa-2a or alfa-2b, plus ribavirin (placebo group) for 12 weeks, followed by just peginterferon alfa-2a or alfa-2b plus ribavirin. Total treatment duration was 24 weeks or 48 weeks (SIM group) based on criteria for response-guided therapy or 48 weeks (placebo). SVR was seen in 81% of the patients in the SIM group and 50% in the placebo group, clearly establishing that the addition of SIM improved SVR 12 in HCV GT1 treatment-naïve patients [188]. In a phase 2b study of treatment-experienced GT 1 patients in whom the two regimens were compared, with 12, 24, or 48 weeks of SIM versus placebo plus peginterferon and ribavirin, with all patients receiving 48 weeks of total therapy, the SIM recipients had higher SVR12 rates, and there were increasingly high rates of SVR12 in null responders, partial responders, and relapsers, respectively [189].

A distinctive feature of the SIM development program emerging from the studies on simeprevir was the finding that the Q80K polymorphism in the protease domain, present in up to 50% of US GT1a patients but a smaller percentage of European patients, impaired the chance of SVR with the triple regimen of PEG IFN, ribavirin, and SIM, but only in GT1a patients (the polymorphism is much less common in GT1b). An inkling of this had emerged in the phase 2 program but became quantitatively better established in phase 3. This polymorphism results in a modest loss of antiviral activity in in vitro assays. The clinical findings led to the first

approval of a regimen for HCV infection bearing the stipulation that baseline resistance testing was required for a subgroup of patients, i.e., those with GT1a, to identify patients in whom a suboptimal response could be expected.

Had SIM been the first protease inhibitor developed for HCV infection, it would likely have dominated the landscape for treatment of GT1 patients during the interlude between PR therapy and interferon-free DAA therapy. It had efficacy that easily matched that of its two forerunners, and its tolerability was superior, with the major adverse effects including photosensitivity and a benign effect on bilirubin transporters that caused occasional hyperbilirubinemia which seldom required discontinuation of therapy. As it happened, its major contribution to patient care was in combination with SOF without interferon in the interval lasting through most of 2014, before NS5A inhibitor-containing therapy was approved (see below).

The culmination of the interferon era, albeit too late in that era to enjoy more than a brief period of use, was the combination of pegylated interferon, ribavirin, and SOF. In the phase 3 NEUTRINO clinical trial, subjects previously untreated with chronic HCV infection with genotypes 1, 4, 5, or 6 were enrolled in an open-label single-treatment group with pegylated interferon alfa-2a and weight-based ribavirin and SOF for 12 weeks. The overall SVR rate was 90%, the highest SVR rate, with the shortest duration of treatment, for any interferon-based regimen [190]. In the simultaneously published FISSION study, 24 weeks of peginterferon alfa-2a and ribavirin 800 mg was compared to 12 weeks of SOF and ribavirin in treatment-naive patients with genotypes 2 and 3, with SVR rates of 67% in each group [190].

As these important refinements of PEG IFN-based therapy for HCV were being made, the development of DAA therapy had been moving forward rapidly. Ironically, the most attractive interferon-based regimen in the history of the field in terms of efficacy, tolerability, and shortened duration of therapy, the combination of peginterferon, ribavirin, and SOF, quickly lost its relevance as the development of interferon-free DAA-based therapy bore fruit less than 5 years after the earliest glimmerings of what such therapy could achieve.

7 A Historic Proof of Concept: Curability of HCV Without Interferon

In 2010, the first demonstration of potent viral suppression with a non-interferon containing DAA combination regimen was published from the INFORM-1 trial [155]. Treatment of 73 patients for 13 days with a combination of 2 oral DAAs, the nucleoside polymerase inhibitor (RG7128, meracitabine) and an NS3/4A PI (danoprevir), without peginterferon or ribavirin profoundly suppressed HCV RNA levels in patients with genotype 1 infection. The median change in HCV RNA concentration from baseline to day 14 ranged from −3.7 to −5.2 log(10) IU/mL in the cohorts that received 13 days of combination treatment. At the highest combination doses, the median change in HCV RNA concentration from baseline to day 14 was −5.1 log(10) IU/mL in treatment-naïve patients and −4.9 log(10) IU/mL in previous standard of care null responders to interferon-based therapy versus an

increase of 0.1 log(10) IU/mL in the placebo group. Minority PI-resistant variants present at baseline were suppressed by mericitabine [156]. However, the later INFORM-SVR study of this regimen with or without ribavirin for 24 weeks yielded low rates of SVR with 24 weeks of therapy [191].

Subsequently, Gane and colleagues evaluated SOF-based interferon-free regimens for untreated patient with HCV genotype 2 and 3 in the ELECTRON study, which spawned a number of arms before its ultimate completion. At the first presentation by Dr. Gane of the findings, many who were present recall to this day the several overflow rooms required to accommodate an audience correctly sensing it was witnessing a milestone in the history of medicine [192]. Forty patients were randomly assigned to four groups; all four groups received SOF plus ribavirin for 12 weeks. Three of these groups also received peginterferon alfa-2a for 4, 8, or 12 weeks. Two additional groups of previously untreated patients with HCV genotype 2 or 3 infection received SOF monotherapy for 12 weeks or SOF plus peginterferon alfa-2a and ribavirin for 8 weeks. Two groups of patients with HCV genotype 1 infection received SOF and ribavirin for 12 weeks: 10 patients with no response to prior treatment and 25 with no previous treatment. Of the 40 patients who underwent randomization, 100% who received SOF plus ribavirin without interferon and 100% who received SOF plus ribavirin for 12 weeks and interferon for 4, 8, or 12 weeks had a sustained virologic response at 24 weeks. For the other patients with HCV genotype 2 or 3 infection, 100% of the patients who received SOF plus peginterferon alfa-2a and ribavirin for 8 weeks had a sustained virologic response at 24 weeks, as did 60% who received SOF monotherapy. Among patients with HCV genotype 1 infection, 84% previously untreated patients had a sustained virologic response at 24 weeks. However, only 10% of HCV GT1 prior null responders to interferon and ribavirin had SVR [193], one of several early studies with DAA therapy that showed a deleterious impact of prior interferon nonresponse on response to non-interferon-based DAA therapy, a gap that was ultimately overcome with combination regimens.

Another landmark proof of concept study, performed by Lok and colleagues, was an open-label, phase 2a study in patients with HCV genotype 1a or 1b without cirrhosis who had not had a response to therapy with peginterferon and ribavirin. Patients were assigned in a 1:1 ratio to receive DCV (NS5A inhibitor) and asunaprevir (NS3 PI) for 24 weeks (11 patients) or DCV, asunaprevir, peginterferon alfa-2a, and ribavirin (10 patients). Coadministration of DCV and asunaprevir alone to 11 patients led to a rapid reduction in HCV RNA. Of these 11 patients, five had undetectable HCV RNA at the end of the treatment period and four had sustained virologic response at weeks 12 and 24 after treatment. SVR24 occurred in 9 of the 10 quadruple regimen patients, but it was the four patients with SVR to interferon-free DAA therapy who provided proof-of-concept for interferon-free cure [194]. These data a glimmering of what became a major theme in subsequent years, namely, the difference in resistance barrier of first-generation protease and especially NS5A inhibitors to genotype subtypes 1a versus 1b. Patients with the latter subtype were less prone to develop resistance during exposure to the first-generation members of

these two classes because of differences in the number of nucleotide substitutions at critical loci needed to generate resistance – fewer for 1a than for 1b [195].

8 Further Early Studies of DAA Combination Therapy

With the proof of concept for curability of HCV infection without interferon now established, intense activity in the field fueled a number of development programs that not only affirmed the concept of curability but soon resulted in the stunning realization that extraordinarily high rates of cure could be attained with first-generation antiviral regimens. Many had anticipated that progress in the field would be incremental and that it would take years for cure rates in most patients could occur, but within a 2-year period, it became clear that history would record a quantum leap forward.

An early trial of combination therapy with daclatasvir, the first-in-class NS5A inhibitor to be tested in patients, and SOF was one of the first studies to establish that very high rates of SVR could be attained in most patients. In a trial by Sulkowski et al., 44 previously untreated patients with HCV genotype 1 infection and 44 patients with HCV genotype 2 or 3 were randomly assigned to DCV plus SOF daily, with or without ribavirin, for 24 weeks. The study was expanded to include 123 additional patients with genotype 1 infection who were randomly assigned to daclatasvir plus sofosbuvir, with or without ribavirin, for 12 weeks (82 previously untreated patients) or 24 weeks (41 patients who had previous virologic failure with TVR or BOC plus peginterferon alfa and ribavirin). Among patients with genotype 1 infection, 98% of 126 previously untreated patients had SVR, as did 98% of 41 patients who had not attained SVR to HCV protease inhibitors in combination with peginterferon and ribavirin. A total of 92% of 26 patients with genotype 2 infection and 89% of 18 patients with genotype 3 infection had a sustained virologic response at week 12 [196].

The theme of an incipient quantum leap in HCV curability was not limited to nucleotide-containing regimens. The AVIATOR trial evaluated a combination of paritaprevir (protease inhibitor) with low-dose ritonavir boosting, ombitasvir (NS5A inhibitor), and dasabuvir (a non-nucleotide polymerase inhibitor) in several hundred noncirrhotic treatment-naïve patients who received a variety of two or three drug combinations with or without ribavirin. Of the nine arms, SVR rates varied between 85% and 99%. Two 8-week regimens fell just short of 90% SVR, and the highest rates of SVR were attained in treatment-naïve patients who received 12 weeks of the three-drug regimen plus ribavirin (99%) and 24 weeks of the same regimen in prior interferon null responders (98%) [197].

The FISSION (treatment-naïve) and POSITRON (treatment-experienced) trials were instrumental in providing a portent, contrary to the expectations arising from the ELECTRON study, that genotype 3 would emerge as the “problem child” in the

early era of DAA therapy, with lower SVR rates for GT3 patients, particularly those with cirrhosis, when treated with SOF and RBV for 12 weeks. In cirrhotic patients, SVR12 was achieved at a rate of 34% for treatment-naïve and 21% for treatment-experienced patients. By extending this regimen to 16 weeks, SVR12 rates could be increased to 61% in treatment-experienced patients with HCV GT3 infection [198]. The subsequent VALENCE trial confirmed that high SVR12 rates could be achieved in HCV GT2 patients with cirrhosis after 12 weeks of therapy (100% for treatment-naïve, 88% for treatment-experienced). Extending treatment to 24 weeks for HCV GT3 patients allowed for an improvement to 92% in treatment-naïve patients, but those who were treatment-experienced remained at 62% [199]. As a result of the cumulative studies up to that time, the initial approval of SOF and ribavirin for GT3 entailed a recommended treatment duration of 24 weeks rather than the 12-week approval garnered for GT2.

9 The Era of Approved Interferon-Free Therapy Begins

The first approval of oral, interferon-free treatment occurred in late 2013 for sofosbuvir and ribavirin in HCV genotypes 2 and 3 infection. Contemporaneously, sofosbuvir and ribavirin combined with peginterferon was approved for all genotypes based on data showing SVR rates of about 90%, with a treatment duration of for 12 weeks for all patients. Also around the same time, the protease inhibitor simeprevir (SIM) was approved with peginterferon and ribavirin in combination for genotype 1. These three seemingly disparate developments proved fateful because, as the year 2014 dawned, it was apparent to HCV treaters in countries where SIM and SOF had each been approved with interferon that it would be more effective to combine these agents with each other and leave interferon and even ribavirin aside.

By the time peginterferon, ribavirin, and simeprevir were approved in combination, the phase 2 COSMOS trial had shown the combination of SIM and SOF to confer very high rates of SVR with excellent tolerability. This was a phase 2, four-arm trial evaluating SMV+SOF without or with ribavirin and for 12 versus 24 weeks in genotype 1 patients across the fibrosis range of F0–F4. The trial demonstrated SVR rates over 90% in all arms [200]. Based on the COSMOS data, many clinicians prescribed the regimen for their patients with excellent results that generally emulated the trial, despite initial concerns about whether payers would cover the combination regimen in the absence of FDA approval for the two drugs together. By the time the combination of SIM and SOF was approved in late 2014 in the United States, thousands of patients had benefitted from the "head start" they had been given on the opportunity to cure their HCV infections with SIM and SOF in combination.

Atypically, the publication of the phase 3 trials of the combination of SIM and SOF was released *after* the US FDA had already approved it based on the results of COSMOS in the context of the pressing unmet need for interferon-free therapy and higher rates of SVR. Subsequently, in the phase 3 OPTIMIST-1 trial, a randomized open-label study assessed the efficacy and safety of 12 and 8 weeks of simeprevir

and sofosbuvir in HCV GT1-infected treatment-naïve and treatment-experienced patients without cirrhosis. Patients were randomly assigned to simeprevir 150 mg once daily and sofosbuvir 400 mg once daily for 12 or 8 weeks with primary endpoint of SVR12. Superiority in SVR12 was assessed for SIM and SOF at 12 and 8 weeks versus a composite historical control SVR rate. SVR12 with SIM and SOF for 12 weeks was 97% versus 83% in the 8-week arm. Patients in the 8-week arm with GT1a and the Q80K polymorphism had lower SVR rates [201]. OPTIMIST-2 evaluated the combination of SIM and SOF in GT1 treatment-naïve or treatment-experienced cirrhotic patients for 12 weeks, with SVR in 83% overall (88% and 79% in naïve and experienced patients, respectively). Patients with GT1a infection and the Q80K polymorphism had lower rates of SVR than those without Q80K [202].

Nearly contemporaneous with the approval of simeprevir and sofosbuvir in combination was the approval of ledipasvir/sofosbuvir (LDV/SOF) in late 2014 based upon a very large phase 3 development program. The phase 3 ION-1 and ION-2 studies evaluated the fixed-dose combination (FDC) of SOF and the first-generation NS5A inhibitor LDV in GT1 treatment-naïve (ION-1) and treatment-naïve and treatment-experienced (ION-2) patients without or with cirrhosis. Each trial contained four arms, featuring LDV/SOF without or with ribavirin for 12 or 24 weeks. SVR12 rates in ION-1 were 99%, 97%, 98%, and 99% with 12 weeks of LDV/SOF without ribavirin and with ribavirin and 24 weeks without and with ribavirin, respectively [203]. ION-2 included treatment-experienced patients who achieved SVR12 rates after 12 weeks of treatment of 82–86% (with or without ribavirin, respectively) and 100% in each of the 24-week arms, respectively. In both studies the inclusion of RBV appeared to make no difference to the overall SVR rates in cirrhosis, nor was there a difference in results between genotype 1a and 1b patients. Results in ION-2 were similar in patients with or without exposure to a protease inhibitor combined with PEG IFN and RBV [204].

The pivotal phase 3 ION-3 LDV/SOF study reflected the widespread interest in shortening duration of DAA therapy without significantly compromising the chance of SVR. With three arms containing LDV/SOF for 8 weeks with or without ribavirin or for 12 weeks without ribavirin, all in treatment-naïve noncirrhotic patients with GT1, SVR rates varied between 93% and 95% with no significant differences among them. Retrospective analysis indicated that relapse rates were higher in the 8-week ribavirin-free arm when patients had baseline viral load of >6,000,000 IU/mL, accounting for about 30% of GT1 patients [205]. The AASLD/IDSA guidelines subsequently recommended against the adoption of the 8-week regimen in African–Americans and HIV-/HCV-coinfected patients based on data extrapolated from other studies [206]. In one of the clearest examples of the impact of real-world post-marketing studies with DAA regimens, a high proportion of such studies vindicated the hypothesis that treatment in GT1 patients with “low” baseline viral level was equally effective for 8 as for 12 weeks [207–210].

Subsequently, the SIRIUS trial randomized 155 HCV-1 patients with compensated cirrhosis who had failed PI therapy to either LDV/SOF FDC plus RBV for 12 weeks, or 24 weeks of the FDC alone, and found similar SVR12 rates

between the two regimens (96% versus 97%) [211]. Concomitantly, a pooled analysis of all phase 2b and phase 3 trials that included cirrhotic patients with HCV-1 treated with this DAA combination ($n = 513$), including the SIRIUS population, demonstrated that RBV may improve SVR12 rates in treatment-experienced patients receiving 12 weeks of therapy (96% versus 90%). There was no difference seen in SVR12 rates between those receiving the LDV/SOF with RBV for 12 weeks and those receiving 24 weeks of FDC without RBV (96% versus 98%) [212], an unexpected finding after the earlier and smaller ION-2 study [205].

Nearly simultaneous with approval of LDV/SOF came the approval of the first nucleotide-free regimen: paritaprevir/r (ritonavir boosting)/ombitasvir and dasabuvir. Paritaprevir, a protease inhibitor, was the first drug, and remains the only drug to date, in the HCV armamentarium to be co-administered with ritonavir for pharmacologic boosting of the PI, a concept borrowed from the HIV field. It was formulated in a single-tablet regimen with the NS5A inhibitor, ombitasvir, and a non-nucleotide inhibitor, dasabuvir, was administered as a separate tablet. This regimen has been replaced by the pangenotypic combination of glecaprevir and pibrentasvir in many countries (see below) but retains an important place in the history of the first generation of DAA regimens.

In the SAPPHIRE-1 phase 3 trial, the three-drug regimen was evaluated in previously untreated patients with HCV genotype 1 infection and no cirrhosis. Treatment with this regimen included: single-tablet coformulation of ABT-450 (paritaprevir)/r–ombitasvir and dasabuvir (250 mg twice daily) with ribavirin. The overall rate of sustained virologic response in this group was 96.2%. The response rates in this group were 95.3% among patients with HCV genotype 1a infection and 98.0% among those with HCV genotype 1b infection [213].

In the SAPPHIRE-2 trial, patients with HCV genotype 1 infection and no cirrhosis, who had been previously treated with peginterferon–ribavirin, were randomly assigned to receive co-formulated paritaprevir/ritonavir/ombitasvir and dasabuvir with ribavirin or to matching placebos during the 12-week double-blind period. In the active treatment group, an overall rate of 96.3% virologic response at posttreatment week 12 was seen. This rate was superior to the historical control rate. Rates were 95.3% among patients with a prior relapse, 100% among patients with a prior partial response, and 95.2% among patients with a prior null response [214].

The role of ribavirin with this triple regimen was investigated extensively in two phase 3 trials known as PEARL-III and PEARL-IV. Patients with HCV genotype 1b infection (PEARL-III) and HCV genotype 1a infection (PEARL-IV) were randomized to 12 weeks of paritaprevir/r–ombitasvir, dasabuvir, and ribavirin or to matching placebo for ribavirin. The rate of SVR among patients with HCV genotype 1b infection was 99.5% with ribavirin and 99.0% without ribavirin, and among those with genotype 1a infection was 97.0% and 90.2%, respectively. Response rates in all treatment groups were superior to the historical response rate with a peginterferon-containing TVR-based regimen [215].

The phase 3 TURQUOISE-II trial evaluated the above regimen with ribavirin in treatment-naïve or treatment-experienced patients with compensated HCV GT1 cirrhosis and compared 12 to 24 weeks of treatment. In this study, SVR12 rates were 92% and 96% following 12 and 24 weeks therapy, respectively. Results varied according to HCV GT1 subtype, higher in HCV GT1b with SVR12 of 98.5% and 100%, compared with 89% and 94% in HCV GT1a subtype following 12 and 24 weeks, respectively [216]. With these results in HCV GT1b cirrhosis, the phase 3b TURQUOISE-III study evaluated the three DAA regimens without RBV in HCV GT1b compensated cirrhosis. One hundred percent of the enrolled patients achieved SVR12 including 33 patients with prior PegIFN/RBV treatment experience. Based on these results, for all HCV GT1 cirrhosis patients, except prior HCV GT1a null responders who needed 24 weeks, 12 weeks of ritonavir-boosted paritaprevir, dasabuvir, and ombitasvir was sufficient, with RBV still needed in those with HCV GT1a [217].

In late 2014 the regimen of paritaprevir/ritonavir, ombitasvir, and dasabuvir with and without RBV were approved to treat HCV GT1 patients in the United States. Following approval of this regimen, post-marketing surveillance identified several patients with cirrhosis who developed hepatic decompensation and/or liver failure while receiving this therapy. This led to the US FDA issuing a warning that treatment with ritonavir-boosted paritaprevir, dasabuvir, and ombitasvir can cause serious liver injury in patients with advanced liver disease (www.fda.gov/drugs/drugsafety/ulm468634.htm).

The first DAA regimen approved for treatment of genotype 3 without ribavirin in the United States was daclatasvir and sofosbuvir (2015), followed in early 2016 by expanded approval for use with or without ribavirin in genotype 1 patients, including patients with cirrhosis, post-liver transplant HCV, and HIV coinfection [218, 219]. The ALLY-3 study evaluated 12 weeks of DCV plus SOF in treatment-naïve and treatment-experienced GT3 patients without or with cirrhosis. SVR occurred in 96% of the noncirrhotic patients but in only 63% of those with cirrhosis [220]. Other studies demonstrated substantial improvement in SVR rates in GT3 cirrhotic patients with 24 weeks of treatment, with no augmentation with RBV [221]. Daclatasvir was an important drug in the evolution of HCV therapy but suffered from the lack of a companion drug.

In 2016 another DAA regimens were approved by the FDA: elbasvir (EBR), an NS5A inhibitor, and grazoprevir (GZR), a NS3/4A protease inhibitor, co-formulated in a single tablet. The phase 3 C-EDGE treatment-naïve (TN) trial evaluated chronic HCV genotype 1, 4, and 6 treatment-naïve with and without cirrhosis given EBR/GZR 50/100 mg tab daily for 12 weeks. The overall SVR rate was 95%. The SVR rate for GT1a was 92% and 99% for GT1b. Lower SVR12 rates occurred in patients with baseline NS5A resistance-associated substitutions (RASs) associated with >fivefold loss of EBR susceptibility [222]. These included substitutions at the 28, 30, 31, and 93 positions of the NS5A molecule. The phase 3 open-label trial C-EDGE treatment-experienced (TE) for HCV GT1 peginterferon plus RBV failures with and without cirrhosis evaluated fixed-dose elbasvir–grazoprevir daily

for 12 or 16 weeks with or without ribavirin. There were four treatment arms, EBR/GZR × 12 weeks, EBR/GZR + RBV × 12 weeks, EBR/GZR × 16 weeks, and EBR/GZR + RBV × 16 weeks. SVR rates were 92.4%, 94.2%, 92.4%, and 98.1%, respectively. Virologic failure occurred only in prior nonresponders, not relapsers. No virologic failures occurred in patients treated for 16 weeks with ribavirin [223].

An analysis of six clinical trials assessed the safety and efficacy of EBR/GZR in patients with compensated cirrhosis and compared 12 versus 16–18 weeks of treatment without or with ribavirin. Ribavirin did not add significantly to the efficacy of 12 weeks of treatment. Among treatment-experienced patients, only those treated for 16–18 weeks with ribavirin had no virologic failures. In genotype 1a patients, baseline RASs were the major driver of virologic failure [224].

The cumulative data on this regimen led to GZR/EBR for 12 weeks in treatment-naïve or treatment-experienced genotype 1a patients with and without compensated cirrhosis without NS5A RAVs and GZR/EBR + RBV for 16 weeks in GT1a patients with NS5A RASs. For genotype 1b patients with and without compensated cirrhosis, treatment-naïve or treatment-experienced, GZR/EBR for 12 weeks without RAS testing was recommended based on data across a broad spectrum of patient populations, except for decompensated cirrhotics in whom no protease inhibitor is recommended [225].

10 The Issue of NS5A Inhibitor Resistance

Resistance to NS5A inhibitors emerged as a major theme during the era of the first-generation DAA regimens. Most patients who failed to have SVR on such regimens had NS5A resistance-associated substitutions (RASs) in their viral populations at the time of virologic failure, which usually took the form of posttreatment relapse rather than on-treatment breakthrough or failure to suppress HCV RNA to undetectable levels. Most of the relevant RASs were in the 28, 30, 31, and 93 positions. Approximately 15% of patients had such variants at baseline as detected by population sequencing, which required a threshold of roughly 15–20% of the viral population within an individual patient to be detected; deep or “next-generation” sequencing had a lower threshold in the range of 1% but proved to have lower predictive value for virologic failure [226].

Most of what was learned about the impact of baseline RASs, and the need for adjustment of the regimen prior to treatment initiation, was gleaned from retrospective analyses of data from studies in which patients were not stratified by the presence or absence of baseline RASs. This proved to be most impactful for the regimen of elbasvir/grazoprevir, the phase 3 trials of which had arms with or without ribavirin for treatment durations of 12 or 16 weeks. It emerged that in genotype 1a patients the chance of SVR was significantly impacted by RASs in the four positions cited above and that this adverse impact was overcome by the addition of ribavirin

and extension to 16 weeks in patients with genotype 1 (the regimen was approved only for genotypes 1 and 4). This resulted in the regimen being the only one with a stipulation in its package insert in the United States that baseline RAS testing was advised before treatment of genotype 1a patients, with adjustment of the regimen accordingly if it was to be used at all in such patients with baseline RASs. Although a signal of an impact of baseline RASs could be shown with other genotype 1 regimens, e.g., LDV/SOF in some populations [227], the impact was not such as to lead to advice to obtain RAS testing in the package insert nor in the AASLD or EASL guidelines [228]. In genotype 3 patients, however, the regimen of SOF/VEL generated recommendations for baseline RAS testing to assess for the presence of the Y93H variant in interferon-experienced or cirrhotic patients with genotype 3 and the addition of ribavirin should this variant, which confers substantial resistance to VEL, be present (see below) [228].

11 The Advent of Pangenotypic DAA Regimens

The era of pangenotypic HCV DAA therapy was ushered in with publication of the double-blind, placebo-controlled ASTRAL-1 study involving untreated and previously interferon-treated patients with chronic HCV ($n = 624$) with genotypes 1a, 1b, 2, 4, 5, or 6 infection, including those with compensated cirrhosis (19%) and treatment-experienced (32%), who received the nucleotide polymerase inhibitor sofosbuvir and the NS5A inhibitor velpatasvir in a once-daily, fixed-dose combination tablet or matching placebo for 12 weeks. The rate of SVR 12 among patients receiving sofosbuvir–velpatasvir (SOF/VEL) was 99% with only 2 virologic failures, both in genotype 1, and a small number of nonvirologic failures [229].

The ASTRAL-2 study was a randomized, phase 3 studies for patients HCV genotype 2 treatment-naïve and treatment-experienced, including patients with compensated cirrhosis. In one of the trials, patients with HCV GT2 were randomly assigned to sofosbuvir–velpatasvir or sofosbuvir plus weight-based ribavirin for 12 weeks. The SVR rate was 99% in the sofosbuvir–velpatasvir group versus 94% in the sofosbuvir–ribavirin group, with no virologic failures in the SOF/VEL group [230].

The same regimen for HCV genotype 3 was evaluated separately in the ASTRAL-3 study. This phase 3 study evaluated 12 weeks of SOF/VEL without RBV versus 24 weeks of sofosbuvir plus RBV, including patients with compensated cirrhosis. In patients without cirrhosis, treatment-naïve patients had SVR in 98% versus 91% of interferon-experienced patients. Among the patients with cirrhosis receiving SOF/VEL, SVR12 rates were 93% in treatment-naïve patients and 89% in those with prior treatment failure. Overall, the rate of sustained virologic response in the SOF/VEL group was 95% and 80% in the sofosbuvir–ribavirin group [230]. Cumulatively, ASTRAL-1, ASTRAL-2, and ASTRAL-3 and the subsequent approval of SOF/VEL in 2016 signaled the end of the era of combination therapy with sofosbuvir and ribavirin alone for any patients with hepatitis C.

Voxilaprevir (VOX) is a second-generation HCV protease inhibitor with coverage across genotypes and against most PI-resistant variants. A triple regimen of SOF/VEL/VOX appeared highly promising in phase 2 trials when given for 8 or 12 weeks and was subjected to a series of four trials called the POLARIS studies in phase 3. POLARIS-2 and POLARIS-3 evaluated DAA-naïve patients, including both noncirrhotic and cirrhotic patients except genotype 3 patients with cirrhosis. POLARIS-2, the larger of the two trials, was designed to assess the efficacy of 8 weeks of SOF/VEL/VOX versus 12 weeks of SOF/VEL single in DAA treatment-naïve subjects. SVR was 95% versus 98% of subjects, respectively, with genotype 1a driving the SVR rate in the 8-week regimen to below the noninferiority endpoint established in the protocol [231]. POLARIS-3, the trial in genotype 3 patients with cirrhosis, yielded identical SVR rates by intent-to-treat analysis of 96% in each group [231]. Since SOF/VEL performed well in these trials, SOF/VEL/VOX did not garner FDA approval in an 8-week regimen in DAA-naïve patients, although it did succeed in doing so in Europe.

Glecaprevir (GLE) and pibrentasvir (PIB) are a second-generation NSA 3/4A protease inhibitor and NS5A inhibitor, respectively. These are pangenotypic drugs that cover a broad range of RASs associated with the first-generation protease inhibitors and NS5A inhibitors. In a study in which the resistance profiles of the HCV NS5A inhibitors were evaluated in an independent laboratory, PIB had the broadest range of coverage within the NS5A class but was still susceptible to resistance in the setting of certain dual variants [232].

Zeuzem and colleagues conducted a randomized trial in over 600 patients (ENDURANCE-1) with genotype 1 infection randomly assigned in a 1:1 ratio to receive once-daily GLE/PIB for either 8 or 12 weeks. The rate of sustained virologic response at 12 weeks among genotype 1-infected patients was 99.1% in the 8-week group and 99.7% in the 12-week group, with only one virologic failure in the 8-week group and none in the 12-week group [233]. To establish the clinical pangenotypic efficacy expected from the in vitro properties of these drugs, the GLE/PIB combination was evaluated in three open-label studies (SURVEYOR-II, Part 4, ENDURANCE-4, ENDURANCE-5,6) and a randomized, double-blind, placebo-controlled study (ENDURANCE-2). In the ENDURANCE-2 study, adult patients with untreated or previously treated HCV genotype 2 infection without cirrhosis were randomly assigned (2:1) to groups given once-daily oral glecaprevir/pibrentasvir or placebo for 12 weeks. In the SURVEYOR-II, Part 4, and ENDURANCE-4 studies, adult patients with untreated or previously treated patients with HCV genotype 2, 4, 5, or 6 infection, without cirrhosis, were given once-daily oral GLE/PIB for 12 or 8 weeks, respectively. Among patients receiving GLE/PIB for 8 weeks, rates of SVR12 were 98% in those infected with HCV genotype 2 and 93% in those infected with HCV genotypes 4, 5, or 6. Among patients receiving GLE/PIB for 12 weeks, rates of SVR12 were 99.5% (95% CI, 98.5–100) in those infected with HCV genotype 2 and 99% (95% CI, 97.6–100) in those infected with HCV genotype 4, 5, or 6. In the 8 week treated patients, no virologic failures occurred in the patients with genotypes 4, 5, or 6 [234]. Similarly high rates of success, with rare virologic

failure, were observed in ENDURANCE-5,6 with 8 weeks of treatment for HCV GT5 and 6 without cirrhosis and 12 weeks with cirrhosis [235].

For HCV genotype 3 patients, the ENDURANCE-3 study enrolled 505 treatment-naïve patients without cirrhosis and randomized 2:1 to receive 12 weeks of once-daily therapy to three arms, consisting of GLE/PIB for 12 weeks, sofosbuvir + daclatasvir (SOF + DCV) for 12 weeks, or GLE/PIB for 8 weeks. SVR 12 was achieved in 95%, 97%, and 95% in each arm, respectively, with SVR 12 from GLE/PIB for 8 weeks meeting noninferiority compared to the other two arms. However, there were arithmetically greater numbers of patients with virologic failure in the 8- and 12-week GLE/PIB arms, particularly the former [233]. The clinical significance of this is unclear, and the 8-week regimen was approved for GT3 noncirrhotic patients, along with all other genotypes in noncirrhotics, in the United States in 2017. There were a small number of patients in ENDURANCE-3 with a baseline A30 RAS, and these patients had a lower rate of SVR, but the significance of this, too, is unclear, and there has been no recommendation for baseline RAS testing with this regimen [228, 236].

The EXPEDITION-1 study evaluated 12 weeks of GLE/PIB in patients with compensated cirrhosis across genotypes 1–6; no attempt was made in the phase 3 program to compare 8 versus 12 weeks in cirrhotic patients. Uniformly high rates of SVR12 ($\geq$98%) were seen in this study, establishing 12 weeks as the approved treatment duration in this population when the GLE/PIB regimen was approved [237]. As with other protease inhibitor-containing regimens, GLE/PIB is not recommended for use in patients with decompensated cirrhosis.

12 Special Populations

12.1 Decompensated Cirrhosis and Pre-liver Transplant (LT)

In patients with HCV infection awaiting LT, the primary aim of antiviral therapy is to prevent recurrent HCV infection of the new liver, which is associated with reduced graft and patient survival [238]. A key study that set the tone for what has followed in transplant candidates with HCV infection was conducted in patients with HCV genotypes 1–4 awaiting LT for HCC who were treated with sofosbuvir and RBV. Seventy percent of those with an undetectable HCV RNA at the time of transplantation achieved a posttransplant virologic response, defined as a negative HCV RNA 12 weeks after LT. Those with an undetectable HCV RNA on treatment for >30 days prior to LT had a low risk of viral relapse and recurrent HCV infection in the graft [239]. A contemporaneously reported retrospective database study showed improved posttransplant survival in recipients with a listing diagnosis of hepatitis C who were HCV RNA negative at the time of transplantation [240]. Another study showed that there has been improvement in posttransplant survival in the DAA era compared to the pre-DAA era attributable to DAA-associated SVR, whether attained on the wait list or after transplantation

[241]. Even in the absence of liver transplantation, the attainment of SVR in patients with decompensated cirrhosis may improve liver function and, in some cases, reduce portal hypertension [242–247].

The SOLAR program evaluated LDV/SOF and RBV for 12–24 weeks in patients with HCV genotypes 1 and 4 infection (mostly genotype 1) and decompensated cirrhosis. In the US SOLAR-1 trial, SVR rates of 87% were achieved after 12 weeks of treatment and 89% after 24 weeks in patients who had not undergone transplantation, with similar response rates in patients with Child–Pugh B or C [242]. There was improvement in synthetic liver function in the majority of patients and subsequent increases in both MELD and CTP scores. The international SOLAR-2 trial investigated the same regimens in similar cohorts. In GT1 non-transplanted patients with decompensated cirrhosis, SVR was achieved in 87% and 96% of the Child–Pugh B patients and 85% and 78% of the CP B patients treated for 12 and 24 weeks, respectively [243].

Neither SOLAR-1 nor SOLAR-2 evaluated ribavirin-free therapy in patients with decompensated cirrhosis. The ASTRAL-4 phase 3 study filled this gap by evaluating SOF/VEL with and without RBV for 12 weeks or without ribavirin for 24 weeks, in previously treated and untreated patients with HCV genotypes 1–6 and decompensated cirrhosis. Overall rates of SVR12 were 83% in those receiving 12 weeks of the FDC, 94% in those receiving 12 weeks of FDC plus RBV, and 86% in those receiving 24 weeks of FDC without RBV. The difference between 12 weeks of SOF/VEL and ribavirin and 24 weeks of SOF/VEL was relatively small in HCV GT1 but much larger in HCV GT3, with SVR rates of 86% and 50%, respectively [244].

12.2 Post-liver Transplant

Although SVR was sometimes attainable with interferon-based therapy in post-liver patients, with greater frequency after the protease inhibitors were introduced, toxicity was a major problem. The introduction of interferon-free DAA therapy radically transformed the therapeutic landscape for posttransplant patients. Dramatic evidence for this came from a study early in the DAA era demonstrating sometimes striking clinical improvement with sofosbuvir and ribavirin in a group of posttransplant patients with decompensated cirrhosis [248]. In posttransplant patients without cirrhosis in SOLAR-1, SVR was attained in 96% and 98% with 12 or 24 weeks of treatment. Child–Pugh A patients had similar rates of SVR, but there were lower response rates in Child–Pugh B and C patients: 85–88% and 60–75% in those with CTP B and C cirrhosis, respectively [242]. In the cohort of GT1 transplanted patients in SOLAR-2, 93–100% achieved SVR whether noncirrhotic or cirrhotic with Child–Pugh scores of A or B, regardless of duration of treatment. All five patients with fibrosing cholestatic hepatitis had SVR [243]. The use of ritonavir-boosted paritaprevir/r, ombitasvir, and dasabuvir achieved an SVR rate of 97% in noncirrhotic patients with recurrent HCV GT1 infection [249]. Daclatasvir and sofosbuvir also showed high levels of efficacy in both decompensated hepatitis C

cirrhosis and patients with post-liver transplantation HCV infection recurrence [218, 250]. The MAGELLAN-2 trial evaluated the safety and efficacy of GLE/PIB in liver or renal transplant adults with chronic hepatitis C genotype 1–6 infection. GLE/PIB in liver- or kidney-transplanted patients for 12 weeks achieved 99% SVR, and the treatment was tolerated well [251], thereby earning this newest regimen a firm place in the therapeutic armamentarium for post-liver transplant HC-infected patients.

Other published "real-world" studies have similarly shown high rates of SVR in patients post-liver transplantation [252]. As a result, many centers have adopted a policy of withholding antiviral therapy until after transplantation, lest viral eradication in an advanced decompensated cirrhotic delay transplantation by blunting the progression of the MELD score and/or precluding access to an HCV-positive organ [253]. This approach is most often adopted in patients with MELD scores of over 20 or those with CTP C [254]. For all regimens used after liver transplantation or, increasingly, after transplantation of other HCV-positive organs to facilitate access to organ transplant (see below), attention must be paid to potential drug–drug interactions, which have been extensively studied and for which specific information is available in the package inserts.

12.3 Renal Failure

Patients with HCV and chronic kidney disease have historically not had good treatment options. Ribavirin is associated with a high incidence of hemolytic anemia because of drug accumulation in these patients. Interferon-based antiviral therapy was highly problematic in patients after renal transplantation because of the risk of graft rejection with interferon. In the era of DAA therapy, the potential use of SOF in the renal failure population has been considered potentially problematic because of the up to 20-fold accumulation of the major metabolite of SOF, which undergoes renal excretion. Although such toxicity has not been recognized in several case series, the use of SOF in this population has not been recommended.

As a result of the restrictions on SOF use in this population, two major trials were performed with nucleotide-free therapy that changed the paradigm for these patients. The C-SURFER trial was a phase 3 randomized study of safety and observational study of efficacy; patients with HCV genotype 1 infection and chronic kidney disease (stage 4–5 with or without hemodialysis dependence) were randomly assigned to receive GZR and EBR or placebo once daily for 12 weeks. SVR12 in the combined immediate treatment group by per protocol analysis, leaving out a small number of nonvirologic failures, was 99% [255]. The subsequent EXPEDITION-4 study of GLE/PIB, including over 100 treated patients, demonstrated a similarly high SVR rate of 98%, with the only two failures representing nonvirologic failure [256].

The "other side of the coin" in patients with renal failure historically has been the difficulty in treating these patients after kidney transplantation because of the high risk of interferon-induced rejection of the graft. As a result, patients had to be treated pretransplant, but this led to patients being deprived of the opportunity to receive an

HCV-positive kidney, waiting times for which have been significantly shorter in many geographic areas than waiting times for HCV-negative organs. This changed dramatically with a phase 2, open-label clinical trial that evaluated the safety and efficacy of the daily fixed-dose combination of LDV/SOF in 114 kidney transplant recipients who were more than 6 months posttransplant enrolled patients that had genotype 1 (91%) or 4 infection; 69% were treatment-naïve and 15% had compensated cirrhosis. Patients were randomized to 12 weeks or 24 weeks of LDV/SOF. Median eGFR prior to treatment was 50 mL/min for patients in the 12-week study arm and 60 mL/min for those in the 24-week arm. Overall SVR12 was 100% excluding nonvirologic failures. Adverse events were common (64%), and serious adverse events occurred in 11% of the patients. Four patients with an eGFR >40 mL/min at baseline experienced a decrease to 30 mL/min at the last visit recorded; one patient who had interrupted study treatment had a final value of 14.4 mL/min. All but one of the six patients with compensated cirrhosis whose eGFR decreased to <40 mL/min continued study treatment without interruption [257].

Given the simplification of HCV treatment in the last few years and the efficacy of the new regimens, a major paradigm shift has occurred in end-stage renal disease patients as a result of the prolonged kidney transplant wait times for HCV-negative organs in some parts of the United States. In 2017, Goldberg et al. reported the THINKER pilot trial evaluating the safety and efficacy of transplantation of the kidneys from HCV genotype 1-viremic donors into HCV-negative patients, followed by 12 weeks of elbasvir–grazoprevir upon the appearance of viremia soon after transplantation. All ten recipients achieved SVR 12 [258]. An additional ten GT1 patients were subsequently treated successfully by the same group. Nineteen of the 20 patients in total had detectable HCV RNA at days 2–4 postoperatively and the remaining patient on day 5. Seventeen of the patients received 12 weeks of treatment, while 3 received 16 weeks including ribavirin because of baseline NS5A RASs [259]. Another group treated ten patients with one dose of elbasvir–grazoprevir pretransplant and 12 weeks of follow-up therapy (GT1) with sofosbuvir added for patients with GT2 and 3, again with 100% SVR [260]. Based on these and other emerging studies, transplant centers around the United States are offering HCV-infected kidney organs to HCV-negative recipients in hopes to decrease the waiting times for transplantation and time on dialysis in most cases. Recently, this concept has been extended to other transplants, including liver, cardiac, and lung transplantation [261–263].

12.4 HIV Coinfection

Coinfection with HIV-1 and hepatitis C virus (HCV) appears to accelerate the course of HCV-associated liver disease [264]. Historically, as discussed earlier HIV-/HCV-coinfected patients did not respond as well to interferon-based therapy compared to HCV infection alone. This discordance in ability to respond faded in the interferon-free DAA era. Nearly all the development programs for the current DAA regimens

included separate trials dedicated to HIV-/HCV-coinfected patients, although occasional trials included HIV-infected subjects within the larger study population [233].

The ION-4 open-label study involved patients coinfected with HIV-1 and genotype 1 or 4 HCV receiving an antiretroviral regimen of tenofovir and emtricitabine with efavirenz, rilpivirine, or raltegravir. All patients received LDV/SOF as a single fixed-dose combination for 12 weeks. Overall, 96% had a sustained virologic response at 12 weeks after the end of therapy, including rates of 96% in patients with HCV genotype 1a, 96% in those with HCV genotype 1b, and 100% in those with HCV GT4. Rates of sustained virologic response were similar regardless of previous treatment or the presence of cirrhosis [265]. However, black race and the TT allele at the IL28B locus were associated with virologic relapse, one of the few DAA studies with a signal of such an impact, and, with only 12 weeks having been studied, likely contributing to the stipulation in the AASLD Guidance that black patients and those with HIV coinfection should not receive 8 weeks of LDV/SOF [206].

The C-EDGE CO-INFECTION study assessed the efficacy, safety, and tolerability of GZR/EBR in patients with HCV and HIV coinfection. In this phase 3, open-label, single-arm study, treatment-naïve patients with chronic HCV genotype 1, 4, or 6 infection and HIV coinfection, with or without cirrhosis, were enrolled from 37 centers in nine countries across Europe, the United States, and Australia. Patients were either naïve to treatment with any antiretroviral therapy (ART) or stable on ART for at least 8 weeks. All patients received EBR/GZR in a fixed-dose combination tablet once daily for 12 weeks. SVR12 was achieved in 96% of patients. All patients with cirrhosis achieved SVR12 [266].

The ASTRAL-5 study evaluated SOF/VEL for 12 weeks in a cohort of 106 patients with HIV-HCV coinfection across genotypes 1–4. SVR12 was attained in 101/106 (95%), including 19 of 19 patients with cirrhosis. Three of the five subjects who failed to attain SVR were nonvirologic failures [267].

The EXPEDITION-2 trial, evaluated an 8-week regimen of GLE/PIB for people with both HIV and hepatitis C. About two-thirds of the patients had HCV genotype 1 (mostly with harder-to-treat subtype 1a), followed by genotypes 3 (17%) and 4 (11%); only a small number had genotypes 2 or 6. Sixteen patients (10%) had cirrhosis. Study participants had well-controlled HIV infection with a median CD4 count of nearly 600 cells/mm^3. Participants without cirrhosis received GLE/PIB for 8 weeks, while those with cirrhosis were treated for 12 weeks. Ninety-eight percent of participants achieved SVR 12 and 99%, with no virologic failures, for those without cirrhosis who were treated for 8 weeks [268].

The rates of SVR after treatment have been in line with HCV-monoinfected patients, thus resulting in harmonization of treatment recommendations of regimens for HCV-monoinfected and HIV-/HCV-coinfected patients [269]. However, one consideration in treating these patients with DAAs is potential drug–drug interaction with HIV antiretrovirals. The clinical trial development programs involved investigation of the potential interactions between HCV DAAs and HIV antiretrovirals. Careful consideration to avoid such drug–drug interactions in this population has to be given when choosing regimens, and modification of the antiretroviral regimen may be required.

12.5 DAA Failures

Combination regimens of direct-acting antiviral agents (DAAs) provide rates of sustained virologic response exceeding 90%, regardless of HCV genotype, disease stage, or treatment history. Treatment options for patients who failed previous DAA-containing regimens, particularly those with nonstructural protein 5A inhibitors, had been limited, with no FDA-approved regimens for this populations until mid-2017. This changed with the advent of the two pangenotypic regimens SOF/VEL/VOX and GLE/PIB.

Two phase 3 trials evaluated patients who had been previously treated with a DAA-containing regimen. In POLARIS-1, patients with HCV genotype 1 infection who had previously received a regimen containing an NS5A inhibitor were randomly assigned in a 1:1 ratio to receive SOF/VEL/VOX ($n = 150$) or matching placebo ($n = 150$) once daily for 12 weeks. Patients who were infected with HCV of other genotypes (114 patients) were enrolled in the SOF/VEL/VOX group. In POLARIS-4, patients with HCV genotype 1, 2, or 3 infection who had previously received a DAA regimen without an NS5A inhibitor were randomly assigned in a 1:1 ratio to receive SOF/VEL/VOX ($n = 163$) or SOF/VEL ($n = 151$) for 12 weeks. An additional 19 patients with HCV genotype 4 infection were enrolled in the SOF/VEL/VOX group. In POLARIS-1, the rate of sustained virologic response was 96% with SOF/VEL/VOX, as compared with 0% with placebo. Baseline RASs did not appear to affect response. In POLARIS-4, the rate of response was 98% with SOF/VEL/VOX and 90% with SOF/VEL. The overall rate of SVR in the SOF/VEL group was driven down by patients with GT1a and GT3, where there was no clear advantage over SOF/VEL alone [270]. Accordingly, SOF/VEL/VOX became approved in the United States for patients with GT1-6 who have failed a regimen with an NS5A inhibitor and for GT1b, 2, 4, 5, and 6 if the patient failed sofosbuvir without an NS5A inhibitor.

A phase 2, open-label study (MAGELLAN-1) evaluated the efficacy and safety lower dose GLE/PIB without RBV ($n = 6$), higher dose GLE/PIB plus RBV ($n = 22$), or higher-dose GLE/PIB without RBV ($n = 22$). By intent-to-treat analysis, sustained virologic response at posttreatment week 12 was achieved in 100% (6/6, 95% confidence interval 61–100), 95% (21/22, 95% confidence interval 78–99), and 86% (19/22, 95% confidence interval 67–95) of patients in arms A, B, and C, respectively [271]. There were 0, 1 and 1 virologic failures, respectively.

In the MAGELLAN-1 part 2 study, GLE/PIB was given to patients with HCV genotype 1 or 4 and prior DAA treatment failure for 12 or 16 weeks. In this study patients with prior failure to PI-containing regimens (NS5A inhibitor naïve) had an SVR of 100% with both 12 and 16 weeks of GLE/PIB. In patients with prior failure to NS5A inhibitors but NS3/4A PI-naïve there was a 94% SVR 12 rate with 16 weeks of GLE/PIB and slightly lower with 12 weeks. SVR rates were lower in patients with prior exposure to both PI's and NS5A inhibitors, leading to FDA approval of the G/P regimen only for genotype 1 patients with prior exposure to NS5A inhibitors (16 weeks) or PI inhibitors (12 weeks) alone, but not both [272].

13 Conclusion

The development of HCV therapy ranks among the great achievements of medicine in the era spanning the close of the twentieth century and the opening of the twenty-first century. The conceptual framework for the development of direct-acting antiviral therapy was provided by the advances in treatment of HIV that occurred in the last decade of the millennium, with vital contributions from the fields of virology and medicinal chemistry. The lack of genomic archiving for HCV has made it possible to cure, rather than suppress, a human viral infection for the first time. We now have treatment that is almost universally capable of effecting virologic cure across viral genotypes, and we have salvage therapy that can cure most of the few who fail an initial course of treatment. It is even possible that our salvage regimens can be used, for a longer duration or with ribavirin, to cure the approximately 0.1% of patients who fail repeated courses of therapy, including one of the currently approved salvage regimens, despite being compliant with treatment, or that elements from different regimens can be combined to accomplish the same goal.

The extraordinary success in treating HCV infection has been richly complemented by a large and growing body of literature, dating back to the interferon era and being amplified in the DAA era, demonstrating improved clinical outcomes following virologic cure. Not only does cure prevent the progression of hepatic fibrosis and decompensation [273–278] but, as in other liver diseases in which the offending agent or pathologic process has been suppressed or treated, regression of fibrosis or even cirrhosis can ensue, as can reduction in portal hypertension [274, 279, 280]. Overwhelming evidence indicates that the risk of hepatocellular carcinoma in patients with advanced fibrosis or cirrhosis is markedly reduced, although not to the point of obviating the need for ongoing screening [276, 281–284]. Patients who have been cured virologically have higher rates of overall survival [241, 275, 276], as well as improved outcomes after transplantation [285–287]. Extrahepatic conditions associated with HCV infection can also be ameliorated or prevented, such as de novo diabetes [288–290], cryoglobulinemia [291], non-Hodgkin's lymphoma [292–294], and renal and cardiovascular or cerebrovascular disease [295]. Improvement in patient-reported outcomes and health-related quality of life has been well documented [296, 297].

With the advent of the recent pangenotypic regimens, a high bar has been set for further development of antiviral regimens. It remains possible that we will see the development of novel regimens that will be capable of curing patients with a shorter duration of therapy requiring only one prescription, or even, perhaps, with the parenteral administration of a drug with established or novel mechanisms of action, with or without a short oral course of agents in the existing classes. For the most part, however, the focus on hepatitis C has shifted toward the realm of social science and public health policy, with identification of infected people and affordable access to treatment dominating the landscape on an international scale.

Compliance with Ethical Standards

Conflict of Interest: Ira M. Jacobson has received research grants from Assembly, BMS, Gilead, Janssen, Enanta, Merck, and Genfit. He has received a honorarium from Novo Nordisk, Siemens, Gilead, Springbank, Janssen, AbbVie, and Intercept for consulting.

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The Clinical Development of Ledipasvir/Sofosbuvir (LDV/SOF, Harvoni®)

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Abstract The fixed-dose combination tablet of ledipasvir (LDV), an HCV NS5A inhibitor, and sofosbuvir (SOF), an HCV nucleotide analog NS5B polymerase inhibitor, was the first all-oral (one-pill once daily), interferon-free and ribavirin-free regimen approved for the treatment of patients with chronic hepatitis C. With over 5,900 HCV-infected patients enrolled in LDV/SOF clinical trials through late 2017, the accelerated clinical development program was able to generate safety and efficacy data across a broad range of patient populations. The initial registration trials demonstrated that 12 weeks of treatment with LDV/SOF resulted in high cure rates of over 95% in HCV genotype 1 patients regardless of historical negative treatment predictors including cirrhosis or prior treatment history. The program subsequently expanded to include other HCV genotypes and special populations with significant unmet medical need including but not limited to decompensated liver disease, HIV/HCV coinfection, posttransplantation, and children. With favorable pharmacokinetic properties, good safety profile, and high efficacy rates, the approval of LDV/SOF (Harvoni®) ushered in a new era of treatment and management for the millions of HCV-infected patients globally.

Keywords Direct-acting antivirals, HCV genotype 1 infection, Hepatitis C virus, NS5A inhibitors, NS5B nucleotide inhibitors

Abbreviations

DAAs	Direct-acting antivirals
FDC	Fixed-dose combination
HCV	Hepatitis C virus
HIV-1	Human immunodeficiency virus type 1
IFN	Interferon
LDV	Ledipasvir
Peg-IFN	Pegylated interferon
RBV	Ribavirin
SOF	Sofosbuvir
SVR	Sustained virologic response

1 Introduction

Globally, chronic hepatitis C virus (HCV) infection remains a significant public health challenge, with over 80 million persons infected [1]. In 2011, the first class of direct-acting antivirals (DAAs), namely, HCV nonstructural protein (NS) 3/4A protease inhibitors, was approved [2] . While these treatments were successful in up to 75% in specific populations such as treatment-naive patients, these regimens were less effective in treatment-experienced patients, especially in those with

advanced liver disease [3]. In addition, these treatments were given in combination with pegylated interferon (Peg-IFN) and ribavirin (RBV) for up to 48 weeks and were associated with additional serious side effects including anemia, rash, serious infection, decompensation, high discontinuation rates, and complicated response-guided therapy algorithms with high pill burdens. It was estimated that up to 50% of patients with HCV infection were not eligible for these treatments due to relative or absolute contraindications to Peg-IFN [4].

There remained a significant unmet medical need for simplified treatment regimens that were more effective with improved safety and tolerability profiles. As such, the goal of the LDV/SOF clinical development program was to develop an IFN-free, RBV-free all-oral regimen for the treatment of chronic HCV infection by combining two potent DAAs, ledipasvir (LDV), an NS5A inhibitor, and sofosbuvir (SOF), a pangenotypic nucleotide NS5B polymerase inhibitor, into a fixed-dose combination (FDC) tablet. This regimen would obviate toxicity, tolerability, as well as contraindications, associated with Peg-IFN and/or RBV which were components of approved therapy.

The clinical development program was initially focused on genotype 1 HCV infection which represents the majority of all cases of chronic HCV infection in the United States and Europe [5]; however, the program was expanded rapidly to include all HCV genotypes and distinct patient populations across the globe with a significant unmet medical need.

2 Clinical Pharmacology of Sofosbuvir, Ledipasvir, and Ledipasvir/Sofosbuvir

Clinical pharmacology studies were conducted with either LDV/SOF or the components, SOF and LDV, as individual agents, alone or in combination, including coadministration with other DAAs. The SOF and LDV clinical development programs run in parallel with the development of the LDV/SOF fixed-dose combination (FDC) tablet prior to initiation of the LDV/SOF phase 3 clinical studies.

2.1 Clinical Pharmacology of Sofosbuvir

The pharmacokinetic (PK) properties of sofosbuvir (SOF) were evaluated extensively in healthy adult subjects and in patients with chronic hepatitis C infection [6, 7]. Following oral administration, SOF is absorbed quickly, and peak plasma concentration is observed ~0.5–2 h post-dose, regardless of dose level. SOF is extensively metabolized in the liver to form the pharmacologically active nucleoside analog triphosphate GS-461203 which undergoes subsequent dephosphorylation to

yield GS-331007, the pharmacologically inactive, primary circulating nucleoside metabolite responsible for over 85% of systemic drug exposure. Peak plasma concentration of GS-331007 is observed between 2 and 4 h post-dose. The terminal half-life is 0.4 h for SOF and 27 h for GS-331007 which supports once-daily dosing. Consumption of a moderate- or high-fat meal increases SOF AUC by 2-fold and C_{max} by 1.3-fold, while the exposure of GS-331007 is not altered. These observed increases in SOF levels are not considered clinically meaningful, and SOF can be administered without regard to food [8]. Sofosbuvir is approximately 61–65% bound to human plasma proteins, while GS-331007 has minimal binding.

Population pharmacokinetic analysis in HCV-infected patients demonstrated that race, gender, age, and baseline body mass index (BMI) had no clinically relevant effect on the exposure of SOF or GS-331007. Due to the hepatic metabolism of SOF and concerns around the use of DAAs with hepatic impairment, a PK study was conducted in HCV-infected patients with hepatic impairment. This study showed C_{max} and AUC values of SOF were ~80% and 130% higher in cirrhotic compared to non-cirrhotic patients; however, these differences were not considered clinically significant. As such, no dose adjustment of sofosbuvir is recommended for patients with mild, moderate, and severe hepatic impairment [9]. Renal clearance is the major elimination pathway for GS-331007, and a PK study was conducted in HCV-negative subjects with renal impairment. This study showed that the AUC of GS-331007 was increased 55%, 88%, and 451% in subjects with mild, moderate, and severe renal impairment, respectively. Based on these findings, dose adjustment for patients with mild or moderate renal impairment is not required; however, sofosbuvir is currently not approved for use in patients with severe renal impairment [10, 11]. There are ongoing studies evaluating the safety and efficacy of LDV/SOF in HCV-infected patients with end-stage renal disease on dialysis.

SOF has limited clinically relevant drug-drug interactions as both SOF and GS-331007 are not substrates, inducers, or inhibitors of drug-metabolizing enzymes such as cytochrome P450 (CYP) or UDP-glucuronosyltransferase (UGT) A1 enzymes. In PK studies, coadministration of SOF with several drugs that impact CYP enzymes such as contraceptives, methadone, immunosuppressants, and antiretrovirals have not been shown to have clinically relevant effects on PK parameters. However, SOF is a substrate of drug transporters P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), while GS-331007 is not; therefore, inhibitors or inducers of these transporters may alter plasma concentrations of SOF. For example, coadministration with P-gp inducers such as rifampin and St John's wort can decrease SOF concentration affecting efficacy and should be avoided [12, 13].

2.2 Clinical Pharmacology of Ledipasvir/Sofosbuvir

2.2.1 Clinical Pharmacology of LDV/SOF in Adults

Pharmacokinetic properties of ledipasvir, sofosbuvir in combination, and GS-331007 were studied in healthy adult subjects and patients with chronic hepatitis

C infection [14]. While AUC and C_{max} of SOF and GS-331007 were similar in healthy subjects and those with chronic HCV infection, AUC and C_{max} of LDV were 24% and 32% lower, respectively, in HCV-infected patients. Following oral administration of LDV/SOF, median peak concentration is observed 4–4.5 h post-dose. LDV concentrations are not affected by food supporting the recommendation that LDV/SOF can be administered without regard to food. Ledipasvir is minimally metabolized by the liver, highly protein-bound (more than 99%), and is primarily eliminated in the feces as an unchanged drug through biliary excretion. Studies conducted in subjects with renal insufficiency or hepatic impairment demonstrated that no dose adjustment is required in patients with end-stage renal disease or those with severe hepatic impairment. Population pharmacokinetic analysis in HCV-infected patients indicated that race, gender, and age had no clinically relevant effect on the exposure of LDV, similar to observations with SOF.

Ledipasvir is not a substrate, inducers, or inhibitors of traditional drug-metabolizing enzymes, e.g., CYP- or UGT1A1-mediated drug-drug interactions. In vitro, LDV is a substrate and an inhibitor of P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) and as such may alter the absorption of substrates of these transporters. Several drug interaction studies were conducted with LDV/SOF to assess for clinically meaningful drug interactions. Potent inducers of P-gp such as rifampin, St. John's wort, and carbamazepine will reduce plasma concentrations of SOF and/or LDV and should be avoided. Immunosuppressants such as cyclosporine and tacrolimus, opiate substitution therapy, and oral contraceptives can be safely coadministered with LDV/SOF. Tipranavir, an antiretroviral can lead to reduced SOF and LDV levels and should be avoided with LDV/SOF coadministration. Rosuvastatin exposure can increase with LDV/SOF coadministration, potentially increasing the risk of rhabdomyolysis, and is contraindicated [14].

Ledipasvir solubility decreases as pH increases; therefore, medications that increase gastric pH may result in a decreased concentration of LDV. This effect can be managed with specific dosing instructions: antacid dosing should be separated by at least 4 h from LDV/SOF; H_2-receptor antagonists should be given simultaneously or staggered apart from LDV/SOF at a dose no higher than famotidine 40 mg twice daily or equivalent; and proton-pump inhibitors at doses comparable to omeprazole 20 mg or lower can be administered simultaneously.

2.2.2 Clinical Pharmacology of LDV/SOF in Adolescent Patients

The pharmacokinetic properties of SOF, GS-331007, and LDV were evaluated in adolescent patients (12 to <18 years of age) in study GS-US-337-1116 (Group 1), who received the adult dose of LDV/SOF (90/400 mg) [15]. No clinically relevant differences in the exposure of SOF, GS-331007, and LDV were observed in the adolescent population compared with adult patients in the phase 2 and 3 studies, confirming the appropriateness of LDV/SOF (90/400 mg) for use in adolescents ages 12 to <18 years.

3 Dose Selection of Sofosbuvir, Ledipasvir, and Ledipasvir/Sofosbuvir

3.1 Dose Selection of Sofosbuvir

Data from dose-ranging phase 1b and phase 2 studies of SOF conducted within the SOF development program as either monotherapy or combination therapy with Peg-IFN and RBV revealed exposure-response relationships that supported the dose selection of SOF 400 mg for the treatment of HCV infection. The phase 1b study P7851-1102 assessed once-daily doses GS-9851 from 50 to 400 mg (also known as PSI-7851) 50:50 diasteromeric mixture of SOF and GS-491241 (also known as PSI-7976) (*an HCV RNA NS5B inhibitor*) administered for 3 consecutive days to treatment-naive patients with chronic genotype 1 HCV infection. When GS-9851 enters into the liver cell, both GS-491241 and SOF molecules rapidly get converted into the same active triphosphate. Mean maximal decreases from baseline in HCV RNA were 0.09, 0.49, 0.56, 1.15, and 1.95 $\log_{10}$ IU/mL for placebo and GS-9851 50, 100, 200, and 400 mg doses, respectively [13]. The GS-9851 400 mg dose had the earliest and most potent antiviral effect in the greatest percentage of patients, with the majority having a continued reduction in HCV RNA ($\geq$1.0 $\log_{10}$) 2 days after the last dose of GS-9851 (Fig. 1).

Subsequent data from two phase 2 dose-finding studies P7977-0221 and P7977-0422 (*PROTON*) confirmed the selection of the SOF 400 mg dose. In study P7977-0221, a total of 64 treatment-naive patients with genotype 1 HCV infection were randomized to receive SOF doses of 100, 200, or 400 mg or matching placebo for 27 days in combination with Peg-IFN + RBV for 48 weeks [16]. From days 0 to 27 (SOF/placebo treatment period), mean HCV RNA levels rapidly declined with all

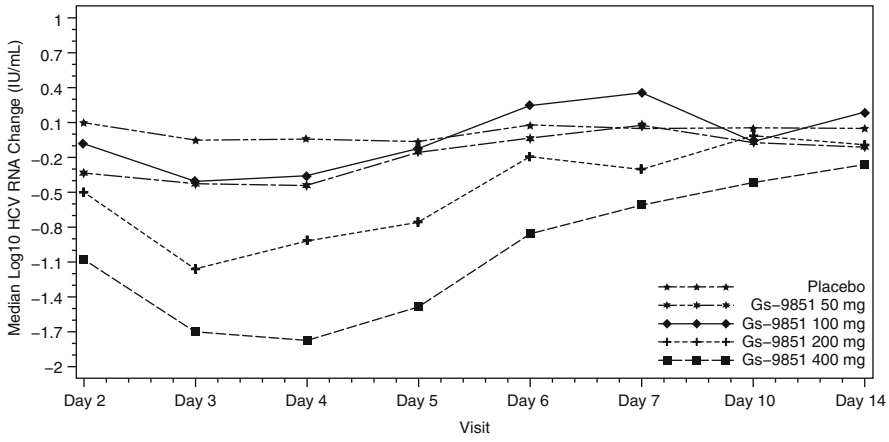

Fig. 1 Median $\log_{10}$ HCV RNA change from baseline following multiple doses of GS-9851: phase 1 study P7851-1102. GS-9851 (also known as PSI-7851) is a mixture of two phosphate diastereoisomers (GS-491241 (also known as PSI-7976) and SOF)

doses of SOF with Peg-IFN + RBV compared with placebo given with Peg-IFN + RBV. The sustained virologic response 12 weeks after treatment completion (SVR12) rates were higher in the SOF 200 mg and 400 mg groups (72% and 80%, respectively) compared to the SOF 100 mg and placebo groups (56% and 50%, respectively). Based on the lower rates of virologic failure, SOF 200 and 400 mg were the therapeutic doses selected for further evaluation in the phase 2 study P7977-0422 (*PROTON*).

In study P7977-0422, 122 treatment-naive patients with genotype 1 HCV infection were randomized to receive SOF 200 mg, SOF 400 mg, or matching placebo once daily with Peg-IFN + RBV for 12 weeks [17]. In addition, 25 treatment-naive genotype 2 or 3 HCV-infected patients received open-label SOF 400 mg once daily in combination with Peg-IFN + RBV for 12 weeks. Sofosbuvir 200 and 400 mg in combination with Peg-IFN + RBV for 12 weeks led to high SVR12 rates of 90–92% in patients with genotype 1, 2, or 3 HCV infection. On-treatment failures occurred in the SOF 200 mg group but not in the SOF 400 mg group.

In both phase 2 studies, all doses of SOF were well tolerated. The adverse event and laboratory profiles were similar for Peg-IFN + RBV, SOF 200 mg with Peg-IFN + RBV, and SOF 400 mg with Peg-IFN + RBV, consistent with the expected safety profile of Peg-IFN and RBV. There were no new adverse events or laboratory abnormalities attributable to SOF. Based on the totality of the safety, pharmacokinetic, and antiviral activity, the SOF 400 mg dose was selected to move forward for phase 3 evaluation in the SOF clinical development program and in combination with LDV in phase 2 trials in the LDV clinical development program.

3.2 LDV/SOF Dose Selection

The phase 1 study GS-US-256-0102 assessed once-daily doses of LDV from 1 to 90 mg administered for 3 consecutive days in treatment-naive patients with chronic genotype 1 HCV infection [18]. A dose-dependent response was observed for LDV doses of 3 mg through 30 mg. Ledipasvir resulted in rapid reductions in plasma HCV RNA of $\geq$2 $\log_{10}$ IU/mL as early as 8 h and reductions $>$3 $\log_{10}$ IU/mL on day 2 (36 h) following administration of 3 through 90 mg (Fig. 2). Similar and maximal antiviral responses (median approximately 3 log reduction) were observed following LDV doses of 10, 30, or 90 mg. Maximum effect (E_{max}) modeling indicated that exposures achieved following administration of LDV doses $\geq$30 mg provided $>$95% of maximal antiviral responses in genotype 1a HCV-infected patients. There was no evidence of additional antiviral activity at the 90 mg dose based on median reductions in HCV RNA; however, HCV RNA suppression was sustained for a longer period compared with the 30 mg dose. Based on these data, LDV 30 mg and 90 mg once-daily doses were selected for clinical evaluation in phase 2 trials.

Study GS-US-248-0120 was a phase 2, dose-finding trial that evaluated the safety, tolerability, and antiviral efficacy of LDV 30 mg or 90 mg, administered in combination with the NS3 protease inhibitor vedoprevir, non-nucleoside NS5B

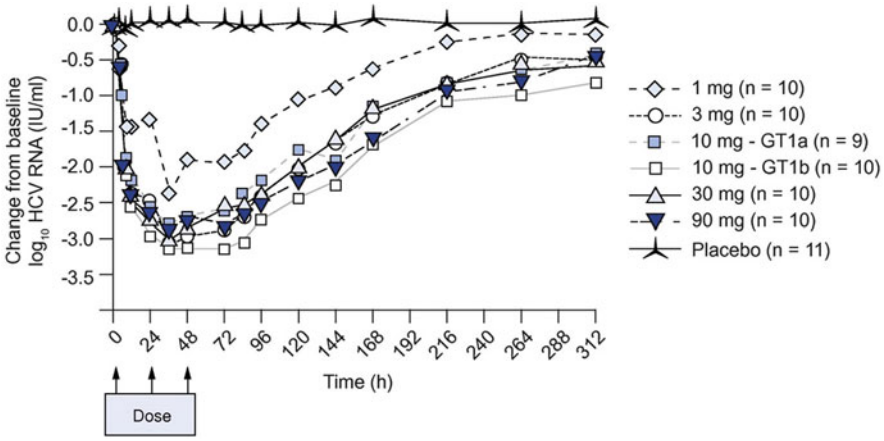

Fig. 2 Median log $_{10}$ HCV RNA change from baseline following administration of LDV (phase 1 study GS-US-256-0102). Patients received LDV or placebo once daily for 3 days. Arrows indicate time of dosing

inhibitor tegobuvir, and RBV in 234 treatment-naive patients with chronic genotype 1 HCV infection. Treatment with LDV 90 mg in combination with other DAAs and RBV for 12 or 24 weeks resulted in higher SVR24 rates compared to LDV 30 mg in combination with DAAs and RBV (58.5% vs 47.8%). In addition, the incidence of virologic breakthrough was lower in the LDV 90 mg group compared to the LDV 30 mg group (10.6% vs 19.6%, respectively).

Treatment with LDV in combination with other DAAs and RBV was generally well tolerated, and increasing the LDV dose did not alter the safety profile of the regimens in terms of overall frequency or severity of AEs or laboratory abnormalities. Based on efficacy and the favorable safety and tolerability profile, the 90 mg dose of LDV was selected for coformulation in the LDV/SOF fixed-dose combination.

4 Safety and Efficacy of Ledipasvir/Sofosbuvir in Phase 2 Trials

The LDV/SOF clinical development program was initiated once phase 2 clinical data became available showing that SOF in combination with RBV ± Peg-IFN resulted in high efficacy across all HCV genotypes and were well tolerated as compared to available standard of care [17, 19, 20] (Table 1). The goal of the phase 2 trials in the LDV/SOF clinical development program was to evaluate the combination of SOF

Table 1 Key phase 2 trials in the sofosbuvir clinical development program

Trial	Population (with genotype)	Number of patients	Regimen	Duration (weeks)	SVR12 (%)
PROTON	HCV 1, treatment naive◊	48	SOF + Peg-IFN + RBV	12[a]	90
	HCV 1, treatment naive	47	SOF + Peg-IFN + RBV	12[a]	91
	HCV 1, treatment naive	26	Placebo + Peg-IFN + RBV	48	58
	HCV 2 or 3, treatment naive	25	SOF + Peg-IFN + RBV	12	92
ATOMIC	HCV 1, treatment naive	52	SOF + Peg-IFN + RBV	12	90
	HCV 1, treatment naive	125	SOF + Peg-IFN + RBV	24	93
	HCV 1, 4, or 6, treatment naive	155	SOF + Peg-IFN + RBV	12[b]	91
ELECTRON					
Part 1 (randomized)	HCV 2 or 3 treatment naive	10	SOF + RBV	12	100
	HCV 2 or 3 treatment naive	9	SOF + Peg-IFN[c] + RBV	12	100
	HCV 2 or 3 treatment naive	10	SOF + Peg-IFN[d] + RBV	12	100
	HCV 2 or 3 treatment naive	11	SOF + Peg-IFN + RBV	12	100
Part 2	HCV 2 or 3 treatment naive	10	SOF	12	60
	HCV 2 or 3 treatment naive	11	SOF + Peg-IFN + RBV	8	100
	HCV 1 treatment experienced	10	SOF + RBV	12	10
Part 3	HCV 1, treatment naive	25	SOF + RBV	12	84
	HCV 2 or 3 treatment experienced	25	SOF + RBV	12	68

◊SOF 200 mg

[a]SOF in combination with Peg-IFN and RBV was followed by an additional course of Peg-IFN and RBV for 12 or 36 weeks according to the virological response on treatment

[b]The patients in this arm were further randomized to receive an additional course of SOF alone or SOF plus RBV for 12 weeks

[c]Peg-IFN was administered just for 4 weeks

[d]Peg-IFN was administered just for 8 weeks

400 mg with LDV 90 mg or LDV/SOF (90 mg/400 mg) FDC with or without RBV in a broad population of HCV patients irrespective of treatment history and fibrosis status. Safety, efficacy, and pharmacokinetic data generated from these studies are described below.

4.1 Study P7977-0523 (ELECTRON)

Study P7977-0523 (ELECTRON) was a phase 2 multicenter, open-label trial to evaluate the safety, tolerability, and antiviral efficacy of SOF-containing treatment regimens in HCV patients [21, 22]. This study had multiple arms and was the first to evaluate the use of sofosbuvir in combination with ledipasvir. The latter arms of the study utilized the fixed-dose combination tablet of LDV/SOF. The study was conducted at two sites in New Zealand and commenced enrollment of patients in June 2012; only results relevant to the combination of SOF and LDV or LDV/SOF are described below (Table 2).

Part 4 (Groups 12 and 13) of the study enrolled nine patients with genotype 1 HCV infection who had documented null response following previous treatment with Peg-IFN and RBV for $\geq$12 weeks and 25 treatment-naive patients with genotype 1 HCV infection who received SOF 400 mg once daily + LDV 90 mg once daily and weight-based RBV (1,000–1,200 mg/day divided twice daily) for 12 weeks. The latter arms of the study included Part 6 (Groups 16 and 17) which randomized 19 patients with genotype 1 HCV infection who were prior null responders to Peg-IFN therapy with cirrhosis to receive either LDV/SOF (90 mg/400 mg) once daily or LDV/SOF once daily in combination with weight-based RBV for 12 weeks; Part 16 (Group 18) enrolled ten non-cirrhotic treatment-naive patients with genotype 2 or 3 HCV infection to receive LDV/SOF once daily for 12 weeks; Group 20 enrolled 14 patients with genotype 1 HCV infection and hemophilia to receive LDV/SOF once daily in combination with weight-based RBV for 12 weeks, while Group 21 enrolled 25 treatment-naive patients with genotype 1 HCV infection to receive LDV/SOF once daily in combination with weight-based RBV for 6 weeks.

This study showed that in treatment-naive and null-responder patients with genotype 1 HCV infection, treatment with SOF (400 mg) with LDV (90 mg) and RBV for 12 weeks provided a high virologic response rate with an SVR12 rate of 100% compared with patients who received only SOF + RBV, where 84% and 10% of treatment-naive and null-responder patients, respectively, achieved SVR12. In patients who were null responders with genotype 1 HCV infection and cirrhosis, treatment with LDV/SOF or LDV/SOF + RBV for 12 weeks led to high virologic responses with SVR12 rates of 70% and 100%, respectively. Patients with multiple negative predictors of response such as prior null response and cirrhosis achieved high SVR rates with LDV/SOF with or without RBV. Treatment-naive patients with genotype 1 HCV infection who received LDV/SOF with RBV for 6 weeks had a lower SVR12 rate of 68% compared with 100% in those who received 12 weeks of LDV/SOF with RBV, indicating that 6 weeks of LDV/SOF with RBV was likely to be too short a duration of treatment to achieve an optimal response rate.

The most common reported adverse events were headache, fatigue, and nausea. Most of the adverse events were mild in severity. Overall, five patients experienced severe adverse events; of these, the only severe event considered related to treatment was grade 3 hemolytic anemia, a known side effect of RBV. One patient discontinued treatment after 7 weeks due to an adverse event (spontaneous

Table 2 Efficacy rates of LDV/SOF in study P7977-0523 (ELECTRON)

	GT-1 null responder	GT-1 treatment naive	GT-1 null responder with cirrhosis		GT-2 or GT-3 treatment naive	GT-1 hemophilia	GT-1 treatment naive
	SOF + LDV + RBV 12 weeks ($N = 9$)	SOF + LDV + RBV 12 weeks ($N = 25$)	LDV/SOF 12 weeks ($N = 10$)	LDV/SOF + RBV 12 weeks ($N = 9$)	LDV/SOF 12 weeks ($N = 10$)	LDV/SOF + RBV 12 weeks ($N = 10$)	LDV/SOF 6 weeks ($N = 25$)
SVR12 (n/N)	9/9 (100.0%)	25/25 (100.0%)	7/10 (70.0%)	9/9 (100.0%)	8/10 (80.0%)	14/14 (100.0%)	17/25 (68.0%)
95% CI	66.4–100.0	86.3–100.0	34.8–93.3	66.4–100.0	44.4–97.5	76.8–100.0	46.5–85.1

perforation of a colonic diverticulum, assessed as not related to treatment); this patient went on to achieve SVR. The RBV-free groups had lower rates of adverse events and laboratory abnormalities compared to the RBV-containing groups.

4.2 Study GS-US-337-0118 (LONESTAR)

Study GS-US-337-0118 (LONESTAR) was a phase 2, randomized open-label trial to evaluate LDV/SOF with or without RBV in patients with HCV genotype 1 infection [23]. The study was conducted at a single center in the United States and enrolled patients from Nov 2012 to Dec 2012 (Table 3).

In Cohort A, 60 non-cirrhotic, treatment-naive patients were randomly assigned (1:1:1; stratified by HCV genotype [1a vs 1b]) to receive LDV/SOF once daily for 8 weeks (Group 1), LDV/SOF and weight-based ribavirin for 8 weeks (Group 2), or LDV/SOF for 12 weeks (Group 3). In Cohort B, 40 patients with a history of virological failure after receiving a protease inhibitor regimen were randomly allocated (1:1; stratified by genotype and presence or absence of cirrhosis) to receive LDV/SOF for 12 weeks (Group 4) or LDV/SOF and weight-based ribavirin for 12 weeks (Group 5).

This study showed that 8 or 12 weeks of LDV/SOF with or without RBV in patients with genotype 1 HCV infection (including approximately 50% with compensated cirrhosis) resulted in an overall SVR12 rate of 97%. The SVR12 rate was 100% in treatment-naive patients who received LDV/SOF + RBV for 8 weeks and 95% in patients who received LDV/SOF for 8 or 12 weeks. In treatment-experienced patients, SVR12 was achieved by 100% and 95% of patients who received LDV/SOF with or without RBV for 12 weeks, respectively.

Patients who were receiving LDV/SOF + RBV had the higher rates of adverse events compared to those receiving LDV/SOF. The most common adverse events were nausea, anemia, upper respiratory tract infection, and headache, with most of these events assessed as mild in severity. Anemia was noted only in patients receiving RBV with eight patients requiring RBV dose reductions to manage anemia; all eight achieved SVR12. No patient discontinued treatment because of an adverse event. Four patients had serious adverse events, of which anemia was the only serious adverse event considered related to study treatment. The only

Table 3 Efficacy rates of LDV/SOF in study GS-US-337-0118 (LONESTAR)

	GT-1 treatment naive			GT-1 treatment experienced	
	LDV/SOF 8 weeks ($N = 20$)	LDV/SOF + RBV 8 weeks ($N = 21$)	LDV/SOF 12 weeks ($N = 19$)	LDV/SOF 12 weeks ($N = 19$)	LDV/SOF + RBV 12 weeks ($N = 21$)
SVR12 (n/N)	19/20 (95.0%)	21/21 (100.0%)	18/19 (94.7%)	18/19 (94.7%)	21/21 (100.0%)
95% CI (%)	75.1–99.9	83.9–100.0	74.0–99.9	74.0–99.9	83.9–100.0

Table 4 Efficacy rates of LDV/SOF in study GS-US-337-0122 (ELECTRON-2)

	GT-3 treatment naive		GT-3 treatment experienced
	LDV/SOF 12 weeks ($N = 25$)	LDV/SOF + RBV 12 weeks ($N = 26$)	LDV/SOF + RBV 12 weeks ($N = 50$)
SVR12 (n/N)	16/25 (64.0%)	26/26 (100.0%)	26/26 (100.0%)
95% CI (%)	42.5–82.0	86.8–100.0	86.8–100.0

grade 3 or 4 hematological abnormality that occurred during treatment was decreased hemoglobin in four patients, all of whom had received RBV.

4.3 Study GS-US-337-012 (ELECTRON-2)

Study GS-US-337-0122 was a phase 2 multicenter, open-label trial to evaluate the efficacy and safety of sofosbuvir-containing regimens for the treatment of chronic HCV infection [24]. This multi-cohort study was the first to evaluate the use of LDV/SOF in non-genotype 1 HCV infection (Table 4).

In Cohort 2 (Groups 3 and 4), 51 treatment-naive, HCV genotype 3 patients were randomly assigned to receive either LDV/SOF once daily for 12 weeks (Group 3) or LDV/SOF and weight-based ribavirin for 12 weeks (Group 4). Cohort 2 (Group 5) evaluated the safety and efficacy of LDV/SOF in 25 patients with genotype 6 HCV infection. Additional details in patients with HCV genotype 3 and 6 infection are presented in Sects. 6.2.2 and 6.2.5 respectively.

4.4 Safety of Ledipasvir/Sofosbuvir in Phase 2 Trials

Across the phase 2 clinical trials, treatment with LDV/SOF with or without RBV was safe and well tolerated. Importantly, a higher proportion of patients in the RBV-containing treatment groups had adverse events, treatment-related adverse events, or adverse events leading to dose modification or interruption of any study drug than patients in the RBV-free treatment groups. The groups receiving RBV also had a higher incidence of laboratory abnormalities that were consistent with the expected toxicity profile of ribavirin, namely, decreases in hemoglobin and lymphocytes and increases in total bilirubin.

5 Safety and Efficacy of Ledipasvir/Sofosbuvir in Phase 3 Registrational Trials

The LDV/SOF phase 3 clinical development program was designed to further evaluate the safety and efficacy of LDV/SOF in a diverse population of patients with HCV genotype 1 infection irrespective of baseline and demographic characteristics. In late 2012, there were several ongoing phase 3 studies in the SOF clinical development program; however, the standard of care in patients with HCV genotype 1 infection was still an HCV protease inhibitor (boceprevir or telaprevir) combined with Peg-IFN and RBV for 24–48 weeks [2, 3]. Based on the existing medical need and the safety data generated from over 1,000 patients treated with LDV in combination with other DAAs, the LDV/SOF phase 3 program was initiated. The phase 3 registrational trials had innovative study designs that helped to significantly accelerate the clinical development program. In the LDV/SOF phase 3 program supporting initial registration, three large multicenter studies (two in treatment naive and one in treatment experienced) were conducted and are described below.

5.1 Efficacy of Ledipasvir/Sofosbuvir in Treatment-Naive Genotype 1 Patients

5.1.1 ION-1 (Study GS-US-337-0102)

The ION-1 trial was designed to assess the efficacy and safety of 12 or 24 weeks of the fixed-dose combination of LDV/SOF with or without RBV in previously untreated patients with chronic HCV genotype 1 infection, including those with compensated cirrhosis [25].

This was a multicenter, randomized, open-label trial that enrolled patients at 99 sites in the United States and Europe from October 17, 2012, to May 17, 2013. Eligible patients had chronic HCV genotype 1 infection and had not received treatment for HCV infection previously. All patients received LDV/SOF. Ribavirin was administered orally twice daily, with the dose determined according to body weight (1,000 mg daily in patients with a body weight $<$75 kg and 1,200 mg daily in patients with a body weight $\geq$75 kg). Patients were randomly assigned in a 1:1:1:1 ratio to one of four treatment groups: LDV/SOF for 12 weeks, LDV/SOF plus RBV for 12 weeks, LDV/SOF for 24 weeks, or LDV/SOF plus RBV for 24 weeks. Randomization was stratified according to HCV genotype 1 subtype (1a or 1b) and the presence or absence of cirrhosis.

Subject enrollment occurred in two parts. Part A enrolled and randomized approximately 200 patients (50 per treatment group), and enrollment was halted in all four treatment groups once Part A was fully enrolled. After patients in 12-week treatment groups reached posttreatment Week 4, the data monitoring committee (DMC) reviewed safety and SVR4 efficacy data from the first 12 weeks of dosing for

all patients in the 12-week treatment group. Futility in the 12-week treatment groups was assessed using an interim futility stopping procedure that utilized a conditional power approach under the observed trend. Stopping for futility was triggered when the conditional power was less than 5% (which was equivalent to an observed response rate of 60% or less). If the predefined interim futility criteria were met, the 12-week treatment groups were to be discontinued. As futility criteria were not met, the study was continued as planned. Part B commenced enrollment after this interim futility analysis was complete. Approximately 600 additional patients (approximately 150 per group) were enrolled in Part B.

In the final analysis, a total of 870 patients were randomized, of which 865 received at least 1 dose of study drug. Of the 865 randomized and treated patients, 27 (3.1%) prematurely discontinued study treatment. In general, patients were representative of a treatment-naive population, and demographics and baseline characteristics were generally balanced across the four treatment groups. Overall, 67% of the patients had HCV genotype 1a infection, 12% were black, 70% had the non-CC *IL28B* genotype, and 16% had cirrhosis.

The SVR12 rates observed in all four treatment groups were superior to the historical rate of 60% ($P < 0.001$ for all comparisons) (Table 5). The SVR12 rates were high across all treatment groups (LDV/SOF 12-week group, 99%; LDV/SOF + RBV 12-week group, 97%; LDV/SOF 24-week group, 98%; and LDV/SOF + RBV 24-week group, 99%). Of the 865 patients who were treated, only 3 had virologic failure (1 virologic breakthrough and 2 relapses). The addition of RBV or extending the treatment duration of LDV/SOF from 12 to 24 weeks did not significantly improve the SVR12 rates. High response rates were observed in all patient subgroups, including patients with characteristics historically associated with a poor response to treatment including older age, cirrhosis, high BMI, high HCV RNA levels, and IL-28B non-CC genotype.

Population and deep sequencing of the HCV NS5A and NS5B genes were performed from pretreatment samples and from posttreatment samples from all patients with virologic failure. The prevalence of pretreatment NS5A resistance-associated variants (RAVs) detected with a 1% cutoff was 16% (140/861) overall, of which 135 (96%) achieved SVR12, suggesting that the presence of NS5A RAVs did

Table 5 Virologic outcomes in ION-1 (study GS-US-337-0102)

	LDV/SOF 12 weeks ($N = 214$)	LDV/SOF + RBV 12 weeks ($N = 217$)	LDV/SOF 24 weeks ($N = 217$)	LDV/SOF + RBV 24 weeks ($N = 217$)
SVR12	211 (99)	211 (97)	212 (98)	215 (99)
Virologic failure				
Relapse	1 (<1)	0	1 (<1)	0
On-treatment virologic failure	0	0	1 (<1)	0
Other	4 (2)	6 (3)	3 (1)	2 (1)

Other lost to follow up, withdrew consent

not impact treatment outcome. The two patients that relapsed had preexisting NS5A RAVs at baseline.

The innovative study design and built-in futility analysis of the ION-1 study were crucial in saving considerable amounts of time in the development program and bringing the drug to the market at a much earlier date than initially envisioned.

5.1.2 ION-3 (Study GS-US-337-0109)

The ION-3 trial was conducted primarily to explore the feasibility of shortening treatment duration in previously untreated patients with HCV genotype 1 infection without cirrhosis [26].

This was a multicenter, randomized, open-label trial that enrolled patients at 58 sites in the United States from May 20, 2013, to June 19, 2013. Eligible patients had chronic HCV genotype 1 infection without cirrhosis and had not received treatment for HCV infection previously. All patients received LDV/SOF with or without weight-based RBV (1,000 or 1,200 mg divided twice daily). Patients were randomly assigned in a 1:1:1 ratio to one of three treatment groups: LDV/SOF for 8 weeks, LDV/SOF plus RBV for 8 weeks or LDV/SOF for 12 weeks. Randomization was stratified according to HCV genotype (1a or 1b).

A total of 677 patients were randomized or which 8 (1.3%) prematurely discontinued study treatment. The population was representative of the population of patients with HCV infection in the United States. Demographic and baseline characteristics were generally balanced across the three treatment groups. Overall, 80% had HCV genotype 1a infection, 19% were black, 6% were Hispanic, and 74% had a non-CC *IL28B* genotype.

The SVR12 rates observed in all three treatment groups were superior to the adjusted historical rate of 60% ($P < 0.001$ for all comparisons) (Table 6). The SVR12 rate was 94% with 8 weeks of LDV/SOF, 93% with 8 weeks of LDV/SOF + RBV, and 95% with 12 weeks of LDV/SOF. Importantly, the SVR12 rate in patients who received 8 weeks of LDV/SOF without ribavirin was noninferior to the response rates in the other two treatment groups. This showed that the addition of RBV to the 8-week regimen of LDV/SOF or extending the treatment duration to

Table 6 Virologic outcomes in ION-3 (study GS-US-337-0108)

	LDV/SOF 8 weeks ($N = 215$)	LDV/SOF + RBV 8 weeks ($N = 216$)	LDV/SOF 12 weeks ($N = 216$)
SVR12	202 (94)	201 (93)	206 (95)
Virologic failure			
Relapse	11 (5)	9 (4)	3 (1)
On-treatment virologic failure	0	0	0
Other	2 (1)	6 (3)	7 (3)

Other lost to follow up, withdrew consent

12 weeks in genotype 1, non-cirrhotic patients did not result in improved SVR rates. Furthermore, once again the SVR rates did not vary significantly according to patients' demographic or clinical characteristics, including those historically associated with a poor response to IFN-based treatment.

Population and deep sequencing of the HCV NS5A and NS5B genes were performed from pretreatment samples and from posttreatment samples from all patients with virologic failure.

Overall, 116 of 647 (17.9%) patients were identified as having at least one baseline NS5A RAV with a 1% assay cutoff. Of these, 104 (89.7%) patients with baseline NS5A RAVs achieved SVR12 following treatment. Importantly of the 116 patients with baseline NS5A RAVs, 80 (69%) patients had at least 1 NS5A RAV conferring >100-fold reduced susceptibility to LDV in vitro. Despite the presence of these NS5A RAVs, 69 of these 80 (86.3%) patients achieved SVR12.

5.2 *Efficacy of Ledipasvir/Sofosbuvir in Treatment-Experienced Genotype 1 Patients*

5.2.1 ION-2 (Study GS-US-337-0108)

The ION-2 trial was designed to assess the efficacy and safety of 12 or 24 weeks of LDV/SOF with or without RBV in patients with chronic HCV genotype 1 infection who had been previously treated, including those with compensated cirrhosis [27].

This was a multicenter, randomized, open-label trial that enrolled patients at 64 sites in the United States from January 3, 2013, to February 26, 2013. Eligible patients had chronic HCV genotype 1 infection and had failed prior treatment with either Peg-IFN and RBV or an NS3/4A protease inhibitor combined with Peg-IFN and RBV. All patients received LDV/SOF with or without weight-based RBV (1,000 or 1,200 mg divided twice daily).

Patients were randomly assigned in a 1:1:1:1 ratio to one of four treatment groups: LDV/SOF for 12 weeks, LDV/SOF plus RBV for 12 weeks, LDV/SOF for 24 weeks, or LDV/SOF plus RBV for 24 weeks. Randomization was stratified according to genotype (1a vs 1b), presence or absence of cirrhosis, and response to prior therapy (relapse or virologic breakthrough vs no response.

A total of 441 patients were randomized, of which 440 received at least 1 dose of study drug. In general, patients were representative of a treatment-experienced population: 88% had the non-CC *IL28B* genotype, 52% had received prior treatment with a protease inhibitor regimen, and 20% had cirrhosis. Demographic and baseline characteristics were generally well balanced across the four treatment groups.

The SVR12 rates observed in all four treatment groups were superior to the adjusted historical rate of 25% ($P < 0.001$ for all comparisons) (Table 7). The SVR12 rates were high across all treatment groups (LDV/SOF 12-week group, 93.6%; LDV/SOF + RBV 12-week group, 96.4%; LDV/SOF 24-week group,

Table 7 Virologic outcomes in ION-2 (study GS-US-337-0109)

	LDV/SOF 12 weeks ($N = 109$)	LDV/SOF 12 weeks ($N = 111$)	LDV/SOF 24 weeks ($N = 109$)	LDV/SOF 24 weeks ($N = 111$)
SVR12	102 (94)	107 (96)	108 (99)	110 (99)
Virologic failure				
Relapse	7 (6)	4 (4)	0	0
On-treatment virologic failure	0	0	0	1 (1)
Other	0	0	1 (1)	0

Other lost to follow up, withdrew consent

99.1%; and LDV/SOF + RBV 24-week group, 99.1%). The addition of RBV to or extending the treatment duration of LDV/SOF from 12 to 24 weeks did not appreciably enhance the observed SVR12 rates. High response rates were observed in all patient subgroups, including patients with characteristics historically associated with a poor response to treatment including older age, cirrhosis, high BMI, high HCV RNA levels, and IL-28B non-CC genotype.

A total of 62 of 439 (14.1%) patients with successful NS5A sequencing were identified as having baseline NS5A RAVs. Of these, 54 (87.1%) patients with baseline NS5A RAVs achieved SVR12. Variants associated with resistance to NS3/4A protease inhibitors were detected at baseline in 163 of the 228 patients (71%) who underwent successful sequencing and had received prior treatment with a protease inhibitor regimen. Of these 159 (98%) patients with baseline NS3/4A RAVs achieved SVR12.

5.3 *Safety of Ledipasvir/Sofosbuvir in Phase 3 Registrational Trials*

Treatment with LDV/SOF with or without RBV was safe and well tolerated across the phase 3 program [25–27]. There were no placebo-controlled regimens; however, the safety profile observed was generally similar to that observed in the placebo group of a prior trial with SOF and RBV in HCV-infected patients [28].

The integrated phase 3 safety population of 1,952 patients provided a large safety dataset to support the safety of LDV/SOF (Table 8). The most frequently reported adverse events were fatigue (29.3%), headache (23.1%), and nausea (13.5%), which were all reported more commonly in the patients receiving RBV-containing regimens. The majority of adverse events were mild, with <5% (91 patients) experiencing a grade 3 or 4 adverse event.

Thirteen (0.7%) patients receiving LDV/SOF with or without RBV had an adverse event leading to discontinuation of LDV/SOF. A total of 51 (2.6%) patients had at least 1 serious adverse event (SAE), with only 5 (0.3%) patients experiencing

Table 8 Summary of adverse events in the LDV-/SOF-integrated phase 3 safety population

	RBV-free regimens				RBV-containing regimens			
	LDV/SOF 8 weeks ($N = 215$)	LDV/SOF 12 weeks ($N = 539$)	LDV/SOF 24 weeks ($N = 326$)	LDV/SOF overall ($N = 1{,}080$)	LDV/SOF + RBV 8 weeks ($N = 216$)	LDV/SOF + RBV 12 weeks ($N = 328$)	LDV/SOF + RBV 24 weeks ($N = 328$)	LDV/SOF + RBV overall ($N = 872$)
Adverse event	145 (67.4%)	390 (72.4%)	265 (81.3%)	800 (74.1%)	165 (76.4%)	280 (85.4%)	300 (91.5%)	745 (85.4%)
Grade 3 or 4 adverse event	2 (0.9%)	13 (2.4%)	31 (9.5%)	46 (4.3%)	8 (3.7%)	17 (5.2%)	20 (6.1%)	45 (5.2%)
Serious adverse event	4 (1.9%)	6 (1.1%)	24 (7.4%)	34 (3.1%)	1 (0.5%)	7 (2.1%)	9 (2.7%)	17 (1.9%)
Treatment-related serious adverse event	0	0	4 (1.2%)	4 (0.4%)	0	1 (0.3%)	0	1 (0.1%)
Adverse event leading to permanent discontinuation of any study drug	0	2 (0.4%)	4 (1.2%)	6 (0.6%)	2 (0.9%)	1 (0.3%)	8 (2.4%)	11 (1.3%)
Adverse event leading to permanent discontinuation of SOF/LDV	0	2 (0.4%)	4 (1.2%)	6 (0.6%)	1 (0.5%)	0	6 (1.8%)	7 (0.8%)
Treatment-emergent death	0	0	0	0	0	0	0	0
Fatigue	45 (20.9%)	116 (21.5%)	79 (24.2%)	240 (22.2%)	75 (34.7%)	124 (37.8%)	132 (40.2%)	331 (38.0%)
Headache	30 (14.0%)	113 (21.0%)	79 (24.2%)	222 (20.6%)	54 (25.0%)	75 (22.9%)	99 (30.2%)	228 (26.1%)
Nausea	15 (7.0%)	61 (11.3%)	36 (11.0%)	112 (10.4%)	38 (17.6%)	57 (17.4%)	57 (17.4%)	152 (17.4%)
Insomnia	11 (5.1%)	41 (7.6%)	30 (9.2%)	82 (7.6%)	26 (12.0%)	63 (19.2%)	66 (20.1%)	155 (17.8%)

Data were included to last dose Date of any study drug +30 days

a treatment-related serious adverse event (anemia, factor VIII inhibition, mesenteric vein thrombosis, salpingitis, and headache). One patient died of liver failure secondary to HCV infection and alcohol use 121 days after treatment completion.

Importantly, the difference in the adverse event profile of RBV-free (LDV/SOF) and RBV-containing (LDV/SOF + RBV) treatment groups was also evaluated. The addition of RBV to the treatment regimen was associated with an increase in the total incidence of adverse events, treatment-related adverse events, and serious adverse events compared to patients receiving RBV-free regimens for all treatment durations. Consistent with the frequent need for RBV dose modification, a higher proportion of patients in the RBV-containing (LDV/SOF + RBV) treatment groups (13.5%) had AEs leading to dose modification or interruption of any study drug than patients in the RBV-free (LDV/SOF) treatment groups (0.6%).

In the integrated phase 3 safety population, approximately 75% of patients had at least one laboratory abnormality with the majority (66.8%) being only grade 1 or 2 laboratory abnormalities. The percentage of patients receiving LDV/SOF + RBV who had a grade 3 laboratory abnormality (11.4%) was approximately twofold higher than patients receiving LDV/SOF (5.4%). Few patients had grade 4 laboratory abnormalities. The groups receiving RBV had a higher incidence of laboratory abnormalities that are consistent with the expected toxicity profile of ribavirin, namely, decreases in hemoglobin and lymphocytes and increases in total bilirubin.

5.4 Summary of Phase 3 Data Supporting Initial Registration of Ledipasvir/Sofosbuvir

LDV/SOF (Harvoni®) was the first all-oral, single-tablet, IFN-free and RBV-free treatment approved for the vast majority of patients infected with HCV. Across the phase 3 registrational trials, treatment with LDV/SOF offered a short, simple, well-tolerated regimen with significantly shorter treatment durations without the need for response-guided treatment algorithms compared with up to 48 weeks of standard of care treatment algorithms.

The phase 3 program showed that 12 weeks of LDV/SOF was a highly effective treatment for patients with HCV genotype 1 infection across a broad range of demographic and baseline characteristics. LDV/SOF was the first Peg-IFN-free, RBV-free treatment to demonstrate SVR rates >90% in genotype 1 HCV-infected patients who had failed the current standard of care. In addition, factors that had been traditionally associated with relapse (e.g., age ≥65 years, black or African-American race, Hispanic or Latino ethnicity, high BMI, genotype 1a, high viral load, non-CC IL28B allele) had no notable impact on SVR12 rates nor did the presence of baseline LDV-associated NS5A RAVs in a subset of patients.

No additional benefit appeared to be associated with the addition of ribavirin or with extension of the duration of treatment to 24 weeks. In addition, the 8-week regimen of LDV/SOF was highly efficacious among non-cirrhotic genotype 1

patients who had not been treated previously. At the time of approval, emerging data indicated that in patients who experience relapse with SOF treatment, retreatment with LDV/SOF would be a viable option.

Treatment with LDV/SOF with or without RBV was generally well tolerated, with no treatment-emergent deaths and few permanent discontinuations of study drug due to AEs, SAEs, grade 3 or 4 AEs, or grade 3 or 4 laboratory abnormalities. Importantly, the exclusion of RBV significantly reduces the incidence of AEs and clinically significant laboratory abnormalities experienced by patients. It was observed that increasing treatment duration from 8 to 12 weeks resulted in small but consistent increases in the incidence of AEs but did not change the observed AE profile.

These data and others presented in Sect. 6 supported the approval of LDV/SOF (Harvoni®) in the United States on October 10, 2014, as the first all-oral, single-tablet, IFN-free and RBV-free treatment for HCV genotype 1, 4, 5, and 6 infection in patients with or without cirrhosis. By the end of 2017, Harvoni had been approved in over 80 countries in North and South America, Europe, Asia, Africa, and Australia.

6 Safety and Efficacy of Ledipasvir/Sofosbuvir in Other Patient Populations

The LDV/SOF clinical development program included additional pivotal phase 2 and 3 trials that were designed to assess the efficacy and safety of LDV/SOF in key patient populations with an unmet medical need. These included but were not limited to populations with non-genotype 1 HCV infection, HCV/HIV coinfection, decompensated liver disease, post-liver and kidney transplantation, and children.

6.1 LDV/SOF in Patients with Compensated Cirrhosis Who Failed Prior IFN-Based Treatment

Based on the data from the ION-2 study, a 24-week regimen of LDV/SOF was approved for the treatment of genotype 1 subjects with compensated cirrhosis who had failed prior IFN-based therapy [27]. In that study, 24 weeks of treatment with LDV/SOF ± RBV resulted in a numerically higher SVR rate (100%, 44/44) than 12 weeks of treatment with LDV/SOF ± RBV (84%, 37/44); although it was acknowledged that this difference in SVR was based on a small number of subjects, in the absence of additional data, a conservative duration of 24 weeks was recommended in the initial approval of LDV/SOF. The SIRIUS (GS-US-337-0121) study was conducted to evaluate the potential of shortening treatment duration in this population.

6.1.1 GS-US-337-0121 (SIRIUS)

GS-US-337-0121 was a double-blind, placebo-controlled trial conducted in France, in which 155 cirrhotic subjects were randomized 1:1 to one of two groups: Group 1 ($n = 77$), LDV/SOF once daily for 24 weeks + matched RBV placebo, or Group 2 ($n = 78$), deferred treatment group, matched LDV/SOF placebo once daily and matched RBV placebo (divided dose) for 12 weeks followed by LDV/SOF once daily and RBV for 12 weeks [29]. Randomization was stratified by HCV genotype and response to prior HCV therapy.

In this study, all subjects had prior virologic failure despite prior treatment, with Peg-IFN + RBV, or Peg-IFN + RBV and a protease inhibitor regimen. All subjects had cirrhosis with the exception of one subject randomized to the LDV/SOF + RBV 12-week group. The majority of subjects were male (73.5%) and white (97.4%) with non-CC IL28B alleles (93.5%).

Out of the 155 subjects randomized in this study, 1 subject discontinued treatment due to an AE while taking placebo. Of the remaining 154 subjects, a total of 149 achieved SVR12 across both treatment groups; 97.4% of subjects in the LDV/SOF 24-week group and 96.1% of subjects in the LDV/SOF + RBV 12-week group achieved SVR12 (Table 9). All five subjects who did not achieve SVR12 relapsed, and no subjects experienced on-treatment virologic failure.

Among the 30 subjects with NS5A RAVs at baseline, all 15 subjects (100%) treated with LDV/SOF + RBV achieved SVR12, while 13 of 15 subjects (86.7%) in the LDV/SOF 24-week group achieved SVR12. Both relapse subjects treated with LDV/SOF for 24 weeks had pretreatment NS5A RAVs that were maintained or enriched posttreatment.

LDV/SOF for 24 weeks and LDV/SOF + RBV for 12 weeks were both well tolerated with no subjects discontinuing treatment due to AEs. Comparing these two regimens overall, a higher frequency of AEs and treatment-related AEs were observed with LDV/SOF + RBV for 12 weeks compared with LDV/SOF for 24 weeks. This difference was attributable to a higher incidence in RBV-associated AEs such as pruritus and dyspnea. Importantly, when comparing the three 12-week treatment periods, similar percentages of subjects with any AE were observed during treatment with LDV/SOF (84.6%), placebo (81.8%), and LDV/SOF + RBV (86.8%) suggesting that there is a high background rate of symptoms in HCV-infected patients. Specifically, the only AEs reported more commonly (with an increase in frequency >10%) than placebo were headache and fatigue for LDV/SOF 12 week.

Table 9 Efficacy of LDV/SOF in patients with compensated cirrhosis who have failed on Peg-IFN ± ribavirin ± protease inhibitor

	LDV/SOF + RBV 12 weeks ($N = 77$)	LDV/SOF 24 weeks ($N = 78$)
SVR12	74/77 (96%)	76/78 (97%)
95% CI (%)	88–99	91–100

The comparable efficacy of LDV/SOF + RBV for 12 weeks and LDV/SOF for 24 weeks showed that a shorter course of LDV/SOF, when given with RBV, does not compromise the ability of treatment-experienced patients with cirrhosis to achieve SVR. This data led to the approval of LDV/SOF+ RBV for 12 weeks in previously treated adults with compensated cirrhosis who have failed on Peg-IFN ± ribavirin ± protease inhibitor on November 12, 2015.

6.2 LDV/SOF in Patients with Non-genotype 1 Infection

The most common HCV genotype in the United States and in Europe is genotype 1, while genotypes 2 and 3 HCV infection represent the majority of the remaining cases of chronic HCV infection in United States and in Europe. Genotype 4, 5, and 6 HCV infections are most prevalent in the Middle East, South Africa, and Southeast Asia, respectively [5, 30–32].

At initiation of many of the trials described in Sect. 6.1, there was no approved all-oral, IFN-free, RBV-free therapy for non-genotype 1 HCV infection. The only approved regimen for the treatment of non-genotype 1 HCV infection was SOF + RBV with or without Peg-IFN for 12–24 weeks [21, 31, 33]. While this combination resulted in high SVR rates >90%, there remained a need for simpler, better-tolerated RBV-free regimens given the significant toxicity, tolerability, and adherence issues associated with Peg-IFN and RBV. In patients in whom RBV was relatively or absolutely contraindicated (e.g., cardiac disease, sickle cell disease, thalassemia), there was a critical medical need for a RBV-free regimen. Furthermore, the in vitro activity of LDV across multiple genotypes provided the opportunity to conduct these studies.

6.2.1 Efficacy of LDV/SOF in Patients with Genotype 2 HCV Infection

The efficacy and safety of LDV/SOF in patients with HCV genotype 2 infection was evaluated in two pivotal studies, namely, GS-US-337-1468 and GS-US-337-1903.

GS-US-337-1468 (LEPTON)

Study GS-US-337-1468 was a phase 2 multicenter, open-label trial to evaluate the efficacy and safety of oral regimens for the treatment of HCV infection [34]. Patients were enrolled and treated at two sites in New Zealand from August 2014 through April 2015. In Cohort 2, Group 1, 26 patients with genotype 2 HCV infection received LDV/SOF (90/400 mg) once daily for 12 weeks.

Table 10 Efficacy of LDV/SOF in patients with HCV non-genotype 1 infection

	Number of patients	LDV/SOF 12 weeks	LDV/SOF + RBV 12 weeks
Genotype 2			
GS-US-337-1468 (LEPTON)	26	25/26 (96.2%) 80.4–99.9%	N/A
GS-US-337-1903	106	102/106 (96.2%) 90.6–99.0%	N/A
	25 (IFN ineligible/ intolerant)	24/25 (96.0%) 79.6–99.9%	N/A
Genotype 3			
GS-US-337-0122 (ELECTRON-2)	50 (treatment naive)	16/25 (64%) 43–82%	25/25 (100%) 87–100%
	50 (treatment experienced)	N/A	41/50 (82%) 69–91%
GS-US-337-1701	111	N/A	99/111 (89.2%) 82–94%
Genotype 4			
SYNERGY study	21	20/21 (95.2%) 76–100%	N/A
GS-US-337-1119	44	41/44 (93.2%) 81–99%	N/A
Genotype 5			
GS-US-337-1119	41	39/41 (92.7%) 83–99%	N/A
Genotype 6			
GS-US-337-0122 (ELECTRON-2)	25	24/25 (96%) 80–100%	N/A

Overall 68% of patients were male, 85% were white, and 77% were HCV treatment naive. Two patients had cirrhosis. A total of 25 patients (96.2%) achieved SVR12 (Table 10). No patients experienced on-treatment virologic failure or virologic relapse. The only patient who did not achieve SVR12 withdrew consent and prematurely discontinued from the study after receiving a single dose of LDV/SOF.

Pretreatment NS5A were detected in 16 patients (64%) using a 15% assay cutoff, with L31 M present in 13 of 16 patients. All patients with pretreatment NS5A RAVs achieved SVR12. The NS5B RAV M289I was detected in two genotype 2b patients using a 15% assay cutoff, and both patients achieved SVR12.

GS-US-337-1903

Study GS-US-337-1903 was a phase 3, randomized, multicenter, open-label trial conducted at 40 sites in Japan [35]. This was important in the context of genotype 2 infections accounting for 25–30% of HCV infections in Japan. A total of 239 patients were randomized 1:1 to receive either LDV/SOF 12 weeks or

SOF + RBV 12 weeks (Cohort 1). Twenty-five patients who were ineligible for or intolerant of RBV therapy were treated with LDV/SOF for 12 weeks (Cohort 2).

The median age for the study population was 63 years (range 20–82), although in Cohort 2 the median age was 77 years with a range of 59–82 years. Overall, 34% of patients were treatment experienced, 21% had a non-CC *IL28B* genotype, and 14% had cirrhosis. In Cohort 1, SVR12 rates were 96% with LDV/SOF and 95% with SOF + RBV, thus achieving non-inferiority (Table 10). Among RBV intolerant/ineligible patients in Cohort 2, SVR12 was 96%.

Overall, 92% (118 of 129) of patients treated with LDV/SOF had pretreatment NS5A RAVs using a 15% assay cutoff. SVR12 was achieved in 114/118 (97%) of these patients. A total of six patients (5%) had baseline NS5B NI RAVs, of which one patient relapsed following 12 weeks treatment with LDV/SOF. The high rates of SVR12 in patients with pretreatment NS5A RAVs or NS5B NI RAVs suggest there is little utility of pretreatment resistance testing for patients with genotype 2 HCV infection.

6.2.2 LDV/SOF in Patients with Genotype 3 HCV Infection

Genotype 3 HCV infections account for approximately 20% of all HCV infections globally and 40% of infections in Asia [1, 32]. More recently, genotype 3 HCV infection has been associated with greater risk of steatosis, fibrosis progression, hepatocellular carcinoma, and all-cause mortality [36, 37]. The efficacy of LDV/SOF in patients with HCV genotype 3 infection was evaluated in two pivotal studies, namely, GS-US-337-0122 and GS-US-337-1701.

Study GS-US-337-0122 (ELECTRON-2)

The use of LDV/SOF in genotype 3 HCV infection was evaluated in ELECTRON-2, a phase 2 multicenter, open-label trial to evaluate the safety, tolerability, and antiviral efficacy of SOF-containing treatment regimens in HCV patients. In this study, Cohort 2 (Groups 3 and 4), 51 treatment-naive genotype 3 patients were enrolled to receive LDV/SOF once daily with or without weight-based RBV for 12 weeks; and 50 treatment-experienced patients received LDV/SOF for 12 weeks [24].

Overall, 84% of patients were white and 63% were male. The presence of cirrhosis was more common among treatment-experienced (44%) than treatment-naive (20%) patients. Among treatment-naive patients, the SVR12 results were higher in the LDV/SOF + RBV 12-week treatment group (100%; 26 of 26 patients) compared with the LDV/SOF 12-week treatment group (64%; 16 of 25 patients) (Table 10). Of the nine treatment-naive patients (36%) who did not achieve SVR12 in the LDV/SOF treatment group, eight relapsed and one patient discontinued study treatment. Of the 50 treatment-experienced patients with genotype 3 HCV receiving LDV/SOF + RBV, 41 (82%) achieved SVR12. In those with and without cirrhosis,

the SVR12 was 73% and 89%, respectively. Of the nine treatment-experienced patients who did not achieve SVR12, one experienced virologic breakthrough and eight had virologic relapse.

Common NS5A RAVs detected at baseline included 30A/V/S/R/T. The RAVs L31M and Y93H were observed in only 1 (1%) and 8 (8%) of baseline samples, respectively. Of these only one patient with Y93H RAV at baseline experienced relapse.

Study GS-US-337-1701

Study GS-US-337-1701 was a phase 2 open-label trial of LDV/SOF with RBV in patients with genotype 3 HCV infection [38]. This study was conducted in Canada at 15 sites. A total of 111 patients received LDV/SOF + RBV for 12 weeks, of which 35.1% of patients had cirrhosis at screening.

The majority of patients had genotype 3a HCV infection (94.6%) and non-CC (CT or TT) IL28B alleles (62.2%), and 35.1% had cirrhosis. The study showed that LDV/SOF + RBV in treatment-naive patients with genotype 3 HCV infection resulted in 89.2% of patients achieving SVR12 (Table 10). Overall, 12 patients (10.8%) did not achieve SVR12. Of these, 8 patients (7.2%) relapsed, 3 patients (2.7%) were lost to follow-up, and 1 patient (0.9%) died. No patients had on-treatment virologic failure (i.e., breakthrough, rebound, or nonresponse). Among patients with cirrhosis, the SVR12 rates were lower (79.5%) compared with patients without cirrhosis (94.3%).

Baseline NS5A RAVs were detected in 15 of 106 patients (14.2%), of these 13 patients (86.7%) with baseline NS5A RAVs achieved SVR12. NS5B NI RAVs were detected in 10 of 104 patients (9.6%) with successful NS5B deep sequencing. All patients with baseline NS5B NI RAVs achieved SVR. A total of eight patients experienced viral relapse, of which two had Y93H at baseline (1.0% and 18% of viral population). Y93H was no longer detectable at virologic failure in both patients. Three other patients with Y93H at baseline achieved SVR12. No other NS5A RAVs or NS5B NI RAVs were detected in patients with relapse at baseline or virologic failure. A high percentage of patients achieved SVR12 regardless of the presence of NS5A RAVs at baseline, suggesting a minor impact of these on the treatment outcome. Furthermore, all patients with baseline NS5B NI RAVs achieved SVR12. This suggests that there is little utility of pretreatment resistance testing for patients with genotype 3 HCV infection considering LDV/SOF therapy.

6.2.3 LDV/SOF in Patients with Genotype 4 HCV Infection

Genotype 4 hepatitis C virus (HCV) accounts for an estimated 13% of patients with HCV globally. In several countries in sub-Saharan Africa and the Middle East, genotype 4 accounts for more than half of HCV infections [1, 39]. Historically, genotype 4 HCV has been considered difficult to treat because of its low rate of

response to Peg-IFN and RBV [40]. At initiation of these trials, SOF and the protease inhibitor simeprevir in combination with Peg-IFN and RBV for 12 weeks were approved and had been shown to substantially improve SVR rates in patients with HCV genotype 4 [33]; however, due to the safety profile of Peg-IFN and RBV, there remained a need for IFN- and RBV-free therapy. The efficacy of LDV/SOF in patients with HCV genotype 4 infection was evaluated in two pivotal studies, namely, SYNERGY and GS-US-337-1119.

SYNERGY Trial

The SYNERGY trial (NCT01805882) was a single-center, open-label cohort, nonrandomized phase 2a trial in HCV-infected patients conducted in the United States [41]. In this study, 21 HCV genotype 4 patients were enrolled to receive LDV/SOF once daily for 12 weeks.

Overall 60% were treatment naive and 43% had advanced fibrosis. One patient took the first dose and then withdrew consent. Among the 20 patients who completed treatment, all achieved SVR12 (Table 10).

Study GS-US-337-1119

Study GS-US-337-1119 was a phase 2 multicenter, open-label trial to evaluate the efficacy and safety of LDV/SOF in patients with HCV genotype 4 or 5 infection conducted in France [42]. A total of 44 genotype 4 patients were enrolled to receive LDV/SOF once daily for 12 weeks.

The majority of patients were white (82%) or male (64%). Among treatment-experienced patients, 41% had cirrhosis, while only 5% of treatment-naive patients had cirrhosis. The SVR12 rate was 93.2% (41 of 44) for genotype 4 patients (Table 10). For genotype 4 patients, 21 (95.5%) treatment-naive and 20 (90.9%) treatment-experienced patients achieved SVR12. No patients had on-treatment virologic failure. Each of the three genotype 4 patients who did not achieve SVR12 relapsed.

Pretreatment NS5A RAVs were detected in all 44 patients (100%) with genotype 4 HCV infection. Of the 44 patients with genotype 4 HCV infection and NS5A RAVs, 41 (93%) achieved SVR12. Three of ten patients with genotype 4 HCV infection with triple NS5A RAVs pretreatment had virologic relapse, while all patients with genotype 4 HCV infection with double or single NS5A RAVs pretreatment achieved SVR12. A global prevalence study of NS5A RAVs across 454 patients with genotype 4 HCV infection showed that these specific triple RAVs associated with reduced susceptibility to LDV are found in less than 2.7% (12 of 454) of patients with genotype 4 HCV infection.

6.2.4 LDV/SOF in Genotype 5 HCV Infection

At initiation of study GS-US-337-1119, there was also no approved all-oral, IFN-free, RBV-free therapy for patients with genotype 5 HCV infection. For patients with genotype 5 HCV infection, the only treatment option approved was SOF + Peg-IFN + RBV for 12 weeks or SOF + RBV for 24 weeks in patients ineligible or intolerant to Peg-IFN [33]. Thus, there was an unmet medical need for IFN- and RBV-free treatment regimens, given the significant toxicity, tolerability, and adherence issues associated with these compounds.

Study GS-US-337-1119

The use of LDV/SOF in genotype 5 HCV infection was evaluated in study GS-US-337-1119, a phase 2 multicenter, open-label trial to evaluate the efficacy and safety of LDV/SOF in patients with HCV genotype 4 or 5 infection [43]. A total of 41 genotype 5 patients were enrolled to receive LDV/SOF once daily for 12 weeks.

All patients were white and 51% were male. Among treatment-experienced patients, 30% had cirrhosis, while only 13% of treatment-naive patients had cirrhosis. The SVR12 rate was 92.7% in genotype 5 patients (Table 10). Overall, 19 (90.5%) treatment-naive and 19 (95%) treatment-experienced patients achieved SVR12. No patients had on-treatment virologic failure. Two genotype 5 patients relapsed. One genotype 5 patient who did not achieve SVR12 had HCV RNA $<$ LLOQ at their last on-treatment visit.

Baseline NS5A sequencing was successful and analyzed in 39 of 41 patients. Baseline NS5A RASs were observed in 4 of these 39 patients (10.3%). Following treatment with LDV/SOF for 12 weeks, SVR12 was achieved in three of four patients with baseline NS5A RASs.

6.2.5 LDV/SOF in Genotype 6 HCV Infection

Genotype 6 HCV constitutes about 1% of HCV infections globally and is found mainly in Southeast Asia and Southern China [1]. Genotype 6 HCV is genetically diverse, with 23 subtypes, many of which have not been cloned, limiting in vitro testing of antiviral agents. Due to its genetic diversity and relatively low prevalence, genotype 6 HCV infection was not as well characterized as the other genotypes, but long-term infection appears to be associated with the similar risk of cirrhosis and hepatocellular carcinoma as genotype 1 HCV infection.

GS-US-337-0122 (ELECTRON-2)

The use of LDV/SOF in genotype 6 HCV infection was evaluated in ELECTRON-2 (GS-US-337-0122), a phase 2 multicenter, open-label trial to evaluate the safety, tolerability, and antiviral efficacy of SOF-containing treatment regimens in HCV patients [24]. In this study, Cohort 2 (Group 5) evaluated the safety and efficacy of LDV/SOF in treatment-naive or treatment-experienced patients with genotype 6 HCV infection. A total of 25 patients were enrolled to receive LDV/SOF once daily for 12 weeks [24].

Overall, 84% of patients were Asian and 64% were male. Among treatment-naive and treatment-experienced patients with genotype 6 HCV infection, 24 patients (96%) achieved SVR12 (Table 10). One patient (4.0%), who had discontinued treatment after 8 weeks, relapsed and discontinued the study due to withdrawal of consent.

Importantly, baseline NS5A RAVs were observed in 23 (92%) patients with genotype 6 HCV infection. Following treatment with LDV/SOF for 12 weeks, SVR12 was achieved in 22 of 23 patients with NS5A RAVs. The one patient with NS5A RAVs who did not achieve SVR discontinued treatment early at 8 weeks.

6.2.6 Safety of LDV/SOF in Patients with Non-genotype 1 Infection

Treatment with LDV/SOF was generally safe and well tolerated, and the adverse event profile was similar across the different HCV genotypes. The safety profile associated with the use of LDV/SOF ± RBV in non-genotype 1 HCV infection did not differ, as expected, from the safety profile observed in patients with genotype 1 HCV infection with no new safety signal observed.

These studies supported the use of LDV/SOF (Harvoni®) for the treatment of patients with genotype 4, 5, or 6 HCV infection which was first approved in the United States on November 12, 2015. In addition, LDV/SOF has been approved for the treatment of genotype 2 or 3 HCV infection in certain regions including Canada and the European Union.

6.3 LDV/SOF in Patients with HCV/HIV Coinfection

Globally, it is estimated that 4 to 5 million persons are chronically infected with both human immunodeficiency virus type 1 (HIV-1) and HCV [44]. It has been shown that patients with HCV/HIV coinfection have higher rates of cirrhosis, hepatocellular carcinoma, and hepatic decompensation than patients with HCV monoinfection [45, 46]. However, uptake of HCV treatment in the IFN era was lower in the HCV-/HIV-coinfected population owing to historically lower SVR rates, patient comorbidities, patient and practitioner perceptions, high rates of treatment-related cytopenias, and complex interactions with concomitant antiretroviral drugs [47]. The

Table 11 Efficacy of LDV/SOF in patients with HIV/HCV coinfection

	ERADICATE	GS-US-337-0115
	LDV/SOF 12 weeks ($N = 50$)	LDV/SOF 12 weeks ($N = 335$)
SVR12	49/50 (98%)	321/335 (95.8%)
95% CI (%)	89–100	93.1–97.7

first DAAs approved, namely, the NS3/4A protease inhibitors boceprevir and telaprevir, were not approved by the Food and Drug Administration for patients with HCV/HIV coinfection. The efficacy of LDV/SOF in patients with HCV/HIV coinfection was evaluated in two studies, namely, ERADICATE and GS-US-337-0115.

6.3.1 Study CO-US-337-0115 (ERADICATE)

The ERADICATE trial (NCT01878799) was a single-center, open-label cohort, phase 2b pilot study of previously untreated, non-cirrhotic patients with HCV genotype 1 and HIV coinfection conducted from June 2013 to September 2014 [48]. Eligible patients included those with HCV genotype 1 infection receiving antiretroviral therapy (ART) with HIV RNA values of 50 copies/mL or fewer and a CD4 T-lymphocyte count of $\geq$100 cells/mL or patients with untreated HIV infection with a CD4 T-lymphocyte count of $\geq$500 cells/mL.

Fifty patients with HCV/HIV coinfection were enrolled and received LDV/SOF once daily for 12 weeks. Of the 50 enrolled, 37 were receiving ART, and 13 were not receiving antiretroviral treatment. Patients were predominantly African-American (84%), men (74%), IL28B non-CC genotype (84%), and with genotype 1a infection (74%). Median baseline CD4 count was 576 cells/mm^3 for patients receiving ART and 687 cells/mm^3 for patients not receiving antiretroviral treatment.

Forty-nine of 50 participants (98%) achieved SVR12 and 1 patient experienced relapse (Table 11). Deep sequencing was carried out on one patient who experienced relapse, which showed enrichment of the Y93H mutation (NS5A RAV) that was present at baseline.

6.3.2 Study GS-US-337-0115 (ION-4)

Study GS-US-337-0115 (ION-4) was a phase 3, open-label, multicenter trial that assessed the antiviral efficacy, safety, and tolerability of LDV/SOF administered for 12 weeks in HCV treatment-naive and treatment-experienced (including treatment intolerant) patients with chronic genotype 1 or 4 HCV infection who were coinfected with HIV-1 [49].

This was a multicenter, randomized, open-label trial that enrolled patients between March 2014 and June 2014 and was conducted at 60 sites in the United States, Puerto Rico, Canada, and New Zealand. Eligible patients had chronic HCV

genotype 1 or 4 and HIV-1 coinfection including those with compensated cirrhosis and/or prior treatment failure. On the basis of drug-interaction data in healthy volunteers that was available at study initiation, the antiretroviral drugs allowed in the study included emtricitabine and tenofovir disoproxil fumarate plus efavirenz, raltegravir, or rilpivirine. All patients received LDV/SOF for 12 weeks.

A total of 335 patients were enrolled and received at least 1 dose of study drug. In general, patients were representative of the HIV-infected population. Overall, 75% of the patients had HCV genotype 1a infection, 34% were black, 55% were treatment experienced, and 20% had cirrhosis.

Overall, 322 patients (96%) achieved SVR12 (Table 11). Of the 13 patients who did not achieve SVR, 10 patients relapsed, and 2 patients had on-treatment virologic failure (both in the setting of noncompliance). High SVR12 rates were observed in most subgroups, including patients who were treatment-experienced with cirrhosis (97.9%). High and similar SVR12 rates were also observed irrespective of ARV regimen. In this study, there were 13 treatment-experienced patients enrolled who had failed a SOF + RBV regimen. All 13 of these patients achieved SVR12 and are further described in Sect. 6.5.

Pretreatment NS5A and NS5B deep sequencing data was obtained for all 335 patients enrolled in study GS-US-337-0115 (ION-4). Baseline analyses of NS5A RAVs and NS5B NI RAVs were conducted with a 15% cutoff.

Of 325 patients, 34 (10.5%) had pretreatment NS5A RAVs, of which 31 (91.2%) achieved SVR12. The two patients who experienced on-treatment virologic failure had no NS5A RAVs at baseline and developed NS5A RAVs at the time of virologic failure. Four of the ten patients who experienced virologic relapse had pretreatment NS5A RAVs, and eight of the ten patients who relapsed had posttreatment NS5A RAVs.

6.3.3 Safety of LDV/SOF in Patients with HIV/HCV Coinfection

The use of LDV/SOF in HCV-/HIV-coinfected patients was safe and well tolerated with no discontinuations due to adverse events. The adverse event profile was similar to that observed in HCV-monoinfected patients. There were no clinically significant changes in CD4 cell counts or HIV RNA levels observed. In addition, no renal adverse event signal or trends suggestive of renal toxicity regardless of ARV regimen were identified with intensive renal laboratory monitoring. However, due to the elevated levels of TFV with TDF-containing regimens in the presence of LDV/SOF in patients who have preexisting renal disease, it is recommended that such patients are monitored according to TDF prescribing information.

These studies supported the supplemental indication in the United States for LDV/SOF (Harvoni®) for the treatment of coinfected patients, granted on November 12, 2015.

6.4 LDV/SOF in Patients Who Are Posttransplant or with Decompensated Liver Disease

Prior to 2012, for posttransplantation patients with compensated liver disease, recurrence of HCV infection following transplantation was essentially universal and was associated with poorer graft and patient survival compared with patients undergoing liver transplantation for other causes [50–53].

In patients with decompensated liver disease, the 1-year mortality for patients with Child-Pugh-Turcotte (CPT) B decompensated cirrhosis was approximately 20%, while the 1-year mortality for patients with CPT C decompensated cirrhosis was >50% [54].

When studies in these populations were initiated, there were no approved therapies for the treatment of HCV patients with decompensated liver disease. The poor adverse event profile of IFN-based regimens had limited their use in this sick patient population to specialized centers and clinical trials [52]. Instead, the mainstay of treatment in the United States for patients with decompensated liver disease due to HCV had been liver transplantation. Unfortunately, less than 5% of patients with decompensated liver disease due to HCV in the United States were listed in a given year for transplantation, and <2% received liver transplantations annually [55]. As such, posttransplantation patients with compensated liver disease as well as patients with decompensated liver disease regardless of transplantation status remained populations with a high unmet medical need for treatment. Two studies, namely, GS-US-337-0123 (SOLAR-1) and GS-US-337-0124 (SOLAR-2), were designed to determine the efficacy and safety of LDV/SOF in combination with ribavirin in patients with advanced liver disease including patients who have undergone liver transplantation.

6.4.1 Studies GS-US-337-0123 (SOLAR-1) and GS-US-337-0124 (SOLAR-2)

GS-US-337-0123 and GS-US-337-0124 were phase 2, multicenter, randomized, open-label trials that were conducted at 63 sites in the United States, Europe, Canada, Australia, and New Zealand with patients enrolled between September 2013 and August 2014 [56, 57].

These two studies were identical in study design, including eligibility criteria. A total of 670 patients were enrolled in two cohorts. Cohort A consisted of two groups of patients with advanced cirrhosis Child-Pugh class B and C who had not undergone liver transplantation (Groups 1 and 2, respectively). Cohort B consisted of five groups of patients, all of whom had undergone liver transplantation previously (Group 3, non-cirrhotic; Group 4, compensated cirrhosis (CPT-1); Group 5, Child-Pugh class B; Group 6, Child-Pugh class C; and Group 7, fibrosing cholestatic hepatitis). Patients in each of the seven groups were randomized in a 1:1 ratio to receive either 12 or 24 weeks of LDV/SOF once daily plus RBV. Groups 3, 4, and

7 received weight-based RBV (1,000 mg/day in patients with a body weight of <75 kg and 1,200 mg/day in patients with a body weight ≥75 kg), while in groups 1, 2, 5, and 6, RBV was administered at a starting dose of 600 mg in a divided daily dose and titrated upward as tolerated.

A total of 455 of 670 patients (67.9%) were posttransplantation, while 329 patients (49.1%) had decompensated cirrhosis, regardless of transplantation status. Of these, 78 patients (23.7%) had a MELD score >15. Across all groups, the majority of patients were male (76.9%) and white (91.5%) with a mean age of 58 years (range, 21–81) and a mean (SD) BMI of 27.9 (4.96) kg/m^2.

The SVR12 and relapse rates presented below (Table 12) are from pooled analysis of both studies. Overall, 92.7% (569 of 614) patients with genotype 1 HCV infection and 82.5% (33 of 40) patients with genotype 4 HCV infection achieved SVR12. There were 13 patients (12 genotype 1 HCV infection and 1 genotype 4 HCV infection) who were transplanted prior to their posttreatment Week 12 visit and were excluded from the analysis. Overall, a small number of patients relapsed: 20 of 589 patients (3.4%) with genotype 1 HCV infection and 3 of 36 patients (8.3%) with genotype 4 HCV infection relapsed. In decompensated patients with genotype 1 HCV infection, irrespective of transplantation status, the relapse rates were 8.1% and 4.3% in patients who received LDV/SOF + RBV for 12 or 24 weeks, respectively, resulting in a numerical difference in relapse rates of 3.8% which was not clinically significant (95% CI, −2.1 to 10.2%).

Pretreatment resistance analysis was performed for 622 patients who received LDV/SOF + RBV with NS5A sequencing (587 patients with genotype 1 infection and 35 patients with genotype 4 infection) and for 619 patients with NS5B sequences

Table 12 Efficacy of LDV/SOF in patients who are posttransplant or with decompensated liver disease

	Liver disease status (group)	Duration of treatment (weeks)	SVR12 (n/N)	Relapse (n/N)
Pretransplantation	CPT B cirrhosis (Group 1)	12	48/56 (85.7%)	7/55 (12.7%)
		24	48/52 (92.3%)	2/50 (4.0%)
	CPT C cirrhosis (Group 2)	12	36/43 (83.7%)	3/39 (7.7%)
		24	39/48 (81.3%)	3/42 (7.1%)
Posttransplantation	Stage F0–F3 fibrosis (Group 3)	12	102/107 (95.3%)	3/105 (2.9%)
		24	104/105 (99.0%)	0/104
	CPT A cirrhosis (Group 4)	12	58/60 (96.7%)	0/58
		24	56/58 (96.6%)	0/56
	CPT B cirrhosis (Group 5)	12	43/48 (89.6%)	1/44 (2.3%)
		24	46/49 (93.9%)	0/46
	CPT C cirrhosis (Group 6)	12	4/8 (50.0%)	3/7 (42.9%)
		24	7/9 (77.8%)	1/8 (12.5%)
	Fibrosing cholestatic hepatitis (Group 7)	12	7/7 (100.0%)	0/7
		24	4/4 (100.0%)	0/4

(586 patients with genotype 1 infection and 33 patients with genotype 4 infection). For patients with genotype 1 infection treated for 12 weeks, the SVR rates were 89.4% versus 96.4% in patients with or without NS5A RAVs (1% cutoff), respectively. Among post-liver transplantation patients with compensated liver disease (Groups 3, 4, and 7), the presence of pretreatment NS5A RAVs had minimal, if any, impact on relapse. Among 586 patients with genotype 1 and full-length NS5B sequence, 28 had NS5B NI RAVs (4.8%), of which 27 achieved SVR12 (96.4%; 27 of 28). The lack of significant associations between the presence of either NS5A or NS5B pretreatment RAVs with SVR rates in patients with genotype 1 HCV infection who are posttransplant or have decompensated cirrhosis is consistent with the results of virologic analyses for patients with genotype 1 HCV infection and compensated disease. For genotype 4 patients, there were no patients without RAVs at 1% cutoff.

In this population, it was important to also understand the effects of successful treatment on hepatic outcomes such as CPT and MELD scores. Among the patients who had CPT C at baseline, 61.3% improved to CPT B at posttreatment Week 12, and of the patients who had CPT B at baseline, 33.1% improved to CPT A by posttreatment Week 12. These improvements in CPT score were driven largely by improvements in albumin and bilirubin (64% and 43.6%). Among patients with MELD scores $\geq$15 at baseline, 63.2% had a MELD score $<$15 at posttreatment Week 12. Conversely, among patients with MELD scores $<$15 at baseline, 5.8% had a MELD score $\geq$15 at posttreatment Week 12. Overall improvements in CPT and MELD scores were observed in the majority of patients who achieved SVR12 (66.9% and 59.8%, respectively) suggesting short-term clinical improvements with HCV eradication.

6.4.2 Safety of LDV/SOF in Patients Who Are Posttransplant or with Decompensated Liver Disease

As expected in a patient population with decompensated liver disease and/or patients who were post-liver transplantation, high percentages of patients experienced AEs, Grade 3 or 4 AEs, and serious adverse events were observed. However, few patients (3.0%) experienced treatment-related SAEs or adverse events that led to discontinuation of LDV/SOF. Twenty treatment-emergent deaths were reported; none were considered related to LDV/SOF. The most commonly reported AEs were fatigue (42.5%), anemia (33.6%), and headache (27.3%). Longer treatment with LDV/SOF + RBV for 24 weeks compared with 12 weeks was not associated with an increased safety burden. Additional analyses demonstrated a similar AE profile among patients with decompensated cirrhosis, regardless of transplantation status.

For posttransplantation patients with compensated liver disease, treatment with LDV/SOF + RBV was safe and well tolerated. None of the four treatment-emergent deaths (multifocal leukoencephalitis, myocardial infarction, infection [food poisoning/pneumonia], and graft rejection) were considered related to LDV/SOF. The most clinically relevant safety finding was anemia, a known effect of RBV therapy which

was likely exacerbated by preexisting disease. Hemoglobin reductions were appropriately addressed through monitoring and toxicity management. Furthermore, the decreased hemoglobin levels observed during LDV/SOF + RBV treatment resolved in nearly all patients by posttreatment Week 4, demonstrating the reversibility of the anemia following RBV discontinuation. Despite the lack of drug-drug interactions between LDV/SOF + RBV and common immunosuppressants, it was observed that a common reason for adjustment in the dose or frequency of administration of immunosuppressive agents was improved hepatic function, likely as the result of the suppression of HCV viremia.

For patients with decompensated liver disease, regardless of transplantation status, treatment with LDV/SOF + RBV was also safe and tolerable. All 16 treatment-emergent deaths that occurred were associated with the clinical progression of end-stage liver disease, in some cases potentially exacerbated by immunosuppression (i.e., sepsis, septic shock, multi-organ failure); none were considered to be drug related. Similar to posttransplantation patients with compensated liver disease, anemia was the most clinically relevant safety finding.

The data above led to the approval of LDV/SOF in patients who are posttransplant or with decompensated liver disease on February 12, 2016.

6.5 LDV/SOF in Adolescent Patients

The prevalence of HCV in children varies globally, with estimates of 0.05–0.36% in the United States and Europe and up to 5.8% in regions of Africa [58, 59]. Despite the overall more favorable prognosis compared to adults, approximately 4–6% of children with chronic HCV infection have evidence of advanced fibrosis or cirrhosis, and some children eventually require liver transplantation for end-stage liver disease as a consequence of HCV infection [60, 61].

While there was a transformation in the treatment of HCV infection with the development of DAAs in adults, the standard of care in adolescent patients (12 to <18 years old) was IFN or Peg-IFN with weight-based RBV. Patient acceptance was very low given the requirement for subcutaneous injections for Peg-IFN and the substantial adverse events associated with therapy, including concerns for growth and development in this age group [62]. As such there was a need to address this unmet medical need in the pediatric population.

6.5.1 Study GS-US-337-1116

Study GS-US-337-1116 was a phase 2, multicenter, open-label trial conducted at 24 sites in Europe and the United States, United Kingdom, and Australia from November 2014 to October 2015 [15]. Eligible patients were 12 to <18 years old and had chronic infection with HCV genotype 1.

Table 13 Efficacy of LDV/SOF in adolescents

	Genotype 1 LDV/SOF 12 weeks		
	Treatment naive, with or without cirrhosis ($N = 80$)	Treatment experienced, without cirrhosis ($N = 20$)	Total ($N = 100$)
SVR12	77/80 (96.3%)	20/20 (100.0%)	97/100 (97.0%)
95% CI (%)	89.4–99.2	83.2–100.0	91.5–99.4

A total of 100 patients were enrolled and received LDV/SOF once daily for 12 weeks. The median age of patients was 15 years, and the majority were HCV treatment naive (80%); 84% were infected through perinatal transmission. In this study, 63% of patients were female, 90% were white, 76% had a non-CC *IL-28B* genotype, and 81% had HCV genotype 1a infection. Treatment with LDV/SOF for 12 weeks resulted in a high SVR12 rate of 97% (Table 13). This rate was similar to the SVR12 rates observed in adult patients treated with LDV/SOF in other clinical studies. Of note, the only patient with known cirrhosis achieved SVR12. A total of three patients (3.0%) did not achieve SVR12 and had "other" virologic outcome (due to reasons such as lost to follow-up). No patients experienced virologic failure.

Virologic analyses were performed for the 97 patients who had a posttreatment virologic outcome. NS5A RAVs were detected at baseline in 8.2% and 5.2% of patients with a 1% and 15% detection assay cutoff, respectively. The presence of NS5A and NS5B RAVs did not impact treatment outcome; all patients with RAVs achieved SVR12.

6.5.2 Safety of LDV/SOF in Adolescent Patients

Treatment with LDV/SOF was safe and well tolerated in HCV-infected adolescents, and no new safety signal was detected. The most commonly reported adverse events were headache (27% of patients), diarrhea (14%), and fatigue (13%). No patient experienced serious adverse events or discontinued treatment because of an adverse event. All adverse events were mild or moderate in intensity; no patient experienced grade 3 or 4 adverse events. Most laboratory abnormalities were mild in severity.

In addition, effects on short-term development and growth were evaluated. No clinically relevant effects on development as assessed by changes from baseline in Tanner pubertal stages or growth as assessed by changes from baseline in body height, body height percentiles, body weight, or body weight percentiles to posttreatment were observed. In addition, no clinically relevant changes from baseline in BMI or BMI percentiles were observed.

Treatment with LDV/SOF demonstrated a favorable safety profile, comparable PK exposure, and high efficacy in adolescent patients 12 to <18 years of age. The safety profile of LDV/SOF in adolescents was consistent with that observed for adults treated with LDV/SOF in previous studies in adults ≥ 18 years of age. The data above led to the approval of LDV/SOF in adolescents 12 to <18 years of age on April 7, 2017.

6.6 LDV/SOF in Retreatment of Patients Who Failed Prior SOF Regimens

At the time these studies were developed, there was no approved therapy for patients with HCV infection who had failed a SOF + RBV ± Peg-IFN regimen. This was a growing problem due to the extensive number of patients being treated with these regimens at that time. The available evidence suggested that the primary mode of resistance to SOF is the development of the S282 T mutation. This mutation develops rarely and is rapidly overgrown by wild-type virus, suggesting that patients who have failed SOF can be retreated with a SOF-containing regimen.

Overall, 90 patients who had previously failed on a SOF-based treatment regimen received at least 1 dose of LDV/SOF in the clinical studies described in Table 14 [49, 63, 64]. Among the patients, with or without cirrhosis, who had previously failed a SOF-containing regimen and were treated with LDV/SOF with or without RBV for 12 weeks across these four clinical studies, 56 had failed SOF + RBV, 25 had failed SOF + Peg-IFN + RBV, 8 had failed a prior LDV/SOF + RBV 6-week regimen, and 1 had failed a prior SOF + GS-9669 + RBV 12-week regimen. SVR12

Table 14 Clinical studies and SVR12 rates that support the efficacy of LDV/SOF for retreatment of patients who failed prior SOF regimen

Study number	Study description	SVR12 (n/N)	
		Overall	Patients with cirrhosis
Prior SOF treatment failures ($N = 90$)			
GS-US-337-1118 (Group 1)	Treatment-experienced patients with genotype 1 HCV infection, with or without cirrhosis, who had failed a prior SOF + RBV ± Peg-IFN regimen were retreated with LDV/SOF + RBV for 12 weeks	50/51 (98%)	14/14 (100%)
GS-US-337-0122 (ELECTRON-2; Cohort 1, Group 1)	Treatment-experienced patients with genotype 1 HCV infection, with or without cirrhosis, who had failed a prior SOF-containing regimen were retreated with LDV/SOF + RBV for 12 weeks	19/19 (100%)	1/1 (100%)
GS-US-337-0115 (ION-4; prior SOF failures subset)	Treatment-experienced patients with genotype 1 HCV infection, with or without cirrhosis, who were coinfected with HIV-1 and had failed a prior SOF + RBV regimen were retreated with LDV/SOF for 12 weeks	13/13 (100%)	1/1 (100%)
CO-US-337-0117 (SYNERGY; Group D)	Treatment-experienced patients with genotype 1 HCV infection, with or without cirrhosis, who had failed a prior SOF + RBV regimen were retreated with LDV/SOF for 12 weeks	14/14 (100%)	–

was achieved in all (100%) patients, irrespective of whether these treatment-experienced patients received 12 weeks of LDV/SOF or LDV/SOF + RBV. This rate was comparable with the rates observed in prior Peg-IFN + RBV ± PI failures in the ION studies, where 100% of patients achieved SVR12.

The presence of pretreatment NS3 RAVs, NS5A RAVs, or NS5B NI RAVs had no clinical impact on whether a patient with genotype 1 infection achieved SVR12 as all patients achieved SVR12. The single patient who relapsed was shown to have genotype 3a infection. The safety profile for patients who had previously failed on a SOF-based treatment was consistent with the expected safety profile of LDV/SOF in the previous phase 3 studies. This data led to the approval of LDV/SOF in previously treated adults who have failed on sofosbuvir + ribavirin ± Peg-IFN on April 23, 2017.

6.7 LDV/SOF in Other Key Populations

The clinical development program of LDV/SOF has generated safety and efficacy data in over 5,900 HCV-infected patients from phase 2 and 3 trials through late 2017. This comprehensive program has included studies in special patient populations [22, 65–71] as well as global, regional, and local studies to support the registration of LDV/SOF worldwide [72–77]. Additional trials in key populations are summarized in Table 15.

7 Conclusion

The development of LDV/SOF (Harvoni®) revolutionized the treatment and management of HCV-infected patients globally. The once-daily, single-tablet regimen of LDV/SOF has been shown to be a highly effective, safe, and tolerable treatment option for patients with chronic HCV across a broad range of characterictics and situations. The pace of the initial clinical development program was unprecedented in its speed due to the widespread recognition from patients, providers, and regulators of the unmet medical need for a safe, simple, and effective all-oral treatment for HCV. In addition a significant number of clinical trials have been conducted in the development program since the first approval of LDV/SOF, with consistent results showing that LDV/SOF is safe and effective across unique populations. This has set a new standard for inclusion of vulnerable groups and special populations in clinical trials in a more timely and comprehensive fashion.

Table 15 Clinical studies that support the efficacy of LDV/SOF in other populations

Population	Study description	SVR12 (n/N)
Bleeding disorders	Treatment-naive and treatment-experienced patients with genotype 1 HCV infection and inherited bleeding disorders, with or without cirrhosis, were treated with LDV/SOF for 12 weeks	14/14 (100%)
Sickle cell disease	Treatment-naive and treatment-experienced patients with genotype 1 or 4 HCV infection and sickle cell disease without cirrhosis were treated with LDV/SOF for 12 weeks (24 weeks for cirrhosis)	9/10 (90%)
Kidney transplant	Treatment-naive and treatment-experienced patients with genotype 1 or 4 HCV infection with or without cirrhosis and post-kidney transplant with eGFR $\geq$40 mL were treated with LDV/SOF for 12 or 24 weeks	114/114 (100%)
Peritransplant	Waitlisted patients who were undergoing a first liver transplantation from an HCV-negative donor were treated with LDV/SOF for 4 weeks postoperatively	15/16 (94%)
Hepatitis B coinfection	Treatment-naive and treatment-experienced patients with genotype 1 HCV infection and active HBV infection with or without cirrhosis were treated with LDV/SOF for 12 weeks	111/111 (100%)
Acute HCV infection	Adults with acute HCV infection were treated with LDV/SOF for 6 weeks	20/20 (100%)
Acute HCV infection in HIV-1-coinfected patients	HIV-1-infected patients with acute HCV infection were treated with LDV/SOF for 6 weeks	20/26 (77%)
Severe renal impairment	Treatment-naive and treatment-experienced patients with genotype 1 HCV infection with or without cirrhosis and severe renal impairment with eGFR $\leq$30 mL were treated with LDV/SOF for 12 weeks	18/18 (100%)
Global studies		
Japan	Treatment-naive and treatment-experienced Japanese patients with genotype 1 HCV infection, with or without cirrhosis, were treated with LDV/SOF with or without RBV for 12 weeks	LDV/SOF: 171/171 (100%) LDV/SOF + RBV: 167/170 (98%)
Korea	Treatment-naive and treatment-experienced Korean patients with genotype 1 HCV infection, with or without cirrhosis, were treated with LDV/SOF for 12 weeks	92/93 (99%)
Taiwan	Treatment-naive and treatment-experienced Taiwanese patients with genotype 1 HCV infection, with or without cirrhosis, were treated with LDV/SOF for 12 weeks	83/85 (98%)

(continued)

Table 15 (continued)

Population	Study description	SVR12 (n/N)
China	Treatment-naive and treatment-experienced Taiwanese patients with genotype 1 HCV infection, with or without cirrhosis, were treated with LDV/SOF for 12 weeks	106/106 (100%)
Egypt	Treatment-naive and treatment-experienced Egyptian patients with genotype 4 HCV infection, with or without cirrhosis, were treated with LDV/SOF ± RBV for 8 or 12 weeks	Treatment naive LDV/SOF 8 weeks: 41/43 (95%) LDV/SOF + RBV 8 weeks: 38/42 (91%) LDV/SOF 12 weeks: 42/43 (98%) LDV/SOF+ RBV 12 weeks: 41/42 (98%) Treatment experienced LDV/SOF 12 weeks: 34/36 (94%) LDV/SOF + RBV 12 weeks: 38/38 (100%)
Russia	Treatment-naive HCV-monoinfected and HCV-/HIV-1-coinfected patients without cirrhosis were treated with LDV/SOF for 8 weeks	69/69 (100%) HIV coinfection: 57/59 (97%)

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Compliance with Ethical Standards

Conflict of Interest Anu Osinusi and John G. McHutchison are employees of Gilead Sciences, Inc.

Ethical Approval All procedures performed in the studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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The Clinical Development of Sofosbuvir/Velpatasvir (SOF/VEL, Epclusa®)

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Abstract The single-tablet regimen of sofosbuvir (SOF), an HCV nucleotide analog NS5B polymerase inhibitor, and velpatasvir (VEL), a second-generation HCV NS5A inhibitor, provides a highly efficacious, safe, and simple treatment regimen for patients with genotype 1–6 HCV infection. The clinical development program for SOF/VEL focused on generating safety and efficacy data across a broad range of patient populations to support a single treatment duration for all patients and therapeutic options for patients with compensated and decompensated liver disease. Three Phase 2 studies defined the optimal dose of VEL as 100 mg for a fixed-dose combination tablet with 400 mg of SOF and demonstrated that the treatment duration

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of 12 weeks provided high SVR rates across all genotypes irrespective of cirrhosis status, prior treatment history, or the presence of baseline resistance-associated substitutions (RASs). The Phase 3 studies enrolled and treated over 1,000 genotype 1–6 HCV-infected patients with 12 weeks of SOF/VEL. In patients with compensated cirrhosis, the overall SVR rate was 98%, and with SOF/VEL + RBV in patients with decompensated cirrhosis, the SVR rate was 94%. With minimal drug-drug interactions and no need for on-treatment safety monitoring, SOF/VEL for 12 weeks provides an important treatment option for patients of all genotypes and is ideally suited to address the global epidemic of chronic HCV infection.

Keywords Decompensated cirrhosis, Elimination, Epclusa, HCV, Pangenotypic, SOF/VEL

1 Introduction

Hepatitis C virus infection is a global health challenge with approximately 80 million persons infected worldwide [1]. Even with interferon-based therapy targeting the host immune system, treatment response rates varied based on genotype, likely due to the substantial genetic variability across genotypes. Early direct-acting antivirals (DAAs) were designed for maximal efficacy against genotype 1 reflecting its predominance in North America and Europe, and, importantly, the first in vitro HCV replicons were limited to genotype 1 only. At Gilead, the ultimate goal for hepatitis C treatment was to develop an all-oral, pangenotypic regimen that could be safely and simply administered across a broad population. Based on the success of tenofovir disoproxil fumarate-containing single tablet regimens for HIV treatment in both in the developed and developing world, there was a keen recognition of the need for this simplicity to have the maximal impact globally on chronic HCV infection. With this goal in mind, a pangenotypic NS5A inhibitor was developed to coformulate with sofosbuvir (SOF), a pangenotypic nucleotide analog nonstructural protein (NS) 5B polymerase inhibitor.

2 Phase 1 Studies

Sofosbuvir had been well characterized from a clinical pharmacology perspective at the time velpatasvir (VEL) was developed. Thus, the Phase 1 program focused on studies with VEL alone initially and, then, in combination with SOF to further define drug interactions. The plasma half-life for VEL of approximately 15 h supported once daily dosing. Velpatasvir is absorbed relatively rapidly, with a median time to C_{max} (T_{max}) of 3 h (Gilead). Velpatasvir is highly protein bound (>99.5%) and is minimally metabolized with biliary excretion of unchanged VEL as the major route of elimination accounting for 77% of recovered drug in a clinical absorption, distribution, metabolism, and excretion study (Gilead). Studies conducted in patients

with renal insufficiency or hepatic impairment demonstrated that no dose adjustment is needed for VEL in patients with end-stage renal disease or those with severe hepatic insufficiency (Gilead). Furthermore, population pharmacokinetic analysis in HCV-infected patients indicated that race, gender, age, and BMI have no clinically relevant effect on the exposure of VEL (or SOF or its major metabolite, GS-331007). Velpatasvir exposure increases approximately 30% when coadministered with a meal, supporting the dosing recommendation of SOF/VEL to be administered without regard to food.

In vitro, VEL was determined to be a substrate and an inhibitor of P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), a substrate of CYP2B6, CYP2C8, and CYP3A4 with slow turnover and an inhibitor of OATP1B1, OATP1B3, and OATP2B1. A large number of drug interaction studies were conducted with SOF/VEL to assess for the potential of clinically meaningful drug interactions. Overall, SOF/VEL has a clinical pharmacology and drug interaction profile that make it well suited for a diverse patient population. Potent inducers of P-gp and/or moderate or potent inducers of CYP2B6, CYP2C8, or CYP3A4 (e.g., rifampin, St. John's wort, carbamazepine) will reduce plasma concentrations of SOF and/or VEL and should be avoided. Immunosuppressants such as cyclosporine and tacrolimus can be safely coadministered as can opiate substitution therapy and oral contraceptives. Efavirenz and tipranavir are the two antiretroviral agents that should be avoided with SOF/VEL coadministration, and statin exposure can increase with SOF/VEL coadministration – the risk of rhabdomyolysis may be increased for these patients, and for rosuvastatin, a dose no higher than 10 mg daily should be used. Absorption of VEL is pH-dependent, and therefore acid-reducing agents can lower exposure. This effect can be minimized with specific dosing instructions: antacid dosing should be separated by at least 4 h from SOF/VEL; H_2-receptor antagonists should be given simultaneously or 12 h apart from SOF/VEL at a dose no higher than famotidine 40 mg twice daily or equivalent; the effect of proton-pump inhibitors up to a dose of omeprazole 20 mg daily or its equivalent can be largely mitigated through coadministration of SOF/VEL with food.

3 Phase 1b Study

Once preliminary safety and pharmacokinetic data were obtained from single and multiple doses of VEL ranging from 50 to 450 mg in healthy subjects, a Phase 1b study, GS-US-281-0102, was undertaken to assess the antiviral activity and safety and pharmacokinetic profiles of VEL administration for 3 days at doses of 5–150 mg in genotype 1–4 HCV-infected patients [2]. A total of 11 dosing cohorts were enrolled across 10 sites in the United States and Puerto Rico: five cohorts of patients with genotype 1a HCV infection (5, 25, 50, 100, and 150 mg VEL); one cohort each of patients with genotype 1b, 2, or 4 HCV infection (150 mg VEL); and three cohorts of patients with genotype 3 HCV infection (25, 50, and 150 mg VEL). Within each cohort, patients were randomized in a 4:1 ratio to VEL or placebo except for the

cohort of patients with genotype 4 HCV infection, all of whom received VEL. Patients were excluded from participation if they had cirrhosis or prior exposure to an HCV NS5A inhibitor.

Of the 87 patients treated, 84 completed 3 days of dosing and 2 weeks follow-up (Day 17). One patient discontinued on Day 1 due to an adverse event of nausea, one withdrew consent on Day 4 after completing dosing, and one was lost to follow-up after the Day 7 visit. A total of 61 patients completed 48 weeks of long-term follow-up. Most patients were male (78%), nearly one-third (31%) were black or African-American, and baseline viral load was similar across dosing groups and genotypes with a mean HCV RNA of 6.43 $\log_{10}$ IU/mL. Between Day 1 and Day 17, 21/87 patients (24%) reported at least one adverse event: 18/70 (26%) of the VEL-treated patients and 3/17 (18%) of the placebo-treated patients. All adverse events were mild or moderate in severity with headache being the most frequently reported adverse event (6/87 patients, 7%). No deaths or serious adverse events were reported from Day 1 through week 48 of follow-up. There was no trend in adverse events relative to the dose of VEL and no clinically relevant changes in laboratory values, vital signs, physical examination findings, or ECGs. The pharmacokinetics of VEL were similar to those observed in healthy volunteers and confirmed that VEL is suitable for once-daily dosing in patients with HCV infection.

Administration of three daily doses of VEL resulted in rapid reductions in HCV RNA such that the median maximal decline in HCV RNA across all genotypes at all VEL doses evaluated was >3 $\log_{10}$ IU/mL (Fig. 1). Among patients with genotype 1a HCV infection, the median maximum HCV RNA decline was >3.6 $\log_{10}$ IU/mL at all doses from 5 to 150 mg. Patients with genotype 1b and 2 HCV infection who received VEL 150 mg for 3 days had median (Q1, Q3) maximal viral load reductions of 4.3 (4.2, 4.4) and 4.4 (4.1, 4.8) $\log_{10}$ IU/mL, respectively. The median (Q1, Q3) maximal viral load reductions in patients with genotype 3 HCV infection were 3.2 (1.0, 4.0) $\log_{10}$ IU/mL, 3.1 (1.9, 3.3) $\log_{10}$ IU/mL, and 3.1 (2.9, 3.8) $\log_{10}$ IU/mL for the 25, 50, and 150 mg VEL doses, respectively. The two patients with genotype 4 HCV infection had maximal viral load reductions of 3.9 and 3.0 $\log_{10}$ IU/mL. Patients receiving the 5 mg VEL dose experienced more rapid viral rebound after treatment than patients receiving higher VEL doses although all patients had HCV RNA return to baseline levels during the follow-up period. Analysis of NS5A sequences was also undertaken. At baseline, 22/70 patients (31%) had pretreatment NS5A resistance-associated substitutions (RASs) detected using a cutoff of 1%. Patients with genotype 1 or 3 HCV infection without pretreatment RASs had greater declines in HCV RNA compared to patients with pretreatment RASs. This difference was most notable at the 25 and 50 mg doses of VEL in genotype 3 HCV-infected patients, whereas at the 150 mg dose level, the difference was not observed. Among the patients with 48 weeks of follow-up, RASs that were present at baseline generally persisted through the follow-up period, whereas those that had emerged during treatment tended to decline over time.

Based on the totality of safety, pharmacokinetic, and antiviral activity, 25 and 100 mg doses of VEL were selected to move forward in combination with SOF for Phase 2 trials in HCV-infected patients.

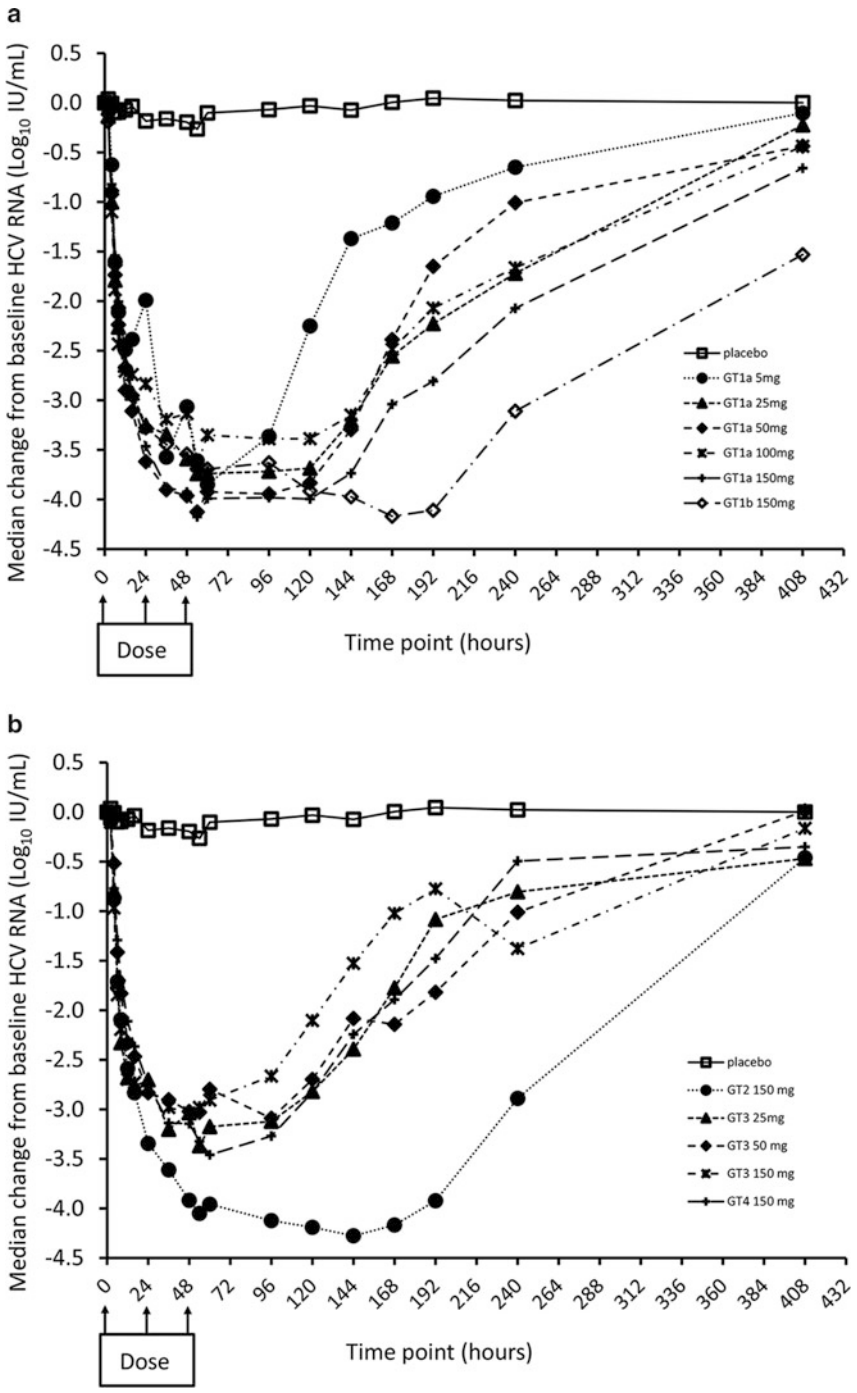

Fig. 1 Viral load reductions over time following three doses of velpatasvir in (**a**) genotype 1 HCV-infected patients and (**b**) genotype 2, 3, or 4 HCV-infected patients (Reproduced from [2])

4 Phase 2 Studies

The potent antiviral activity of VEL across genotypes 1–4 and the previously demonstrated efficacy of SOF as well as the combination of ledipasvir (LDV) and SOF as an approved single-tablet regimen for genotype 1, 4, 5, or 6 HCV infection suggested that the combination of SOF/VEL would be highly efficacious as a therapeutic regimen. Thus, the Phase 2 program was designed to address three fundamental questions. The first was regarding dose selection for VEL (25 mg versus 100 mg); the second was regarding duration of treatment (8 weeks versus 12 weeks); the third was regarding the contribution of ribavirin to safety and efficacy. Recognizing that SOF/VEL had the potential with its pangenotypic activity to be a cornerstone of an HCV elimination strategy globally which would include resource-limited settings, the goal was to determine the optimal dose and duration to provide maximal efficacy and safety across a broad patient population irrespective of genotype, prior treatment history, or fibrosis status to advance into Phase 3 clinical trials and, ultimately, to patients where genotyping would no longer be a necessary component of the HCV treatment algorithm. Safety, efficacy, and pharmacokinetic data were generated from three Phase 2 studies described separately below.

4.1 Study GS-US-342-0102

Study GS-US-342-0102 enrolled treatment-naïve genotype 1–6 HCV-infected patients without cirrhosis [3]. The study was conducted at 48 sites in the United States from August 2013 through August 2014 in two parts. In Part A, genotype 1–6 HCV-infected patients were randomized to receive SOF 400 mg with velpatasvir, 25 or 100 mg, for 12 weeks (groups 1–6). In Part B which was initiated following a review of the safety and efficacy of patients enrolled in Part A, genotype 1 or 2 HCV-infected patients were randomized to receive SOF 400 mg with velpatasvir 25 or 100 mg, with or without weight-based RBV (1,000–1,200 mg daily) for 8 weeks (groups 7–14). Patients were required to have cirrhosis excluded by either liver biopsy within 2 years of screening, a FibroTest score of 0.48 or less and an aspartate aminotransferase-platelet ratio index of 1 or less during screening, or a Fibroscan score of 12.5 kPa or less within 6 months of baseline. Additional exclusion criteria included HIV or HBV coinfection, hepatic decompensation, prior treatment for HCV, and select laboratory abnormalities. The primary endpoint was sustained virologic response 12 weeks after treatment completion (SVR12).

A total of 377 patients were randomized and treated. Table 1 shows demographic, disease, and baseline characteristics by dose and duration. In general, patients were representative of a treatment-naïve population in the United States. Within the different dosing groups, demographic factors were balanced across the different genotypes. All but three patients completed study treatment. One genotype 3 HCV-infected patient receiving SOF + VEL 25 mg for 12 weeks discontinued at

Table 1 Demographic and baseline characteristics in study GS-US-342-0102

	SOF + VEL 25 mg 12 weeks ($N = 77$)	SOF + VEL 100 mg 12 weeks ($N = 77$)	SOF + VEL 25 mg 8 weeks ($N = 56$)	SOF + VEL 25 mg + RBV 8 weeks ($N = 55$)	SOF + VEL 100 mg 8 weeks ($N = 55$)	SOF + VEL 100 mg + RBV 8 weeks ($N = 57$)	Total ($N = 377$)
Mean age (range), years	50 (23–78)	51 (20–70)	51 (19–70)	53 (24–68)	54 (21–71)	51 (18–69)	52 (18–78)
Mean BMI (range), kg/m^2	28 (19–57)	27 (19–48)	26 (19–42)	29 (18–45)	28 (20–43)	30 (21–53)	28 (18–57)
Male, *n* (%)	49 (64)	49 (64)	31 (55)	35 (64)	28 (51)	26 (46)	218 (58)
Race, *n* (%)							
White	64 (83)	67 (87)	48 (86)	50 (91)	48 (87)	49 (86)	326 (87)
Black	10 (13)	2 (3)	6 (11)	5 (9)	7 (13)	5 (9)	35 (9)
HCV genotype, *n* (%)							
Genotype 1	27 (35)	28 (36)	30 (54)	30 (55)	29 (53)	31 (54)	175 (46)
Genotype 1a	22 (29)	21 (27)	25 (45)	25 (46)	24 (44)	25 (44)	142 (38)
Genotype 1b	5 (7)	6 (8)	5 (9)	5 (9)	5 (9)	6 (11)	32 (9)
Genotype 1g	0	1 (1)	0	0	0	0	1 (<1)
Genotype 2	11 (14)	10 (13)	26 (46)	25 (46)	26 (47)	26 (46)	124 (33)
Genotype 3	27 (35)	27 (35)	0	0	0	0	54 (14)
Genotype 4	7 (9)	7 (9)	0	0	0	0	14 (4)
Genotype 5	1 (1)	0	0	0	0	0	1 (<1)
Genotype 6	4 (5)	5 (7)	0	0	0	0	9 (2)
Mean HCV RNA (SD), $\log_{10}$ IU/mL	6.4 (0.82)	6.4 (0.76)	6.5 (0.77)	6.5 (0.71)	6.4 (0.80)	6.7 (0.56)	6.5 (0.75)
HCV RNA ≥800,000 IU/mL, *n* (%)	59 (77)	59 (77)	45 (80)	46 (84)	42 (76)	52 (91)	303 (80)

(continued)

Table 1 (continued)

	SOF + VEL 25 mg 12 weeks ($N = 77$)	SOF + VEL 100 mg 12 weeks ($N = 77$)	SOF + VEL 25 mg 8 weeks ($N = 56$)	SOF + VEL 25 mg + RBV 8 weeks ($N = 55$)	SOF + VEL 100 mg 8 weeks ($N = 55$)	SOF + VEL 100 mg + RBV 8 weeks ($N = 57$)	Total ($N = 377$)
IL28B genotype CC, *n* (%)	25 (33)	31 (40)	18 (32)	23 (42)	22 (40)	16 (28)	135 (36)
Baseline ALT $>1.5 \times$ ULN, *n* (%)	45 (58)	40 (52)	27 (48)	23 (42)	27 (49)	31 (54)	193 (51)

ALT alanine aminotransferase, *BMI* body mass index, *HCV* hepatitis C virus, *RBV* ribavirin, *SOF* sofosbuvir, *ULN* upper limit of normal, *VEL* velpatasvir

week 8 due to virologic failure; one genotype 1 HCV-infected patient receiving SOF + VEL 25 mg for 8 weeks discontinued at Day 6 due to adverse events of abdominal pain, palpitations, and dizziness; and one genotype 1 HCV-infected patient receiving SOF + VEL 100 mg for 8 weeks discontinued due to noncompliance with study drugs.

Overall, among the 377 patients randomized and treated, 337 (89%) achieved SVR12 (Table 2). In part A, assessing 12 weeks of SOF + VEL treatment, the SVR12 rate was 96% (26/27) in those receiving SOF + VEL 25 mg (group 1) and 100% (28/28) in those receiving SOF + VEL 100 mg (group 2). Among patients with genotype 3 HCV infection, the SVR12 rate was 93% (25/27) in those receiving

Table 2 Virologic outcomes in study GS-US-342-0102

			% SVR12 (95% CI)	On-treatment virologic failure, *n* (%)	Relapse, *n* (%)	Other, *n* (%)
Part A 12 weeks	GT1	SOF + VEL 25 mg, $n = 27$	96 (81–100)	0 (0)	1 (4)	0 (0)
		SOF + VEL 100 mg, $n = 28$	100 (88–100)	0 (0)	0 (0)	0 (0)
	GT3	SOF + VEL 25 mg, $n = 27$	93 (76–99)	1 (4)	1 (4)	0 (0)
		SOF + VEL 100 mg, $n = 27$	93 (76–99)	0 (0)	2 (7)	0 (0)
	GT2/ 4/5/6	SOF + VEL 25 mg, $n = 23$	96 (78–100)	0 (0)	0 (0)	1 (4)
		SOF + VEL 100 mg, $n = 22$	95 (77–100)	0 (0)	0 (0)	1 (5)
Part B 8 weeks	GT1	SOF + VEL 25 mg, $n = 30$	87 (69–96)	0 (0)	3 (10)	1 (3)
		SOF + VEL 25 mg + RBV, $n = 30$	83 (65–94)	0 (0)	5 (17)	0 (0)
		SOF + VEL 100 mg, $n = 29$	90 (73–98)	0 (0)	3 (10)	0 (0)
		SOF + VEL 100 mg + RBV, $n = 31$	81 (63–93)	0 (0)	5 (16)	1 (3)
	GT2	SOF + VEL 25 mg, $n = 26$	77 (56–91)	0 (0)	6 (23)	0 (0)
		SOF + VEL 25 mg + RBV, $n = 25$	88 (69–98)	0 (0)	2 (8)	1 (4)
		SOF + VEL 100 mg, $n = 26$	88 (70–98)	0 (0)	3 (12)	0 (0)
		SOF + VEL 100 mg + RBV, $n = 26$	88 (70–98)	0 (0)	3 (12)	0 (0)

SOF + VEL 25 mg (group 3) as well as SOF + VEL 100 mg (group 4). The two patients who did not achieve SVR12 in group 3 experienced virologic failure – one subject had a 5 $\log_{10}$ IU/mL HCV RNA reduction after 4 weeks of treatment but failed to fully suppress by week 8 and thus met a virologic stopping criterion; the other patient relapsed at posttreatment week 4. In group 4, one patient experienced virologic relapse, and one patient had evidence for reinfection with genotype 2b that was not detectable with deep sequencing prior to treatment. The SVR12 rate in patients with genotype 2, 4, 5, or 6 HCV infection receiving SOF + VEL 25 mg (group 5) or SOF + VEL 100 mg was 96% (22/23) and 95% (21/22), respectively. There were no virologic failures in either of these groups; one patient committed suicide prior to posttreatment week 12, and the other patient was lost to follow-up after completing treatment. The high SVR rate and low rate of virologic failure in treatment-naïve, genotype 1–6 HCV-infected patients without cirrhosis treated for 12 weeks with SOF + VEL 25 mg or 100 mg supported assessing a shorter treatment duration.

In order to examine both the 25 and 100 mg doses of VEL and the impact of RBV on an 8-week treatment duration, part B was limited to genotype 1 or 2 patients only. This facilitated enrollment, as well, given the genotype distribution within the United States. The shortened treatment duration of 8 weeks for genotype 3 HCV-infected treatment-naïve patients was assessed in a Phase 2 study conducted in New Zealand and is discussed below. Rates of SVR12 among genotype 1 HCV-infected patients were 87% (26/30) for those receiving VEL 25 mg, 83% (25/30) for those receiving VEL plus RBV, 90% (26/29) for those receiving VEL 100 mg, and 81% (25/31) for those receiving VEL plus RBV. Other than one patient in the SOF + VEL 25 mg group who discontinued treatment on Day 6 and one patient in the SOF + VEL 100 mg group who was lost to follow-up, virologic relapse occurred in patients not achieving SVR12. Among genotype 2 HCV-infected patients, SVR12 rates were 77% (20/26) with VEL 25 mg, 88% (22/25) with VEL 25 mg plus RBV, 88% (23/26) with both VEL 100 mg and VEL 100 mg plus RBV. One patient in the VEL 25 mg plus RBV group did not complete posttreatment assessments, and all other non-SVR12 patients experienced virologic relapse.

Deep sequencing of the HCV NS5A and NS5B genes was performed from pretreatment samples from all patients and from posttreatment samples from all patients with virologic failure. Of the 377 patients enrolled, 375 and 372 had sequencing data for HCV NS5A and NS5B, respectively. The prevalence of pretreatment NS5A RASs detected with a 15% cutoff was 34% (128/375) and 18% (25/142), 23% (7/31), 48% (58/122), and 24% (13/54) in patients with genotype 1a, 1b, 2, and 3 HCV infections, respectively. In contrast, the rates of pretreatment HCV NS5B RASs were much lower with only 5% (17/372) of patients overall having these RASs at baseline. Overall, rates of SVR12 were similar among patients with pretreatment NS5A RASs (90%) as compared to those without pretreatment NS5A RASs (92%). The impact of pretreatment NS5A RASs did not substantially differ based on treatment duration and/or genotype.

Overall, treatment with SOF + VEL with or without RBV was well tolerated, with only one patient (<1%) discontinuing treatment due to an adverse event. This

patient, a 19-year-old white woman with genotype 1 HCV infection, was receiving SOF + VEL 25 mg and experienced mild abdominal pain, mild palpitations, and moderate dizziness on treatment Day 6. The investigator assessed these events as related to study drug, and treatment was discontinued on the following day. All of these events resolved by Day 2 of follow-up. Across all treatment groups, there were low rates of serious adverse events (2%), none of which were assessed by the investigator as related to study drugs, and one death occurred in the study: a 36-year-old man with genotype 2 HCV infection and preexisting psychiatric disease committed suicide after completing 12 weeks of treatment with SOF + VEL 25 mg (group 5). Patients administered with RBV-containing regimens had a higher incidence of RBV-associated toxicities such as fatigue, insomnia, and rash and laboratory abnormalities consistent with hemolysis such as decreased hemoglobin and elevated bilirubin levels. No difference in the type or incidence of adverse events between treatment regimens with respect to dose of VEL or treatment duration was observed. Fatigue and headache were the only adverse events occurring in >10% of patients in the SOF + VEL 100 mg 12-week treatment groups.

Sofosbuvir with VEL 25 or 100 mg for 12 weeks was well tolerated and resulted in high SVR12 rates in noncirrhotic patients infected with genotypes 1–6. With 8 weeks of treatment, higher relapse rates were observed among the genotype 1 or genotype 2 HCV-infected patients at both the 25 and 100 mg dose of VEL, and the addition of RBV did not impact SVR12 rates. These data supported the further development of a fixed-dose combination tablet of SOF/VEL at the 12-week treatment duration.

4.2 Study GS-US-337-0122

The impact of shortening SOF + VEL treatment duration from 12 to 8 weeks in genotype 1 or 2 HCV-infected patients was assessed in GS-US-342-0102, conducted in the United States where genotype 3 HCV infection represents approximately 6% of total HCV-infected patients. In contrast, genotype 3 HCV-infected patients make up over 30% of total HCV infections in New Zealand. Study GS-US-337-0122 (ELECTRON-2) was an ongoing Phase 2 clinical trial at two sites in New Zealand. This trial was amended to assess the safety and efficacy of SOF + VEL 25 or 100 mg with or without RBV for 8 weeks in treatment-naïve genotype 3 HCV-infected patients without cirrhosis [4].

A total of 104 patients were randomized to one of the four treatment groups. Demographic and baseline characteristics are provided in Table 3. The patient population was similar to that enrolled in GS-US-342-0102 with the exception of a higher percentage of native Hawaiian/Pacific Islander patients enrolled in this trial and higher percentage of black patients enrolled in GS-US-342-0102. All but two patients completed treatment. One patient withdrew consent for participation in the study, and one patient discontinued treatment due to a flare of preexisting eczema.

Table 3 Demographic and baseline characteristics in study GS-US-337-0122

	SOF + VEL 25 mg 8 weeks ($N = 27$)	SOF + VEL 25 mg + RBV 8 weeks ($N = 24$)	SOF + VEL 100 mg 8 weeks ($N = 27$)	SOF + VEL 100 mg + RBV 8 weeks ($N = 26$)
Mean age (range), years	48 (29–59)	47 (35–61)	50 (20–63)	47 (29–64)
Mean BMI (range), kg/m^2	25 (20–31)	26 (18–38)	26 (19–33)	26 (18–36)
Male, *n* (%)	17 (63)	18 (75)	17 (63)	11 (42)
Race, *n* (%)				
White	20 (74)	20 (83)	20 (74)	19 (73)
Native Hawaiian/Pacific Islander	5 (19)	2 (8)	3 (11)	6 (23)
HCV genotype, *n* (%)				
Genotype 3	2 (7)	1 (4)	0	0
Genotype 3a	25 (93)	22 (92)	27 (100)	26 (100)
Genotype 3k	0	1 (4)	0	0
Mean HCV RNA (SD), $\log_{10}$ IU/mL	5.9 (0.86)	6.3 (0.69)	6.0 (0.71)	6.2 (0.92)
HCV RNA $\geq$800,000 IU/mL, *n* (%)	13 (48)	14 (58)	16 (59)	19 (73)
IL28B genotype CC, *n* (%)	10 (37)	6 (25)	15 (56)	14 (54)
Baseline ALT $>1.5 \times$ ULN, *n* (%)	10 (37)	14 (58)	13 (48)	12 (46)

ALT alanine aminotransferase, *BMI* body mass index, *HCV* hepatitis C virus, *RBV* ribavirin, *SOF* sofosbuvir, *ULN* upper limit of normal, *VEL* velpatasvir

Table 4 Virologic outcomes in study GS-US-337-0122

8 weeks treatment	% SVR12 (95% CI)	On-treatment virologic failure, *n* (%)	Relapse, *n* (%)	Other, *n* (%)
SOF + VEL 25 mg, $n = 26$	100 (87–100)	0 (0)	0 (0)	0 (0)
SOF + VEL 25 mg + RBV, $n = 24$	88 (68–97)	0 (0)	2 (8)	1 (4)
SOF + VEL 100 mg, $n = 27$	96 (81–100)	0 (0)	0 (0)	1 (4)
SOF + VEL 100 mg + RBV, $n = 26$	100 (87–100)	0 (0)	0 (0)	0 (0)

Virologic outcomes following 8 weeks of SOF + VEL at both dose levels and with or without RBV are presented in Table 4. All patients who received SOF + VEL 25 mg for 8 weeks achieved SVR12. The SVR12 rate was 88% in patients who

received SOF + VEL 25 mg + RBV for 8 weeks with two patients experiencing virologic relapse and one patient discontinuing treatment prior to virologic suppression. In the SOF + VEL 100 mg groups, SVR rates were 96% and 100%, without and with RBV, respectively. There were no virologic failures in either treatment group; one patient withdrew consent from the trial.

The combination of SOF + VEL 25 or 100 mg with or without RBV for 8 weeks was well tolerated. One patient discontinued SOF + VEL 25 mg + RBV treatment due to an exacerbation of facial eczema and eye inflammation. A second patient in the SOF + VEL 100 mg group discontinued RBV only due to dyspepsia and lethargy. Only one serious ADVERSE EVENT occurred in the trial (convulsion) which was assessed by the investigator as unrelated to study drug. No difference in the type or incidence of ADVERSE EVENTs between treatment regimens with respect to dose of VEL or treatment duration was observed.

This Phase 2 study suggested that high SVR12 rates could be achieved in genotype 3 HCV-infected patients without cirrhosis treated for 8 weeks with SOF + VEL. The higher dose of VEL 100 mg was associated with a slightly higher SVR rate – no virologic failures were observed in the SOF + VEL 100 mg treatment groups. The addition of RBV increased hematologic toxicity but did not improve efficacy.

4.3 Study GS-US-342-0109

Study GS-US-342-0109 was conducted in parallel with Study GS-US-342-0102 and enrolled treatment-experienced genotype 1 or 3 HCV-infected patients with or without cirrhosis [5]. The study was conducted at 58 sites in the United States, Australia, and New Zealand from June 2013 through August 2014. Three cohorts of patients were enrolled: treatment-experienced genotype 3 HCV-infected patients without cirrhosis, treatment-experienced genotype 3 HCV-infected patients with cirrhosis, and treatment-experienced genotype 1 HCV-infected patients with or without cirrhosis. For genotype 3 HCV-infected patients, treatment-experienced was defined as having failed prior therapy with an interferon-based regimen, whereas for genotype 1 HCV-infected patients, prior treatment experience was limited to patients who had failed an NS3/4A protease inhibitor in combination with peginterferon and RBV. Within these three cohorts, patients were randomized to one of four treatment groups to receive SOF 400 mg with VEL, 25 or 100 mg, with or without weight-based RBV (1,000–1,200 mg daily) for 12 weeks. Inclusion and exclusion criteria were otherwise similar to GS-US-342-0102, the Phase 2 trial of SOF + VEL in treatment-naïve genotype 1–6 HCV-infected patients. The primary endpoint was SVR12.

A total of 321 patients were randomized and treated. Table 5 shows demographic, disease, and baseline characteristics by dose and duration. In general, patients were representative of a treatment-experienced population. As compared to treatment-naïve patients without cirrhosis enrolled in GS-US-342-0102, these patients were

Table 5 Demographic and baseline characteristics in study GS-US-342-0109

	SOF + VEL 25 mg ($N = 79$)	SOF + VEL 25 mg + RBV ($N = 82$)	SOF + VEL 100 mg ($N = 80$)	SOF + VEL 100 mg + RBV ($N = 80$)	Total ($N = 321$)
Mean age (range), years	55 (22–69)	55 (25–67)	56 (32–68)	55 (41–72)	55 (22–72)
Mean BMI (range), kg/m^2	27 (20–43)	29 (20–45)	29 (20–50)	29 (18–39)	28 (18–50)
Male, *n* (%)	54 (68)	59 (72)	53 (66)	55 (69)	221 (69)
Race, *n* (%)					
White	70 (89)	76 (93)	73 (91)	69 (86)	288 (90)
Black	7 (9)	3 (4)	4 (5)	5 (6)	19 (6)
HCV genotype, *n* (%)					
Genotype 1[a]	27 (34)	29 (35)	27 (34)	28 (35)	111 (35)
Genotype 1a	19 (24)	22 (27)	21 (26)	22 (28)	84 (26)
Genotype 1b	7 (9)	7 (9)	6 (8)	6 (8)	26 (8)
Genotype 3	52 (66)	53 (65)	53 (66)	52 (65)	210 (65)
Mean HCV RNA (SD), $\log_{10}$ IU/mL	6.6 (0.61)	6.6 (0.64)	6.5 (0.63)	6.6 (0.50)	6.6 (0.60)
HCV RNA $\geq$800,000 IU/mL, *n* (%)	65 (82)	69 (84)	68 (85)	73 (91)	275 (86)
IL28B genotype CC, *n* (%)	20 (25)	16 (20)	22 (28)	27 (34)	85 (27)
Baseline ALT $>1.5 \times$ ULN, *n* (%)	49 (62)	51 (62)	51 (64)	50 (63)	201 (63)

ALT alanine aminotransferase, *BMI* body mass index, *HCV* hepatitis C virus, *RBV* ribavirin, *SOF* sofosbuvir, *ULN* upper limit of normal, *VEL* velpatasvir
[a]One genotype 1 patient did not have a confirmable subtype

slightly older, and there was a higher percentage of males, *non-IL28B CC* genotype, higher viral load, and abnormal ALT levels at baseline. Within the different dosing groups, demographic factors were balanced across genotype 1 and genotype 3 patients. Approximately 1/3 of the genotype1 HCV-infected patients had cirrhosis. All but two patients completed study treatment. One genotype 3 HCV-infected patient without cirrhosis receiving SOF + VEL 25 mg + RBV for 12 weeks discontinued treatment due to elevated gamma glutamyltransferase (GGT) and ALT, and one genotype 3 HCV-infected patient with cirrhosis receiving SOF + VEL 25 mg + RBV for 12 weeks discontinued due to noncompliance with study drugs and subsequently withdrew consent.

Table 6 shows SVR12 rates in all treatment groups. Among the treatment-experienced patients with genotype 3 HCV infection without cirrhosis who received SOF plus VEL 25 mg without or with RBV, the SVR12 rates were 85% and 96%, respectively. All treatment-experienced patients with genotype 3 HCV infection without cirrhosis who received SOF plus VEL 100 mg without or with RBV achieved SVR12. Among the treatment-experienced patients with genotype 3 HCV infection and cirrhosis who received SOF plus VEL 25 mg without or with RBV, the SVR12 rates were 58% and 84%, respectively. The SVR12 rates in treatment-experienced patients with genotype 3 HCV infection and cirrhosis who received SOF plus VEL 100 mg without or with RBV were 88% and 96%, respectively. Among patients with genotype 1 HCV infection who had not achieved SVR after previous treatment with a protease inhibitor regimen, SVR 12 rates were 100% and 97% in those treated with SOF plus VEL 25 mg without and with RBV,

Table 6 Virologic outcomes in study GS-US-342-0109

		% SVR12 (95% CI)	Relapse	Other
GT3 No cirrhosis	SOF + VEL 25 mg, $n = 26$	85 (65–96)	4 (15)	0 (0)
	SOF + VEL 25 mg + RBV, $n = 28$	96 (82–100)	1 (4)	
	SOF + VEL 100 mg, $n = 27$	100 (87–100)	0 (0)	0 (0)
	SOF + VEL 100 mg + RBV, $n = 26$	100 (87–100)	0 (0)	0 (0)
GT3 cirrhosis	SOF + VEL 25 mg, $n = 26$	58 (37–77)	11 (42)	0 (0)
	SOF + VEL 25 mg + RBV, $n = 25$	84 (64–96)	3 (12)	1 (4)
	SOF + VEL 100 mg, $n = 26$	89 (70–98)	3 (12)	0 (0)
	SOF + VEL 100 mg + RBV, $n = 26$	96 (80–100)	1 (4)	0 (0)
GT1 No cirrhosis and cirrhosis	SOF + VEL 25 mg, $n = 27$	100 (87–100)	0 (0)	0 (0)
	SOF + VEL 25 mg + RBV, $n = 29$	97 (82–100)	1 (3)	0 (0)
	SOF + VEL 100 mg, $n = 27$	100 (87–100)	0 (0)	0 (0)
	SOF + VEL 100 mg + RBV, $n = 28$	96 (82–100)	0 (0)	0 (0)

respectively, and 100% and 96% in those treated with SOF plus VEL 100 mg without and with RBV, respectively. In contrast to the efficacy data generated in treatment-naïve patients in Study GS-US-342-0102 which didn't differentiate between the two doses of VEL, in the current study including genotype 3 treatment-experienced patients, the VEL 100 mg dose demonstrated higher SVR rates as compared to those observed with SOF + VEL 25 mg.

Deep sequencing of the HCV NS5A and NS5B genes was successfully performed from pretreatment samples for 321 and 318 patients, respectively, and from posttreatment samples from all patients with virologic failure. The prevalence of pretreatment NS5A RASs detected with a 15% cutoff was 17% (53/321) overall: 17% (36/210) in patients with genotype 3 HCV infection and 15% (17/111) in patients with genotype 1 HCV infection. Among patients with genotype 3 HCV infection without cirrhosis, SVR12 rates were similar in patients with and without NS5A RASs. Only 1 of the 11 genotype 3 HCV-infected patients with cirrhosis who relapsed following treatment with SOF plus VEL 25 mg had pretreatment NS5A RASs. The SVR12 rate among genotype 1 HCV-infected patients with RASs was 96% (16/17). Overall, these data suggested that NS5A RASs did not influence treatment outcome even with the lower dose of VEL. The prevalence of HCV NS5B RASs overall was lower with only 4% (11/318) of patients having NS5B RASs at baseline. All but one of these patients achieved SVR12.

Treatment with SOF + VEL with or without RBV was well tolerated, with only one patient (<1%) discontinuing treatment due to an adverse event. This patient, a 58-year-old white woman without cirrhosis and genotype 3 HCV infection, was receiving SOF + VEL 25 mg plus RBV and experienced an elevated ALT and GGT levels on treatment Day 80. The investigator assessed these events as related to a study drug, and treatment was discontinued on the following day; she achieved SVR12. This patient's GGT level returned to pretreatment levels by posttreatment Day 11 and ALT level normalized by posttreatment Day 33. Total bilirubin levels remained normal throughout. Across all treatment groups, there was a low rate of serious adverse events (2%), and none was assessed by the investigator as related to study drugs. Patients administered with RBV-containing regimens had a higher incidence of RBV-associated toxicities such as fatigue, insomnia, and rash and laboratory abnormalities consistent with hemolysis such as decreased hemoglobin and elevated bilirubin levels. No difference in the type or incidence of adverse events between treatment regimens with respect to dose of VEL or treatment duration was observed. Adverse events were similar to those observed in Study GS-US-342-0102 and did not differ based on cirrhosis status.

Among treatment-experienced genotype 1 or 3 HCV-infected patients with or without cirrhosis, SOF with VEL 100 mg for 12 weeks resulted in consistently high SVR12 rates. With the lower dose of VEL, higher relapse rates were observed among the genotype 3 HCV-infected patients with or without cirrhosis; the addition of RBV improved SVR12 rates to some extent in this situation. Given the goal of the SOF/VEL program to have a single-tablet regimen supporting a single treatment duration for patients irrespective of genotype, prior treatment, or cirrhosis status, these data, in combination with those from GS-US-342-0102, supported the further

development of a fixed-dose combination tablet of SOF 400 mg/VEL 100 mg for a 12-week treatment duration.

As described above, these Phase 2 studies assessed the combination of SOF and VEL coadministered as separate agents. It is worth noting that while these trials were ongoing, significant formulation efforts were underway to develop a fixed-dose combination (FDC). Since the dose for Phase 3 was unknown at the time, both the 25 and 100 mg doses of VEL were coformulated with SOF 400 mg. As the SOF/VEL 400/100 mg FDC was selected to move ahead into Phase 3, this formulation was assessed in a bioavailability study comparing the pharmacokinetics of the two drugs coadministered as separate agents as compared to administered as an FDC. The exposure to SOF, SOF metabolites, and VEL was similar across both formulations thus enabling transition to the single-tablet regimen for the registrational Phase 3 trials. This Phase 1 study, 342-0104, also assessed the impact of high-fat or medium-fat meal on the pharmacokinetics of the SOF/VEL FDC and demonstrated that food modestly increased VEL exposure to a degree that would not be anticipated to impact efficacy or safety based on the clinical data. These results enabled coadministration of SOF/VEL without regard to food in the Phase 3 studies.

5 Phase 3 Studies

The dose of VEL (100 mg) in combination with SOF 400 mg and the duration of therapy (12 weeks) were established based on the safety and efficacy results in genotype 1–6 HCV-infected patients enrolled in the three Phase 2 studies. The SOF/VEL Phase 3 studies were designed to evaluate the efficacy and safety of treatment with SOF/VEL in a diverse subject population with respect to HCV genotypes and subtypes, demographic characteristics, and geographical regions. Three multicenter studies evaluated SOF/VEL in subjects without cirrhosis or with compensated cirrhosis, and one multicenter study evaluated regimens of SOF/VEL in subjects with decompensated cirrhosis. At this time (first half of 2014), SOF had been approved, in combination with pegylated interferon for genotype 1 and 4 HCV-infected patients and in combination with RBV for 12 or 24 weeks in genotype 2 or 3 HCV-infected patients, respectively. This treatment landscape informed the study design for each trial, as outlined below. The goal was to demonstrate that SOF/VEL could be a highly effective, single-tablet 12-week treatment regimen for all HCV-infected patients with compensated liver disease, irrespective of HCV genotype or subtype, stage of fibrosis, prior interferon-based treatment, or pretreatment viral resistance. In addition, a Phase 3 study in HCV-infected patients with decompensated cirrhosis was also conducted – a treatment population without any approved treatment options at that time.

5.1 ASTRAL-1

ASTRAL-1 was a Phase 3, double-blind, placebo-controlled study involving untreated and previously treated patients with chronic HCV genotype 1, 2, 4, 5, or 6 infection, including those with compensated cirrhosis [6]. A separate trial with an active comparator group was deemed necessary for patients with genotype 3 HCV infection in light of the special clinical challenges presented in this population, particularly those with cirrhosis and/or prior treatment failure. In ASTRAL-1, patients with HCV genotype 1, 2, 4, or 6 were randomly assigned in a 5:1 ratio to receive SOF/VEL (400 mg/100 mg) in a once-daily, fixed-dose combination tablet or matching placebo for 12 weeks at 81 sites in the United States, Canada, Europe, and Hong Kong from July 18, 2014, through December 19, 2014. Because of the low prevalence of genotype 5 in the study regions, patients with genotype 5 did not undergo randomization but were assigned to the SOF/VEL group. Patients in the placebo group were eligible for deferred treatment with 12 weeks of SOF/VEL. The primary endpoint was SVR12. The protocol allowed enrollment of patients with compensated cirrhosis as well as those who had previously been treated for HCV with a regimen not containing an HCV NS5B inhibitor or NS5A inhibitor. No upper limits were placed on age or body mass index.

Of the 740 patients treated, 35 patients with genotype 5 HCV infection were enrolled directly into the SOF/VEL group, 624 were randomized to receive SOF/VEL, and 116 patients were randomized to receive matching placebo. Demographic and baseline characteristics were generally balanced across these groups (Table 7). In the SOF/VEL group, 34% of the patients had HCV genotype 1a, 19% genotype 1b, 17% genotype 2, 19% genotype 4, 6% genotype 5, and 7% genotype 6. Most patients were white (79%) and male (60%). Nineteen percent of the patients had cirrhosis, 69% had a non-CC *IL28B* genotype (which has been associated with a reduced response to interferon-based HCV treatment), and 32% had received previous unsuccessful treatment for HCV. Of the 201 patients in the SOF/VEL group who had received previous treatment, 28% had received a regimen of peginterferon, RBV, and a protease inhibitor, and 61% had received peginterferon and RBV; 48% of these patients had persistently detectable HCV RNA while receiving previous treatment, and 51% had a virologic relapse or breakthrough. A total of 51% of patients were enrolled in Europe, 46% in North America (Canada and the United States), and 3% in Hong Kong.

Overall, the rate of SVR12 among patients who received 12 weeks of SOF/VEL was 99% (95% confidence interval [CI], 98 to >99), which was significantly superior to the prespecified performance goal of 85% ($P < 0.001$) (Fig. 2). None of the 116 patients in the placebo group had an SVR. Rates of SVR were similar regardless of the HCV genotype: 98% (95% CI, 95 to >99) in patients with genotype 1a infection, 99% (95% CI, 95–100) with genotype 1b, 100% (95% CI, 97–100) with genotype 2, 100% (95% CI, 97–100) with genotype 4, 97% (95% CI, 85 to >99) with genotype 5, and 100% (95% CI, 91–100) with genotype 6. Of the 121 patients with any genotype who had cirrhosis, 120 (99% [95% CI,

Table 7 Demographic and baseline characteristics in ASTRAL-1

	Placebo ($N = 116$)	SOF/VEL ($N = 624$)
Mean age (range), years	53 (25–74)	54 (18–82)
Mean BMI (range), kg/m^2	26 (18–40)	27 (17–57)
Male, *n* (%)	68 (59)	374 (60)
Race, *n* (%)		
White	90 (78)	493 (79)
Black	11 (9)	52 (8)
Region, *n* (%)		
North America	52 (45)	289 (46)
Europe	60 (52)	316 (51)
Hong Kong	4 (3)	19 (3)
HCV genotype, *n* (%)		
Genotype 1a	46 (40)	210 (34)
Genotype 1b	19 (16)	118 (19)
Genotype 2	21 (18)	104 (17)
Genotype 4	22 (19)	116 (19)
Genotype 5	0	35 (6)
Genotype 6	8 (7)	41 (7)
Mean HCV RNA (SD), $\log_{10}$ IU/mL	6.3 (0.58)	6.3 (0.66)
HCV RNA $\geq$800,000 IU/mL, *n* (%)	87 (75)	461 (74)
Compensated cirrhosis, *n* (%)	21 (18)	121 (19)
Previous HCV treatment, *n* (%)	33 (28)	201 (32)
IL28B genotype CC, *n* (%)	36 (31)	186 (30)
Baseline ALT $>1.5 \times$ ULN, *n* (%)	54 (47)	279 (45)

ALT alanine aminotransferase, *BMI* body mass index, *HCV* hepatitis C virus, *RBV* ribavirin, *SOF* sofosbuvir, *ULN* upper limit of normal, *VEL* velpatasvir

95 to >99]) had a SVR. Of the 624 patients who received at least one dose of SOF/VEL, 2 (<1%) had virologic failure: a 56-year-old white man without cirrhosis who had received no previous treatment for genotype 1a HCV infection and a 58-year-old black man with cirrhosis who had persistently detectable HCV RNA during previous peginterferon – RBV treatment for genotype 1b HCV infection. The two men had undetectable serum HCV RNA at week 4 of treatment, and both had a virologic relapse by posttreatment week 4. Four other patients in the SOF/VEL group did not achieve an SVR. Two of the four were lost to follow-up (one did not return after completing 45 days of treatment; the other completed treatment and had undetectable serum HCV RNA at posttreatment week 4 but did not return for the posttreatment week 12 visit), one discontinued treatment because of an adverse event, and one died during follow-up. Rates of SVR in all patient subgroups, including those with cirrhosis (99%) and prior treatment experience (>99%), were high.

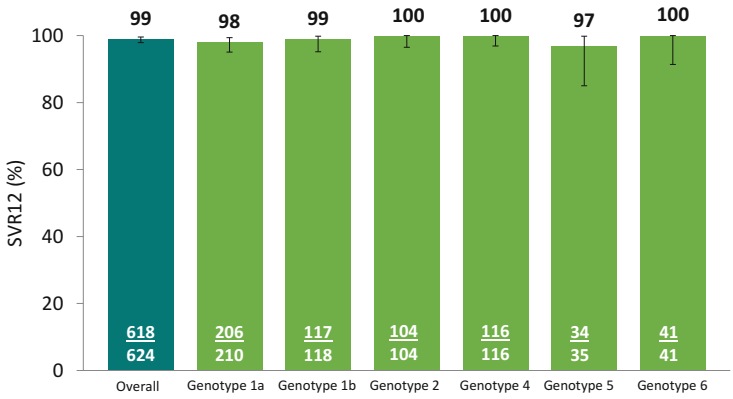

Fig. 2 SVR12 rates in ASTRAL-1 overall and by genotype (Reproduced from [6]). Error bars represent 95% confidence intervals

At baseline, NS5A resistance-associated variants were detected in 257 of 616 patients (42%) for whom sequencing data were available. Of these 257 patients, 255 (99%) had an SVR. The two patients who had virologic failure did not have NS5A-resistant variants at baseline but did so at the time of relapse. The patient with HCV genotype 1a infection who had a relapse had the Y93N variant detected in more than 99% of the viral population. The second patient (with HCV genotype 1b who had a relapse) had the Q30R (in 98.7%) and L31M (in >99%) at baseline and Q30R (in >99%), L31M (in >99%), and Y93H (in 99%) at the time of relapse. The Q30R variant confers an increase by a factor of 2.2 in the 50% effective concentration (EC50) of VEL in the HCV genotype 1a replicon. Arginine (R) variants at position 30 of the NS5A protein were present at baseline in 62 patients in the entire study population: 5 patients with genotype 1, 5 with genotype 2, 50 with genotype 4, and 2 with genotype 5. Of these 62 patients, 60 (97%) had an SVR. Variants associated with resistance to NS5B nucleoside inhibitors were detected at baseline in 54 of the 601 patients (9%) for whom sequencing data were available. No S282 variants were detected. All 54 patients had an SVR.

Twelve weeks of SOF/VEL treatment was well tolerated with the type, frequency, and severity of nonserious adverse events generally similar in both groups (Table 8). Of the 624 patients in the SOF/VEL group, 1 (<1%) discontinued treatment prematurely because of an adverse event. This patient, a 52-year-old white woman with genotype 1a HCV infection without cirrhosis, discontinued treatment because of an anxiety attack on the 13th day of treatment. Of the 116 patients in the placebo group, 2 (2%) discontinued treatment because of an elevated aminotransferase level, a prespecified criterion for discontinuation. A total of 15 patients (2%) in the SOF/VEL group had 19 serious adverse events. No single serious adverse event occurred in more than one patient. There was one death in the SOF/VEL group. This patient, a 55-year-old white man with HCV genotype 5a without cirrhosis who had a history of dyslipidemia for which he was taking

Table 8 Safety of SOF/VEL for 12 weeks in ASTRAL-1

	SOF/VEL 12 weeks ($N = 624$)	Placebo 12 weeks ($N = 116$)
Number (%) of subjects experiencing any		
Treatment-emergent adverse event	485 (78%)	89 (77%)
Grade 3 or above treatment-emergent adverse event	18 (3%)	1 (<1%)
Treatment-emergent serious adverse event	15 (2%)	0
Treatment-emergent treatment-related serious adverse event	0	0
Adverse event leading to premature discontinuation of the study drug	1 (<1%)	2 (2%)
All death	1 (<1%)	0
Common adverse events (≥10% in any group)		
Headache	182 (29%)	33 (28%)
Fatigue	126 (20%)	23 (20%)
Nasopharyngitis	79 (13%)	12 (10%)
Nausea	75 (12%)	13 (11%)

ezetimibe–simvastatin, died during sleep 8 days after the completion of treatment. The cause of death was not determined. The patient was not taking amiodarone. None of the patients in the placebo group had a serious adverse event. There was no significant difference in the rates of any adverse event in the SOF/VEL group and the placebo group (78% and 77%, respectively). The rates of individual adverse events did not differ significantly between the two groups. The most common adverse events were headache, fatigue, nasopharyngitis, and nausea. Hematologic abnormalities were infrequent in the SOF/VEL group, affecting 1% of patients or less. No patients in the placebo group had hematologic abnormalities. No patient in either study group had a Grade 3 or 4 elevation in creatinine (>3.0 mg/dL) or total bilirubin (>2.5 mg/dL).

5.2 ASTRAL-2

After the protocol for ASTRAL-1 was finalized and trial activity had begun, the US Food and Drug Administration requested a separate study be conducted with an active comparator for patients with HCV genotype 2. ASTRAL-2 was a Phase 3, open-label, active comparator trial involving untreated and previously treated patients with chronic HCV genotype 2 infection, including those with compensated cirrhosis [7]. Patients with HCV genotype 2 were randomly assigned in a 1:1 ratio to receive SOF/VEL (400 mg/100 mg) in a once-daily, fixed-dose combination tablet or SOF + RBV for 12 weeks, the standard of care at the time the study was conducted. The primary endpoint was SVR12. The protocol allowed enrollment of patients with compensated cirrhosis as well as those who had previously been treated

for HCV with an interferon-based regimen. Inclusion and exclusion criteria were similar to those in ASTRAL-1 including no upper limits on age or body mass index. Due to the need for RBV coadministration, a creatinine clearance of greater than 50 mL/min at screening was required.

A total of 266 patients were randomized and treated at 51 sites in the United States from October 15, 2014, through December 18, 2014. Randomization was stratified by cirrhosis status and prior treatment history. The demographic and baseline characteristics of patients were generally balanced across treatment groups (Table 9). Most of the patients were white men and had non-CC *IL28B* genotype. A total of 14% of patients had cirrhosis, and 14–15% had received unsuccessful treatment for HCV. All but two patients (<1%), one in each treatment group, completed treatment. One patient in the SOF/VEL group discontinued treatment on Day 1 due to adverse events of difficulty concentrating, headache, and anxiety. One patient in the SOF + RBV group completed the week 10 visit and was subsequently lost to follow-up.

The rate of SVR12 was 99% (95% confidence interval [CI], 96–100) among those who had received SOF/VEL for 12 weeks, as compared with 94% (95% CI, 88–97) among those who had received SOF + RBV for 12 weeks (Fig. 3). The study met its

Table 9 ASTRAL-2 demographic and baseline characteristics

	SOF/VEL 12 weeks ($N = 134$)	SOF + RBV 12 weeks ($N = 132$)	Total ($N = 266$)
Mean age (range), years	57 (26–81)	57 (23–76)	57 (23–81)
Mean BMI (range), kg/m^2	28 (17–45)	29 (19–61)	29 (17–61)
Male, *n* (%)	86 (64)	72 (55)	158 (59)
Race, *n* (%)			
White	124 (93)	111 (84)	235 (88)
Black	6 (5)	12 (9)	18 (7)
Genotype 2 (no confirmed subtype)	13 (9.7%)	12 (9.1%)	25 (9.4%)
Genotype 2a	2 (1.5%)	4 (3.0%)	6 (2.3%)
Genotype 2a/2c	16 (11.9%)	12 (9.1%)	28 (10.5%)
Genotype 2b	103 (76.9%)	104 (78.8%)	207 (77.8%)
Cirrhosis, *n* (%)	19 (14)	19 (14)	38 (14)
Mean HCV RNA (SD), $\log_{10}$ IU/mL	6.5 (0.78)	6.4 (0.74)	6.4 (0.76)
HCV RNA ≥800,000 IU/mL, *n* (%)	111 (83)	101 (77)	212 (80)
Previous HCV treatment, *n* (%)	19 (14)	20 (15)	39 (15)
IL28B genotype CC, *n* (%)	55 (41)	46 (35)	101 (38)
Baseline ALT >1.5 × ULN, *n* (%)	54 (40)	50 (38)	104 (39)

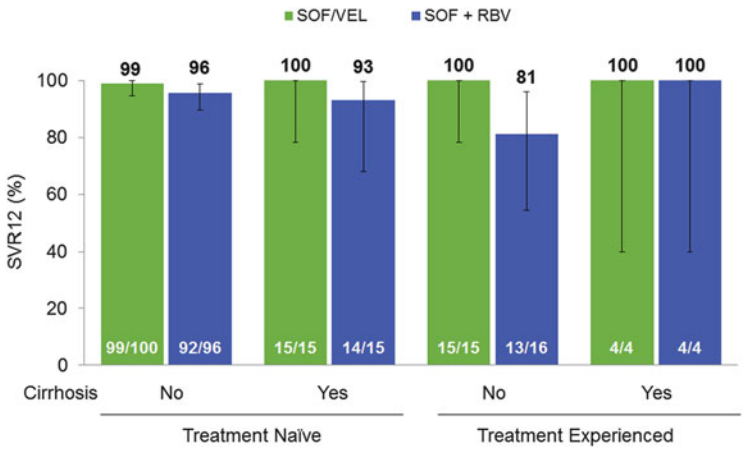

Fig. 3 SVR12 rates following 12 weeks of SOF/VEL or SOF + RBV in ASTRAL-2 by cirrhosis and prior treatment history. Error bars represent 95% confidence intervals

primary statistical endpoint in that those treated with SOF/VEL had an SVR12 rate that was significantly superior to that among patients who had received the standard treatment of SOF + RBV for 12 weeks, with a strata-adjusted absolute difference of 5.2 percentage points (95% CI, 0.2–10.3, $P = 0.02$ with the Cochran–Mantel–Haenszel test stratified according to cirrhosis status and previous treatment). There were no virologic failures among patients receiving SOF/VEL. One 57-year-old black man discontinued study treatment on Day 1 after receiving one dose of the study drug because of adverse events. Of the 132 patients who received SOF + RBV, 6 (5%) had a virologic relapse, and 2 other patients were lost to follow-up. Deep sequencing indicated that approximately 60% of the 134 patients in the SOF/VEL group had NS5A RASs and 10% had NS5B RASs at baseline. The most prevalent NS5A variant observed at baseline was L31M in 52% of the patients. Despite the presence of pretreatment NS5A and NS5B RASs, no patient receiving SOF/VEL had virologic failure.

Overall, treatment with SOF/VEL or SOF + RBV for 12 weeks was generally safe and well tolerated (Table 10). A smaller percentage of subjects in the SOF/VEL 12-week group experienced any adverse event (69%, 92 of 134) compared with the SOF + RBV 12-week group (77%, 101 of 132), including treatment-related adverse events (SOF/VEL, 34%; SOF + RBV, 57%) and adverse events leading to modification or interruption of any study drug (SOF/VEL, 0; SOF + RBV, 10%). The most common adverse events were reported by a smaller percentage of subjects in the SOF/VEL 12-week group compared with the SOF + RBV 12-week group, including fatigue (15% vs 36%), headache (18% vs 22%), nausea (10% vs 14%), and insomnia (5% vs 14%). Most adverse events were Grade 1 (mild) or Grade 2 (moderate) in severity. Grade 3 (severe) adverse events were rare (SOF/VEL, 2%; SOF + RBV, 2%). No Grade 4 (life-threatening) adverse events were reported. Serious adverse events were also rare (2%, four of 266 subjects [2 in each treatment group]). No

Table 10 Safety of SOF/VEL and SOF + RBV for 12 weeks in ASTRAL-2

	SOF/VEL 12 weeks ($N = 134$)	SOF + RBV 12 weeks ($N = 132$)
Number (%) of subjects experiencing any		
Treatment-emergent adverse event	92 (69)	101 (77)
Grade 3 or above treatment-emergent adverse event	3 (2)	3 (2)
Treatment-emergent serious adverse event	2 (2)	2 (2)
Treatment-emergent treatment-related serious adverse event	0	0
Adverse event leading to premature discontinuation of the study drug	1 (<1)	0
All death	2 (2)	0
Common adverse events (≥10% in any group)		
Fatigue	20 (15)	47 (36)
Headache	24 (18)	29 (22)
Nausea	14 (10)	19 (14)
Insomnia	6 (5)	18 (14)

serious adverse event was reported in >1 subject. All serious adverse events were considered by the investigators to be not related to study drug. Two nontreatment-emergent deaths were reported during the study (metastatic lung cancer and cardiac arrest after treatment completion). Only one subject permanently discontinued any study drug (SOF/VEL) due to adverse events. Hematologic laboratory abnormalities consistent with RBV-induced hemolysis were observed in the SOF + RBV arm but not in patients treated with SOF/VEL.

5.3 ASTRAL-3

Before the availability of direct acting antiviral agents, HCV genotypes 2 and 3 were grouped together in treatment guidelines as "easy-to-cure" genotypes. However, in the era of direct acting antivirals, HCV genotype 3 has been associated with fewer available treatment options and lower rates of treatment response. Furthermore, some studies have suggested HCV genotype 3 is associated with more rapid disease progression and a higher rate of complications such as hepatocellular carcinoma. A simple, RBV-free regimen that would be highly effective in patients irrespective of genotypes would be highly desirable. The ASTRAL-3 study was a Phase 3, open-label, active comparator study involving untreated and previously treated patients with chronic HCV genotype 3 infection, including those with compensated cirrhosis [7]. Patients with HCV genotype 3 were randomly assigned in a 1:1 ratio to receive SOF/VEL (400 mg/100 mg) in a once-daily, fixed-dose combination tablet or SOF + RBV for 24 weeks, the standard of care at the time the study was conducted. The primary endpoint was SVR12. The protocol allowed enrollment of patients with

compensated cirrhosis as well as those who had previously been treated for HCV with an interferon-based regimen. Inclusion and exclusion criteria were similar to those in ASTRAL-1 and ASTRAL-2 including no upper limits on age or body mass index. Due to the need for RBV coadministration, a creatinine clearance of greater than 50 ml/min was required at screening.

A total of 552 patients were randomized and treated at 76 sites in the United States, Canada, Europe, Australia, and New Zealand from July 30, 2014, through December 17, 2014. Randomization was stratified by cirrhosis status and prior treatment history. The demographic and baseline characteristics of patients were generally balanced across treatment groups (Table 11). Most of the patients were white men and had non-CC *IL28B* genotype. Nearly a third of patients had cirrhosis, and just over one quarter had undergone unsuccessful treatment.

The rate of SVR12 was 95% (95% CI, 92–98) among those who had received SOF/VEL for 12 weeks, as compared with 80% (95% CI, 75–85) among those who had received 24 weeks of SOF + RBV (Fig. 4). The SVR12 rate with 12 weeks of SOF/VEL was significantly superior to that with 24 weeks of SOF + RBV. The strata-adjusted absolute difference was 14.8 percentage points (95% CI, 9.6–20.0; $P < 0.001$ with the Cochran–Mantel–Haenszel test stratified according to cirrhosis

Table 11 ASTRAL-3 demographic and baseline characteristics

	SOF/VEL 12 weeks ($N = 277$)	SOF + RBV 24 weeks ($N = 275$)	Total ($N = 552$)
Mean age (range), years	49 (21–76)	50 (19–74)	50 (19–76)
Mean BMI (range), kg/m^2	26 (17–48)	27 (17–56)	27 (17–56)
Male, *n* (%)	170 (61)	174 (63)	344 (62)
Race, *n* (%)			
White	250 (90)	239 (87)	489 (89)
Asian	23 (8)	29 (11)	52 (9)
Genotype 3 (no confirmed subtype)	9 (3)	18 (7)	27 (5)
Genotype 3a	265 (96)	250 (91)	515 (93)
Genotype 3b	2 (<1)	5 (2)	7 (1)
Genotype 3h	0	2 (<1)	2 (<1)
Genotype 3k	1 (<1)	0	1 (<1)
Cirrhosis, *n* (%)	80 (29)	83 (30)	163 (30)
Mean HCV RNA (SD), $\log_{10}$ IU/mL	6.2 (0.72)	6.3 (0.71)	6.3 (0.72)
HCV RNA ≥800,000 IU/mL, *n* (%)	191 (69)	194 (71)	385 (70)
Previous HCV treatment, *n* (%)	71 (26)	71 (26)	142 (26)
IL28B genotype CC, *n* (%)	105 (38)	111 (40)	216 (39)
Baseline ALT >1.5 × ULN, *n* (%)	182 (66)	188 (68)	370 (67)

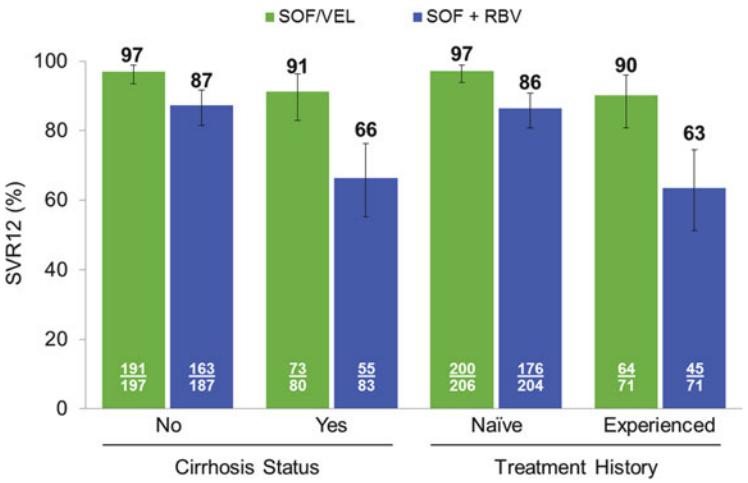

Fig. 4 SVR12 rates following 12 weeks of SOF/VEL or 24 weeks of SOF + RBV in ASTRAL-3 by cirrhosis and prior treatment history. Error bars represent 95% confidence intervals

status and previous treatment). Among the 277 patients who received SOF/VEL, 11 (4%) had virologic failure after the end of treatment, and 2 patients were lost to follow-up. Among the 275 patients who received SOF + RBV, 38 (14%) had a relapse after treatment, 1 had virologic failure during treatment, 6 were lost to follow-up, 4 discontinued treatment because of adverse events, 2 withdrew consent, 2 died, and 1 discontinued treatment before achieving undetectable HCV RNA. Among patients who received SOF/VEL, the SVR rate was 91% among those with cirrhosis, as compared with 97% among those without cirrhosis. Among patients who received SOF + RBV, the rates of SVR among patients with and those without cirrhosis were 66% and 87%, respectively. A similar pattern of response was seen according to whether patients had received previous treatment. Among patients in the SOF/VEL group, the rate of SVR was 90% among those who had received previous HCV treatment, as compared with 97% among those who had received no previous treatment. The corresponding rates among patients in the SOF + RBV group were 63 and 86%. The rate of SVR among patients who had received previous treatment and who had evidence of cirrhosis was 89% in the SOF/VEL group as compared with 58% in the SOF + RBV group. Sustained virologic response did not appear to be correlated with the *IL28B* genotype or early viral kinetics.

Of the 274 patients in the SOF/VEL group who had available data on virologic outcome with deep sequencing data, 43 (16%) had detectable NS5A RASs (A30K, L31M, and Y93H) at baseline. Of these patients, 38 (88%) had an SVR. Of the 25 patients with the Y93H variant at baseline, 21 (84%) had an SVR. Of the 231 patients without NS5A RASs at baseline, 225 (97%) had an SVR. All ten patients with baseline NS5B resistance-associated variants (N142T, L159F, E237G, L320I, and V321A/I) had an SVR.

Treatment with SOF/VEL for 12 weeks was well tolerated in this study and compared favorably with SOF + RBV for 24 weeks (Table 12). Overall, adverse events and Grade 3 and 4 adverse events occurred less frequently in patients in the SOF/VEL 12-week group than in the SOF + RBV 24-week group. For the majority of adverse events that occurred in ≥10% of patients in either treatment group, there was a lower incidence of adverse events in SOF/VEL-treated patients than in SOF + RBV-treated patients. Adverse events associated with the hematological, constitutional, dermatologic, and neuropsychiatric toxicities of RBV were, as expected, markedly less common in the SOF/VEL 12-week group than in the SOF + RBV 24-week group: anemia (0.4% vs 9%), fatigue (26% vs 38%), arthralgia (4% vs 8%), pruritus (3% vs 13%), dry skin (0.7% vs 9%), insomnia (11% vs 27%), irritability (8% vs 15%), and anxiety (3% vs 8%). There were no discontinuations due to adverse events in the SOF/VEL 12-week group compared with nine discontinuations due to adverse events in the SOF + RBV 24-week group, suggesting that the more favorable safety and tolerability profile of the SOF/VEL 12-week group resulted in a higher rate of treatment completion. There were no Grade 4 adverse events or treatment-related serious adverse events in the SOF/VEL 12-week group. Three deaths were reported in the study: one due to gunshot wounds, one due to

Table 12 Safety of SOF/VEL for 12 weeks and SOF + RBV for 24 weeks in ASTRAL-3

	SOF/VEL 12 weeks ($N = 277$)	SOF + RBV 24 weeks ($N = 275$)
Number (%) of subjects experiencing any		
Treatment-emergent adverse event	245 (88)	260 (95)
Grade 3 or above treatment-emergent adverse event	12 (4)	23 (8)
Treatment-emergent serious adverse event	6 (2)	15 (6)
Treatment-emergent treatment-related serious adverse event	0	1 (<1)
Adverse event leading to premature discontinuation of the study drug	0	9 (3)
All death	0	3 (1)
Common adverse events (≥10% in any group)		
Headache	90 (33)	89 (32)
Fatigue	71 (26)	105 (38)
Insomnia	31 (11)	74 (27)
Nausea	46 (17)	58 (21)
Nasopharyngitis	34 (12)	33 (12)
Irritability	23 (8)	40 (15)
Cough	14 (5)	35 (13)
Back pain	25 (9)	20 (7)
Pruritus	8 (3)	35 (13)
Asthenia	16 (6)	26 (10)
Diarrhea	20 (7)	21 (8)
Dyspepsia	9 (3)	30 (11)

natural causes, and one due to an unknown cause; all three patients were in the SOF + RBV 24-week group. There were no clinically meaningful Grade 3 or 4 laboratory abnormalities in the SOF/VEL 12-week group. Consistent with the expected toxicity profile of RBV, decreases in hemoglobin and lymphocytes and increases in reticulocytes, platelets, and total bilirubin were observed in the SOF + RBV 24-week group.

5.4 ASTRAL-4

Prior to SOF-based therapies, the only treatment option for HCV-infected patients who had progressed to decompensated cirrhosis was liver transplantation; interferon-based therapies were associated with poor response rates and unacceptable toxicities including death. The compassionate use program with SOF + RBV demonstrated proof-of-concept that patients with advanced liver disease could be safely and effectively treated. However, treatment durations of 24–48 weeks were required, and efficacy was not optimized. Ledipasvir/sofosbuvir plus RBV for 12 weeks subsequently showed high SVR rates and excellent tolerability in both pretransplant and posttransplant genotype 1 or 4 HCV-infected patients. With these data and the Phase 1 study demonstrating that velpatasvir pharmacokinetics were not substantially altered in severe hepatic impairment, ASTRAL-4 was undertaken to assess the possibility for a pangenotypic treatment option for HCV-infected patients with decompensated cirrhosis. The study set out to address both the impact of treatment duration and the need for RBV in HCV-infected patients with Child-Pugh-Turcotte (CPT) B cirrhosis. The ASTRAL-4 study was a Phase 3, open-label study involving untreated and previously treated patients with chronic HCV genotype 1–6 infection and CPT B cirrhosis [8]. Patients were randomly assigned in a 1:1:1 ratio to receive SOF/VEL once-daily for 12 weeks, SOF/VEL plus weight-based RBV for 12 weeks, or SOF/VEL for 24 weeks. The primary endpoint was SVR12.

A total of 267 patients were randomized and treated at 47 sites in the United States from August 17, 2014, through December 19, 2014. Randomization was stratified by genotype. The demographic and baseline characteristics of patients were generally balanced across treatment groups (Table 13). Overall, 60% of patients had HCV genotype 1a, 18% genotype 1b, 4% genotype 2, 15% genotype 3, 3% genotype 4, and less than 1% genotype 6; no patients had genotype 5. A total of 6% of patients were black, and 55% had received prior treatment for HCV infection. The median baseline CPT score was 8 (range, 5–10), the median baseline MELD score was 10 (range, 6–24), and the median creatinine clearance was 84.7 mL/min (range, 15–198). The majority of patients (95%) had a baseline MELD score of 15 or less. All the patients had CPT class B cirrhosis at screening, but 27 patients (10%) had CPT class A or CPT class C cirrhosis at treatment baseline, which reflects the dynamic changes in CPT scoring in this population.

Rates of SVR were 83% (95% confidence interval [CI], 74–90) in patients who received SOF/VEL for 12 weeks, 94% (95% CI, 87–98) among those who received

Table 13 Demographic and baseline characteristics in ASTRAL-4

	SOF + VEL 12 weeks ($N = 90$)	SOF + VEL + RBV 12 weeks ($N = 87$)	SOF + VEL 24 weeks ($N = 90$)
Mean age (range), years	58 (42–73)	58 (40–71)	58 (46–72)
Mean BMI (range), kg/m^2	31 (17–56)	30 (20–55)	30 (18–50)
Male, *n* (%)	57 (63)	66 (76)	63 (70)
Race, *n* (%)			
White	79 (88)	79 (91)	81 (90)
Black	6 (7)	5 (6)	6 (7)
HCV genotype, *n* (%)			
Genotype 1a	2 (7)	1 (4)	0
Genotype 1b	25 (93)	22 (92)	27 (100)
Genotype 2	0	1 (4)	0
Genotype 3	14 (16)	13 (15)	12 (13)
Genotype 4	4 (4)	2 (2)	2 (2)
Genotype 6	0	0	1 (1)
Mean HCV RNA (SD), $\log_{10}$ IU/mL	6.0 (0.5)	5.8 (0.6)	5.9 (0.6)
HCV RNA ≥800,000 IU/mL, *n* (%)	59 (66)	45 (52)	45 (50)
IL28B genotype CC, *n* (%)	20 (22)	22 (25)	20 (22)
CPT score, *n* (%)			
≤6	3 (3)	6 (7)	7 (8)
7	36 (40)	23 (26)	21 (23)
8	31 (34)	41 (47)	34 (38)
9	19 (21)	13 (15)	22 (24)
10	1 (1)	4 (5)	6 (7)
MELD score, *n* (%)			
<10	36 (40)	29 (33)	26 (29)
10–15	50 (56)	54 (62)	59 (66)
≥16	4 (4)	4 (5)	5 (6)
Ascites, *n* (%)			
None	16 (18)	22 (25)	15 (17)
Mild or moderate	72 (80)	61 (70)	74 (82)
Severe	2 (2)	4 (5)	1 (1)
Mean eGFR (range), mL/min	89 (15–169)	90 (50–167)	90 (43–198)
Prior HCV treatment, *n* (%)	58 (64)	47 (54)	42 (47)

ALT alanine aminotransferase, *BMI* body mass index, *HCV* hepatitis C virus, *RBV* ribavirin, *SOF* sofosbuvir, *ULN* upper limit of normal, *VEL* velpatasvir

SOF/VEL + RBV, and 86% (95% CI, 77–92) among those who received SOF/VEL for 24 weeks (Fig. 5). Post hoc analyses did not detect any significant differences in SVR rates among the three treatment groups. Among patients with HCV genotype 1, the SVR rate was 88% for those who received SOF/VEL for 12 weeks, 96% for those who received SOF/VEL + RBV, and 92% for those who received SOF/VEL for 24 weeks. Among the smaller population of patients with HCV genotype 3, the SVR rate among patients who received SOF/VEL + RBV was 85%, as compared with 50% for the two groups that received SOF/VEL alone. All the patients with HCV genotype 2, 4, or 6 had an SVR except for one patient with HCV genotype 2 who was assigned to receive SOF/VEL for 24 weeks; this patient died of liver failure after completing 28 days of treatment. A total of 22 patients had virologic failure: 11 of 90 patients (12%) who received SOF/VEL for 12 weeks, 3 of 87 patients (3%) who received SOF/VEL + RBV, and 8 of 90 patients (9%) who received SOF/VEL for 24 weeks. Of the 22 patients who had virologic failure, 20 had a relapse, and 2 (both with HCV genotype 3) had virologic breakthrough. One of the patients with virologic breakthrough, a 56-year-old white man who was assigned to receive SOF/VEL + RBV, had undetectable plasma levels of study drugs at the time of virologic failure, which suggests nonadherence. The other patient with virologic breakthrough was a 52-year-old white man with HCV genotype 3a who was assigned to receive SOF/VEL for 24 weeks. This patient had an HCV RNA level of less than 15 IU/mL from week 4 through week 10 with low levels of HCV RNA (26–80 IU/mL) at week 12 and week 16; the patient's participation in the study was terminated early at week 16 because he met the stopping criteria for virologic failure. There was no evidence to suggest nonadherence. Also counted among the patients

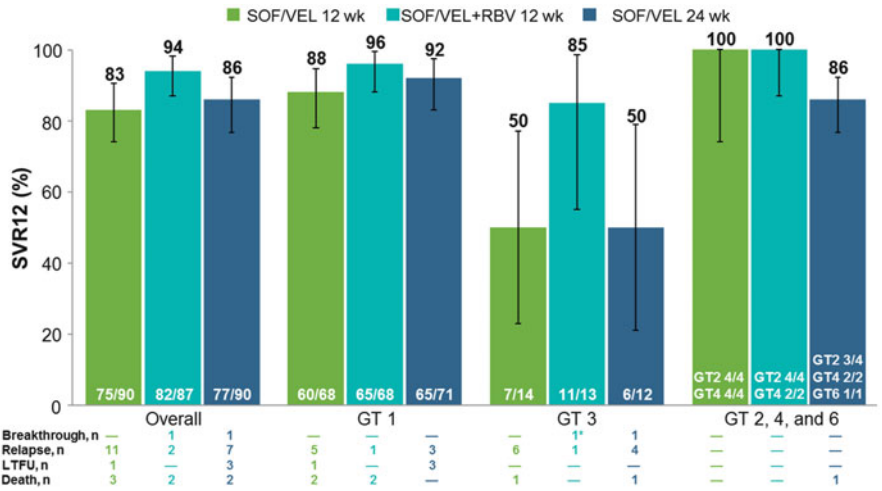

Fig. 5 SVR12 rates and virologic outcomes following 12 weeks of SOF/VEL, SOF/VEL + RBV, or 24 weeks of SOF/VEL in ASTRAL-4 overall and by genotype. *Patient with nondetectable drug levels at time of virologic failure. *LTFU* lost to follow-up

with treatment failure were four who were lost to follow-up and seven who died before the primary endpoint.

Of the 255 patients for whom pretreatment NS5A sequencing data were available, 72 (28%) had pretreatment NS5A RASs. Of these 72 patients, 64 (89%) had an SVR, as compared with 169 of 183 patients (92%) who did not have pretreatment NS5A RASs. Among patients with HCV genotype 1 receiving SOF/VEL + RBV, the SVR rate in those with NS5A RASs was 100%, and the rate without such variants was 98%. Among patients with HCV genotype 1 in the SOF/VEL groups who had pretreatment RASs, the SVR rate was 80% among those who received 12 weeks of treatment and 90% among those who received 24 weeks of treatment; among those who did not have RASs, the rates were 96% and 98%, respectively. An analysis of the effect of resistance on treatment outcome in patients with HCV genotype 3 was limited by the small number (six patients) with RASs in our study. The majority of patients who had virologic failure had NS5A RASs at the time of failure; NS5B RASs were less common and typically observed at low levels. Of the 251 patients for whom pretreatment NS5B deep-sequencing data were available, 8 had pretreatment RASs (at positions N142T, L159F, E237G, and M289I). All eight patients had an SVR.

In this population of patients with decompensated liver disease, understanding whether achieving SVR is associated with improved outcomes is important [9]. Among patients who achieved SVR24 and had CPT and MELD scores available, 54% had an improvement in the CPT score over baseline, 36% had no change in the CPT score, and 10% had a worsening in the CPT score (Table 14). Of the 223 patients with a baseline MELD score of less than 15 for whom MELD data were available at posttreatment week 24, a total of 49% had an improved MELD score, 25% had no change in the MELD score, and 26% had a worsening in the MELD score. Among patients with a baseline MELD score above 15 who achieved SVR24, 72% had an improved MELD score, 4% had no change, and 24% had a worsened MELD score at posttreatment week 24.

A total of 9 patients discontinued study treatment prematurely because of an adverse event: 1 of 90 patients (1%) who received SOF/VEL for 12 weeks, 4 of 87 patients (5%) who received SOF/VEL + RBV, and 4 of 90 patients (4%) who received SOF/VEL for 24 weeks (Table 15). No adverse event that led to discontinuation of a study drug was reported in more than one patient. Serious adverse events occurred in 19% of patients who received SOF/VEL for 12 weeks, 16% of those who received SOF/VEL + RBV, and 18% of those who received SOF/VEL for 24 weeks. The most common serious adverse events were hepatic encephalopathy

Table 14 Shift table of CPT class at baseline and at posttreatment week 24 among patients achieving SVR24 in ASTRAL-4

Baseline CPT class	CPT A (5–6)	CPT B (7–9)	CPT C (10–15)	No assessment
CPT A (5–6)	12/13 (92%)	1/13 (8%)	0/13	3
CPT B (7–9)	50/191 (26%)	138/191 (72%)	3/191 (2%)	19
CPT C (10–15)	2/9 (22%)	5/9 (56%)	2/9 (22%)	1

Table 15 Safety in ASTRAL-4

	SOF/VEL 12 weeks ($N = 90$)	SOF/VEL + RBV 12 weeks ($N = 87$)	SOF/VEL 24 weeks ($N = 90$)
Number (%) of subjects experiencing any			
Treatment-emergent adverse event	73 (81)	79 (91)	73 (81)
Grade 3 or above treatment-emergent adverse event	16 (18)	11 (13)	17 (19)
Treatment-emergent serious adverse event	17 (19)	14 (16)	16 (18)
Treatment-emergent treatment-related serious adverse event	0	1 (1)	1 (1)
Adverse event leading to premature discontinuation of the study drug	1 (1)	4 (5)	4 (4)
All death	3 (3)	3 (3)	3 (3)
Common adverse events ($\geq$10% in any group)			
Fatigue	23 (26)	34 (39)	21 (23)
Nausea	22 (24)	22 (25)	18 (20)
Headache	23 (26)	18 (21)	17 (19)
Anemia	4 (4)	27 (31)	3 (3)
Diarrhea	6 (7)	18 (21)	7 (8)
Insomnia	9 (10)	12 (14)	9 (10)
Pruritus	10 (11)	4 (5)	4 (4)
Muscle spasms	3 (3)	10 (11)	4 (4)
Dyspnea	4 (4)	9 (10)	2 (2)
Cough	2 (2)	9 (10)	2 (2)

and sepsis (with each event occurring in five patients across groups). The most common adverse events in all groups were fatigue (29%), nausea (23%), and headache (22%), although anemia, diarrhea, and insomnia were also common among the patients who received SOF/VEL + RBV. Overall, 81% of patients in the groups who received SOF/VEL alone had at least one adverse event, as compared with 91% of patients receiving SOF/VEL + RBV. Nine deaths occurred during the study. Two patients died after discontinuing study treatment but within 30 days after the end of treatment, and seven patients died more than 30 days after the end of treatment. Most of the deaths were due to complications of end-stage liver disease (i.e., liver failure, sepsis, or multiorgan failure). The nine deaths were evenly divided among the three treatment groups; none were considered to be related to therapy by the investigator. Reductions in hemoglobin, lymphocytes, and platelets were common in all three groups. In the group that received SOF/VEL + RBV, decreases in hemoglobin to less than 10.0 g/dL occurred in 23% of patients and decreases to less than 8.5 g/dL in 7% of patients. In the groups that received SOF/VEL, the rates of decrease in hemoglobin were 8% and 1%, respectively, among those who received 12 weeks and 9% and 1% among those who received 24 weeks. Anemia or reductions in hemoglobin were successfully managed in the majority of patients

with a modification of or interruption in the RBV dose, although one patient was treated with erythropoietin. Two patients who received SOF/VEL for 12 weeks required the infusion of packed red cells for the treatment of gastrointestinal bleeding. Hyperbilirubinemia that was consistent with hemolysis was primarily observed in patients receiving SOF/VEL + RBV.

5.5 *Summary of Phase 3 Data Supporting Initial Registration of SOF/VEL*

A total of 1,035 patients were treated with 12 weeks of SOF/VEL across the three Phase 3 studies in genotype 1–6 patients with compensated liver disease. The overall SVR rate was 98% (1,015/1,035) with a 1% rate of virologic failure. These data conclusively demonstrated that SOF/VEL for 12 weeks provides a simple, highly effective, and safe single-tablet regimen for HCV-infected patients irrespective of genotype or demographic or baseline disease characteristics. This simplicity can enable expansion of treatment to nearly all HCV-infected patients and allow extension of the provider network beyond specialist physicians. Removing the need to genotype reduces overall cost of care, as does the absence of a need for on-treatment laboratory monitoring. Among patients with decompensated cirrhosis, SOF/VEL + RBV for 12 weeks resulted in a 94% SVR rate and was associated with an improvement in CPT and/or MELD scores in the majority of patients enrolled in ASTRAL-4. These data from the four registrational Phase 3 studies supported the approval of SOF/VEL (Epclusa®) in the United States on June 28, 2016, as the first interferon-free pangenotypic regimen and the only pangenotypic regimen indicated for both patients with compensated and decompensated liver disease. Since then, Epclusa has been approved in the European Union and in many other regions worldwide.

5.6 *ASTRAL-5*

HIV-infected patients coinfected with chronic HCV have more rapid progression of liver disease. In the interferon era, response rates to treatment were low, and tolerability was poor such that the decision to treat these patients was challenging and the management complex, involving a coordinated effort across hepatology and infectious disease experts. In light of these differences in efficacy and safety among the HIV/HCV coinfected population, these patients were considered a "special population," and dedicated studies were required in some regions to gain approval for treatment of these patients. The ASTRAL-5 study was a Phase 3 open-label, single-arm trial of 12 weeks of SOF/VEL in HIV/HCV coinfected patients with genotype 1–6 HCV infection [10]. Patients with compensated cirrhosis and/or prior

treatment failure were permitted, and most HIV antiretroviral regimens were allowed, as well as enrollment of subjects not on current treatment for HIV. The primary endpoint of the trial was SVR12.

Of the 106 patients enrolled at 17 sites in the United States, 91 (86%) were men, 48 (45%) were black, and 19 (18%) had cirrhosis. SVR12 was achieved by 101 (95%; 95% CI 89–99) of 106 patients; 74 (95%, 87–99) of 78 with genotype 1, all 11 (100%, 72–100) with genotype 2, 11 (92%, 62–100) of 12 with genotype 3, and all 5 (100%, 48–100) with genotype 4. All 19 patients with cirrhosis had SVR12. Two patients relapsed, two were lost to follow-up, and one withdrew consent. Two discontinued treatment due to adverse events and two had serious adverse events. The most common adverse events were fatigue (25%), headache (13%), arthralgia (8%), and upper respiratory tract infection (8%). These data were consistent with the Phase 3 studies in HCV monoinfected patients and demonstrated that SOF/VEL for 12 weeks was safe and provided high rates of SVR12 in patients with HCV and HIV coinfection. This study supported the supplemental indication in the United States for SOF/VEL for the treatment of coinfected patients, granted on August 2, 2017 [11].

6 Conclusion

The single-tablet regimen of SOF/VEL combines a pangenotypic nucleotide HCV NS5B polymerase inhibitor (SOF) with a pangenotypic HCV NS5A inhibitor (VEL). Large Phase 3 studies in diverse patient populations inclusive of individuals with multiple traditionally negative predictive factors for treatment response (e.g., cirrhosis, prior treatment failure) demonstrated high SVR rates as well as a favorable safety profile. These data support the use of SOF/VEL for 12 weeks in genotype 1–6 HCV-infected patients without cirrhosis or with compensated cirrhosis and SOF/VEL + RBV for 12 weeks in genotype 1–6 HCV-infected patients with decompensated cirrhosis. With SOF/VEL as a treatment option, the only decision a healthcare provider needs to make is regarding the addition of RBV for those patients with decompensated cirrhosis. The simplicity of the regimen (one pill once daily for a single duration for HCV-infected patients with compensated liver disease), limited drug interactions, and a favorable safety profile make it ideally suited to address the global health challenge of chronic HCV and fulfill the World Health Organization's target of HCV elimination by 2030.

Acknowledgment The authors would like to thank the patients and their families as well as study site staff who participated in the clinical trials of Epclusa.

Compliance with Ethical Standards

Conflict of Interest Diana M. Brainard and John G. McHutchison are employees of Gilead Sciences, Inc.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study. The authors would like to thank the patients and their families as well as study site staff who participated in the clinical trials of Epclusa.

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The Clinical Development of Sofosbuvir/Velpatasvir/Voxilaprevir (SOF/VEL/VOX, Vosevi®)

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Abstract The single-tablet regimen of sofosbuvir (SOF), an HCV nucleotide analog NS5B polymerase inhibitor; velpatasvir (VEL), an HCV NS5A inhibitor; and voxilaprevir (VOX), an NS3/4 protease inhibitor, provides a highly efficacious, safe, and salvage regimen for patients with genotype 1 to 6 HCV infection with and without compensated cirrhosis who were previously unsuccessfully treated with direct-acting antivirals (DAAs). The clinical development program for SOF/VEL/VOX focused on generating safety and efficacy data in DAA-experienced patients without retreatment options as well as assessing the possibility of shortening treatment duration for DAA-naive patients. The Phase 3 studies enrolled and treated over 1,000 genotype 1–6 HCV-infected patients with SOF/VEL/VOX. In DAA-experienced patients treated with 12 weeks of SOF/VEL/VOX, the overall SVR rate was 97%, and high SVR rates were observed across all genotypes irrespective of prior DAA regimen, cirrhosis status, or the presence of baseline

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resistance-associated substitutions (RASs), supporting its use as a retreatment regimen. In DAA-naive patients treated with 8 weeks of SOF/VEL/VOX, the overall SVR rate was 95% making it an alternative treatment option for regions in which a shorter duration is of particular interest.

Keywords Hepatitis C virus, Pangenotypic, NS3/4A Protease inhibitor, Salvage therapy, SOF/VEL/VOX

1 Introduction

In 2014, at the time initiation of the clinical development program for VOX, there was much activity in the development of DAA combinations for HCV treatment. However, these combinations were limited to NS5A inhibitors with sofosbuvir or with protease inhibitors. From the outset of the clinical program, the plan for VOX was to develop it with SOF and VEL in a pangenotypic three-DAA combination, with the goal of providing a salvage therapy for the most difficult-to-cure patients who failed prior highly effective DAA therapies and also, potentially, to shorten treatment duration for the relatively easy-to-cure DAA-naive patients. At the time of VOX Phase 1 initiation, the marketed protease inhibitors were only approved for the treatment of HCV genotype 1 and associated with the potential to cause liver injury. Voxilaprevir was specifically designed as a next-generation NS3/4A protease inhibitor with potent antiviral activity against HCV genotypes 1–6, and an improved resistance profile when compared with earlier approved protease inhibitors. In addition, VOX was specifically designed to minimize the potential for drug-induced liver injury (see [1]).

2 Phase 1 Studies

Voxilaprevir was initially characterized as an individual agent in healthy patients in 11 Phase 1 studies. The information from 27 other Phase 1 studies conducted during the initial development of SOF and VEL were supportive of the clinical pharmacology of SOF/VEL/VOX (see [2]). Further, seven Phase 1 studies were performed with SOF/VEL/VOX in a fixed-dose combination, specifically in scenarios in which SOF, VEL, or VOX was a perpetrator of a potential drug-drug interaction or in which using the three drugs in combination was particularly clinically important.

The median peak plasma concentration of VOX was observed 4 h postdose. Voxilaprevir is >99% bound to human plasma proteins and is primarily a substrate of CYP3A4 with slow turnover. Following a single dose of labeled VOX, the majority (approximately 91%) of radioactivity in plasma was parent drug. Biliary excretion of parent drug was determined as the major route of elimination for VOX. The median terminal half-life of VOX following administration of SOF/VEL/VOX was approximately 33 h.

No clinically relevant differences in VOX pharmacokinetics were observed between healthy patients and patients with severe renal impairment. No dose adjustment of SOF/VEL/VOX is warranted for patients with mild or moderate renal impairment. This recommendation for use is guided by its most restrictive component, SOF, and the increased exposure of its major metabolite GS-331007 in renal impairment. Relative to patients with normal hepatic function, the VOX AUC_{inf} values were 299 and 500% higher in patients with moderate and severe hepatic impairment, respectively. Population pharmacokinetic analysis in patients with HCV infection indicated that patients with compensated cirrhosis had 73% higher VOX exposure than those without cirrhosis. No dose adjustment of SOF/VEL/VOX is therefore required for patients with compensated cirrhosis; SOF/VEL/VOX has not been evaluated in patients with decompensated cirrhosis and is not recommended in this population.

Sofosbuvir, VEL, and VOX are substrates of drug transporters P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), while GS-331007 is not (Table 1). Velpatasvir is poorly transported by OATP1B1 and OATP1B3. Voxilaprevir is a substrate of OATP1B1 and OATP1B3. Drugs that are potent inducers of P-gp and/or moderate to potent inducers of CYP2B6, CYP2C8, or CYP3A4 will reduce plasma concentrations of SOF, VEL, and/or VOX, and coadministration of these agents with SOF/VEL/VOX, similar to SOF/VEL, is not recommended. As a decrease in plasma concentration of efavirenz is a result of an interaction with VEL, coadministration of efavirenz is not recommended with SOF/VEL/VOX, again similar to SOF/VEL. However, the addition of VOX to the fixed-dose combination results in a more restrictive drug-drug interaction profile of SOF/VEL/VOX compared to SOF/VEL. With SOF/VEL/VOX, statin exposure is expected to be higher than with SOF/VEL resulting in more restrictions (rosuvastatin is not recommended, and pravastatin dose is not to exceed 40 mg). As the plasma concentration of VOX is increased by OATP inhibitors, coadministration of potent OATP inhibitors, such as cyclosporine A, atazanavir, and lopinavir, with SOF/VEL/VOX is not recommended. Acid-reducing agents do not impact the absorption of VOX, and the dosing recommendations for SOF/VEL/VOX with acid-reducing agents are dependent on the effect on the pH-dependent absorption of VEL. Because the VEL concentration is higher with SOF/VEL/VOX than with SOF/VEL and

Table 1 Mechanisms of drug-drug interaction for SOF, VEL, and VOX

		SOF	VEL	VOX
Drug transporters	P-gp/BCRP	Substrate	Substrate/ inhibitor	Substrate/inhibitor
	OATP1B	–	Substrate/ inhibitor[a]	Substrate/inhibitor
Drug-metabolizing enzymes	CYP3A4	–	Substrate	Substrate
	CYP2C8	–	Substrate	–
	CYP2B6	–	Substrate	–

[a]VEL is also an inhibitor of OATP2B1

because SOF/VEL/VOX is always dosed with food, the concomitant use of SOF/VEL/VOX with proton pump inhibitors is less restrictive.

When SOF/VEL/VOX or its components taken together were administered with food, SOF AUC_{inf} was 64–144% higher; VEL AUC_{inf} was 40–166% higher; and VOX AUC_{inf} was 112–435% higher compared with the exposure under fasted conditions. The increase in VOX exposure when administered with food is the net effect of multiple factors, including increased solubility of VOX and mitigation of the drug-drug interaction in which VOX exposure is decreased by VEL (likely via inhibition of the intestinal uptake transporter OATP2B1). Based on these data, SOF/VEL/VOX was administered with food in the Phase 1b, Phase 2, and Phase 3 studies; collective safety, efficacy, and pharmacokinetic data support administration of SOF/VEL/VOX with food.

3 Phase 1b Study

The pharmacokinetic/pharmacodynamic relationship for antiviral activity was evaluated in a Phase 1b study, GS-US-338-1121, of VOX in patients with HCV infection [3]. The study was double-blind, multicenter, randomized, and placebo-controlled and had an adaptive design to allow testing in a fasted or fed state. In the first completed five cohorts, patients with HCV genotype 1a, HCV genotype 2, and HCV genotype 3 received double-blinded VOX (50, 100, or 300 mg for patients with HCV genotype 1a and 3 and 100 mg for patients with HCV genotype 2) or placebo once daily under fasting conditions for 3 days; VOX 100 mg was administered once daily for 3 days under fasting conditions to patients with HCV genotype 1b and HCV genotype 4.

Of the 67 patients who received treatment, 65 patients completed 10 days of follow-up. Of the two patients who discontinued prior to day 10 of the study, one with genotype 3 infection withdrew consent following treatment with 1 dose of VOX 100 mg, and a second patient with genotype 1a infection treated with VOX 300 mg was lost to follow-up after completion of study treatment. The mean age of study participants was 49 years, and most patients included in this study were male (70%) and white (69%). The viral RNA load was comparable across treatment groups, and the mean viral RNA load at baseline was 6.2 $\log_{10}$ IU/mL. Overall, 11 patients (16%) experienced adverse events, 9 of whom were dosed with VOX (9 of 59; 15%) and 2 of whom received placebo (2 of 8; 25%). No serious adverse events, adverse events leading to study drug discontinuation, or deaths occurred during the study. All adverse events were mild or moderate in severity. The most common adverse events were diarrhea, occurring in 5% (3 of 59) of patients receiving VOX and in 13% (1 of 8) of patients receiving placebo, and headache, occurring in 2% (1 of 59) of patients receiving VOX and in 25% (2 of 8) of patients treated with placebo. The incidence of adverse events was not correlated with the dose of the study drug. There were no clinically significant changes in laboratory abnormalities, vital signs, physical exam findings, or ECGs. Voxilaprevir exhibited linear

pharmacokinetics and was associated with a median half-life of 29–42 h, supporting once-daily dosing.

Administration of VOX daily for 3 days resulted in a rapid decline in HCV RNA from pretreatment levels at all doses and across all genotypes, except among patients with genotype 3a infection who received VOX 50 mg (Fig. 1). Following treatment with 100 mg of VOX, median maximum decline in all groups was >3 log10 IU/mL: the median maximum HCV RNA reduction was 4.5 log10 IU/mL for patients with

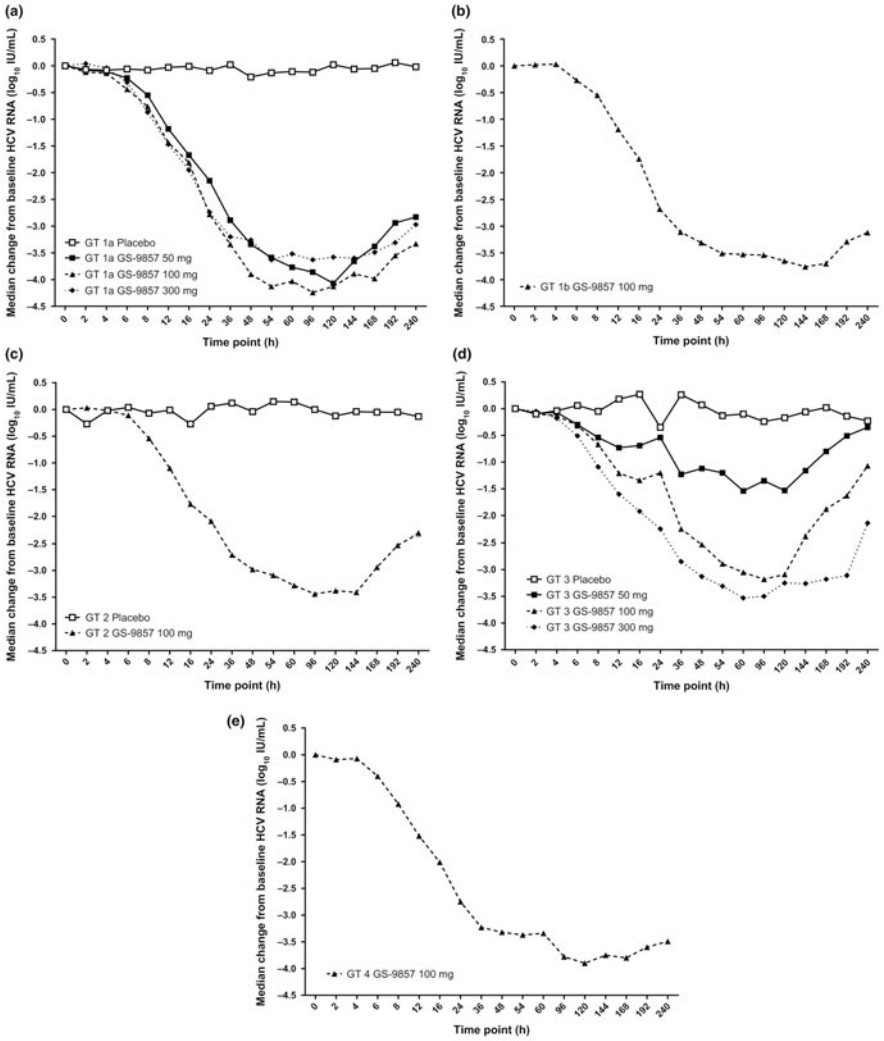

Fig. 1 Median change from baseline HCV RNA over time in patients with genotypes 1–4 HCV infection following administration of VOX (GS-9857) at 0 (day 1), 24 (day 2), and 48 (day 3) hours. (**a**) Genotype 1a. (**b**) Genotype 1b. (**c**) Genotype 2. (**d**) Genotype 3. (**e**) Genotype 4 [3]

HCV genotype 1a, 3.9 log10 IU/mL for patients with HCV genotype 1b infection, 3.6 log10 IU/mL for patients with HCV genotype 2, 3.6 log10 IU/mL for patients with HCV genotype 3a, and 4.1 log10 IU/mL for patients with HCV genotype 4. Patients with HCV genotype 3 receiving VOX 50 mg or 100 mg for 3 days had more rapid virologic rebound after treatment than patients with other HCV genotypes. The presence of NS3 RASs at baseline had no impact on response to 3 days of monotherapy with VOX [4].

The exposure-response relationship with VOX dose in patients with genotype 3 infection could be adequately described using a simple maximal anti-HCV activity (E_{max}) model that used $AUC_{0–24}$ on day 3 of treatment. Using PK and antiviral response data following VOX monotherapy and the known increase in VOX exposure with food, E_{max} modeling predicted VOX exposures at a 100 mg dose when administered as SOF/VEL/VOX with food would achieve near maximal ($\geq$90%) antiviral effect.

Based on the safety, pharmacokinetic, and antiviral activity, the 100 mg dose of VOX with food was selected to move forward in combination with SOF 400 mg and VEL 100 mg in Phase 2 trials with HCV-infected patients.

4 Phase 2 Studies

Based on the potent pangenotypic antiviral activity, improved coverage of clinically important NS3 RASs, and high barrier to resistance (see [1]), VOX was an excellent candidate to pair with SOF and VEL. At the time of Phase 2 initiation of SOF/VEL/VOX clinical development, Phase 3 studies had already demonstrated that the combination of SOF 400 mg and VEL 100 mg administered for 12 weeks was well tolerated and resulted in high SVR rates across all HCV genotypes (see [2]). The goal of adding the third DAA to a fixed-dose combination was to address the two largest remaining questions in the field: How should patients who fail first-line DAA treatment be retreated? What is the shortest duration possible for DAA-based initial HCV treatment?

The primary goal for the SOF/VEL/VOX Phase 2 program was to determine the appropriate duration of treatment based on patient characteristics. The efficacy and safety of SOF/VEL plus VOX or SOF/VEL/VOX for 4–12 weeks of treatment were evaluated in four Phase 2 studies in DAA-experienced and DAA-naive patients with HCV infection with or without cirrhosis, described separately below. Across the SOF/VEL/VOX clinical development program, cirrhosis was determined similarly to other SOF-containing protocols (liver biopsy, FibroTest score >0.75 and AST-platelet ratio index >2, or transient elastography result >12.5 kPa), and key exclusion criteria included hepatic decompensation and coinfection with HBV or HIV.

4.1 Study GS-US-337-1468 (LEPTON)

Study GS-US-337-1468 (LEPTON) was a Phase 2, open-label study conducted at two sites in New Zealand [5]. It enrolled 161 treatment-naive and previously treated patients with genotype 1 and 3 HCV infection between September 2014 and March 2015. Patients were enrolled into one of ten groups, and the duration of therapy with SOF/VEL plus VOX was determined by baseline patient characteristics: 4 or 6 weeks for treatment-naive patients without cirrhosis, 6 weeks for treatment-naive patients with cirrhosis, and 6 or 8 weeks for treatment-experienced patients with or without cirrhosis. Table 2 shows the demographic, disease, and baseline characteristics by treatment group. The DAA-experienced patients with genotype 1 HCV infection had failed prior treatment with DAAs from two classes (protease inhibitor plus non-nucleoside NS5B polymerase inhibitor for six patients, NS5A inhibitor plus NS5B nucleotide polymerase inhibitor for four patients). All patients completed the assigned treatment.

The virologic outcomes are shown in Table 3. No patients experienced virologic breakthrough, and one patient with genotype 3 HCV infection and cirrhosis was lost to follow-up. Among treatment-naive patients with genotype 1 infection without cirrhosis, SVR12 was achieved in 4 of 15 (27%) receiving SOF/VEL plus VOX for 4 weeks and in 14 of 15 (93%) receiving SOF/VEL plus VOX for 6 weeks. Of the 15 treatment-naive patients with genotype 1 HCV and cirrhosis receiving 6 weeks of treatment, 13 (87%) achieved SVR12. Six weeks of treatment led to SVR12 in 20 of 30 (67%) patients with and without cirrhosis who failed previous treatment that contained two DAAs. Eight weeks of SOF/VEL plus VOX led to SVR12 in 17 of 17 (100%) patients with cirrhosis and with genotype 1 HCV who had previously been treated with pegylated interferon plus ribavirin and in 25 of 28 (89%) patients with or without cirrhosis and with genotype 1 HCV who failed a previous protease inhibitor-containing regimen. Among treatment-naive patients with genotype 3 HCV and cirrhosis, SVR12 was achieved by 15 of 18 (83%) receiving 6 weeks of treatment. Eight weeks of SOF/VEL plus VOX led to SVR12 in 19 of 19 (100%) patients with cirrhosis and with genotype 3 HCV who had previously been treated with pegylated interferon plus ribavirin and in 4 of 4 (100%) patients with or without cirrhosis and with genotype 3 who failed a previous DAA-containing regimen.

Overall, RASs forming at least 15% of the viral population in at least one of the three target genes – NS3, NS5A, and NS5B – were detected at baseline in 38% of patients. The SVR12 rate in patients with RASs was 85%, which was similar to the SVR12 rate of 84% in patients without RASs suggesting that baseline resistance did not impact treatment outcome. No treatment-emergent NS3, NS5A, or NS5B RASs were detected at the 15% assay cutoff in the 28 patients who relapsed, consistent with a high barrier to resistance of the regimen.

Adverse events were reported by 80% (128 of 161) of patients overall, and the most common adverse events were headache (23%), nausea (21%), fatigue (17%), and diarrhea (12%). All adverse events were mild or moderate. There were three serious adverse events, none of which were reported by more than one patient and

Table 2 Demographic and baseline characteristics in study GS-US-337-1468 (LEPTON)

	Genotype 1						Genotype 3		
	Treatment naive, no cirrhosis 4 weeks ($N = 15$)	Treatment naive, no cirrhosis 6 weeks ($N = 15$)	Treatment naive with cirrhosis 6 weeks ($N = 15$)	DAA experienced ± cirrhosis 6 weeks ($N = 30$)	PEG+RBV experienced with cirrhosis 8 weeks ($N = 17$)	PI experienced ± cirrhosis 8 weeks ($N = 28$)	Treatment naive with cirrhosis 6 weeks ($N = 18$)	PEG+RBV experienced with cirrhosis 8 weeks ($N = 19$)	DAA experienced ± cirrhosis 8 weeks ($N = 4$)
Mean age, years (range)	54 (40, 64)	50 (24, 65)	59 (51, 66)	55 (35, 73)	58 (48, 70)	57 (39, 66)	52 (39, 64)	55 (44, 66)	56 (43, 62)
Patient sex, *n* (%)									
Male	9 (60)	7 (47)	11 (73)	24 (80)	14 (82)	19 (68)	10 (56)	15 (79)	4 (100)
Female	6 (40)	8 (53)	4 (27)	6 (20)	3 (18)	9 (32)	8 (44)	4 (21)	0
Race, *n* (%)									
White	12 (80)	14 (93)	14 (93)	27 (90)	16 (94)	24 (86)	12 (67)	18 (95)	3 (75)
Asian	2 (13)	1 (7)	0	1 (3)	0	2 (7)	0	0	0
Pacific Islander	1 (7)	0	1 (7)	2 (7)	1 (6)	1 (4)	3 (17)	1 (5)	0
Maori	0	0	0	0	0	1 (4)	3 (17)	0	1 (25)
Other	0	0	0	0	0	1 (4)	3 (17)	0	1 (25)
Mean BMI, kg/m^2 (range)	27 (20, 33)	25 (21, 32)	27 (20, 39)	27 (20, 40)	30 (22, 45)	28 (19, 40)	29 (20, 41)	27 (21, 33)	28 (24, 31)
Mean HCV RNA, $\log_{10}$ IU/mL (SD)	6 (0.5)	6 (0.7)	6 (0.9)	6 (0.5)	6 (0.5)	6 (0.6)	6 (0.7)	6 (0.4)	7 (0.2)
HCV genotype, *n* (%)									
1a	11 (73)	11 (73)	14 (93)	23 (77)	15 (88)	24 (86)	–	–	–
1b	4 (27)	4 (27)	1 (7)	7 (23)	2 (12)	4 (14)	–	–	–
3	–	–	–	–	–	–	18 (100)	19 (100)	4 (100)
IL28B genotype, *n* (%)									
CC	5 (33)	5 (33)	8 (53)	6 (20)	6 (35)	4 (14)	10 (56)	8 (42)	3 (75)
CT	10 (67)	8 (53)	7 (47)	18 (60)	9 (53)	21 (75)	6 (33)	9 (47)	1 (25)
TT	0	2 (13)	0	6 (20)	2 (12)	3 (11)	2 (11)	2 (11)	0
Cirrhosis, *n* (%)	0	0	15 (100)	5 (17)	17 (100)	11 (39)	18 (100)	19 (100)	2 (50)

Table 3 Virologic outcomes in study GS-US-337-1468 (LEPTON)

	Genotype 1						Genotype 3		
	Treatment naive, no cirrhosis 4 weeks ($n = 15$)	Treatment naive, no cirrhosis 6 weeks ($n = 15$)	Treatment naive with cirrhosis 6 weeks ($n = 15$)	DAA experienced ± cirrhosis 6 weeks ($n = 30$)	PEG+RBV experienced with cirrhosis 8 weeks ($n = 17$)	PI experienced ± cirrhosis 8 weeks ($n = 28$)	Treatment naive with cirrhosis 6 weeks ($n = 18$)	PEG+RBV experienced with cirrhosis 8 weeks ($n = 19$)	DAA experienced ± cirrhosis 8 weeks ($n = 4$)
SVR12, *n* (%)	4 (27)	14 (93)	13 (87)	20 (67)	17 (100)	25 (89)	15 (83)	19 (100)	4 (100)
95% CI	8–55	68–>99	60–98	47–83	81–100	72–98	59–96	82–100	40–100
Virologic failure, *n* (%)									
Breakthrough	0	0	0	0	0	0	0	0	0
Relapse	11 (73)	1 (7)	2 (13)	10 (30)	0	3 (11)	2 (11)	0	0
Lost to follow-up, *n* (%)	0	0	0	0	0	0	1 (6)	0	0

none of which were related to study drug. There were no clinically meaningful changes in ALT or total bilirubin values during treatment.

In LEPTON, the three drug combination of SOF/VEL plus VOX demonstrated similar efficacy in patients with genotype 1 or genotype 3 and was well tolerated. Although the 6-week treatment duration achieved SVR rates >90% in treatment-naive genotype 1 patients without cirrhosis, results in more difficult-to-treat patients with cirrhosis and DAA-experienced patients were suboptimal. These data supported the further exploration of the 3-DAA regimen for 6 and 8 weeks of DAA-naive patients and 12 weeks in DAA-experienced patients in other larger Phase 2 studies.

4.2 Studies GS-US-367-1168 and GS-US-367-1169

Study GS-US-367-1168 was a Phase 2, multicenter, open-label study to assess the safety, tolerability, and efficacy of SOF/VEL plus VOX in treatment-naive and DAA-experienced patients with genotype 1 HCV infection [6]. The study enrolled subjects between March and September 2015 at 34 sites in the United States and New Zealand. Treatment-naive patients without cirrhosis received SOF/VEL plus VOX for 6 or 8 weeks, treatment-naive patients with cirrhosis received SOF/VEL plus VOX with or with ribavirin for 8 weeks, and DAA-experienced patients who previously failed an NS5A inhibitor or at least two classes of DAA with or without cirrhosis received SOF/VEL plus VOX for 12 weeks. Study GS-US-342-1169 was a Phase 2, multicenter, open-label study that assessed the safety, tolerability, and efficacy of SOF/VEL plus VOX treatment-naive and treatment-experienced patients with genotype 2, 3, 4, 5, or 6 HCV infection (i.e., non-genotype 1; [7]). Treatment-naive patients without cirrhosis received SOF/VEL plus VOX for 6 weeks, treatment-naive patients with cirrhosis received SOF/VEL plus VOX for 8 weeks, and treatment-experienced patients who previously failed a DAA-based or interferon-based regimen with or without cirrhosis received SOF/VEL plus VOX for 12 weeks. Study GS-US-342-1168 and study GS-US-342-1169 enrolled subjects between March and September 2015 at the same 34 sites in the United States and New Zealand. Based on the similar objectives and design of these two studies, the data will be presented together by prior treatment experience.

The studies enrolled 128 treatment-experienced patients, all but one of whom completed 12 weeks of SOF/VEL plus VOX for 12 weeks. There were 63 patients infected with HCV genotype 1 in GS-US-367-1168 and 65 patients infected with HCV genotype 2, 3, 4, or 6 in GS-US-367-1169, of whom 48% had cirrhosis (Table 4). Among the DAA-experienced patients with genotype 1 infection, 46% previously received a NS5A inhibitor (ledipasvir, 7 patients; daclatasvir, 11 patients; other, 11 patients), and 54% previously received a protease inhibitor and a NS5B polymerase inhibitor (simeprevir with sofosbuvir, 25 patients; other, 9 patients). Among the treatment-experienced patients with non-genotype 1 infection, 42% previously received an interferon-based treatment (27 patients), and 58% had been previously treated with a DAA (38 patients), most of whom with SOF. At baseline,

Table 4 Demographic and baseline characteristics by treatment and duration in study GS-US-367-1168 and GS-US-367-1169

	Treatment-experienced patients	Treatment-naive patients		
	SOF/VEL + VOX 12 weeks ($N = 128$)	SOF/VEL + VOX 6 weeks ($N = 67$)	SOF/VEL + VOX 8 weeks ($N = 99$)	SOF/VEL + VOX + RBV 8 weeks ($N = 31$)
Mean age, years (range)	58 (37–77)	53 (18–72)	55 (19–76)	59 (43–71)
Male, *n* (%)	96 (75)	35 (52)	61 (62)	19 (61)
White, *n* (%)	105 (82)	58 (87)	81 (82)	26 (84)
Mean BMI, kg/m^2 (range)	29 (18–53)	26 (18–42)	29 (19–47)	29 (19–52)
Mean HCV RNA, $\log_{10}$ IU/mL (range)	6 (4–8)	6 (4–8)	6 (5–7)	6 (5–7)
HCV genotype, *n* (%)				
1	63 (49)	35 (52)	69 (70)	31 (100)
2	21 (16)	6 (9)	6 (6)	0
3	35 (27)	21 (31)	18 (18)	0
4	7 (5)	5 (7)	5 (5)	0
5	0	0	0	0
6	2 (2)	0	1 (1)	0
IL28B non-CC genotype, *n* (%)	93 (73)	44 (66)	72 (73)	19 (61)
Cirrhosis, *n* (%)	61 (48)	0	63 (64)	31 (100)
Prior DAA experience, *n* (%)				
None (genotype 2–6 only)	27 (21)	–	–	–
NS5A inhibitor	35 (27)	–	–	–
No NS5A inhibitor	66 (52)	–	–	–

59% had RASs in NS3, NS5A, and/or NS5B using a 15% assay cutoff. Overall, 99% of treatment-experienced patients (127 of 128) who were treated with SOF/VEL plus VOX in the two studies achieved SVR12 (Fig. 2). The only treatment-experienced patient who relapsed had genotype 3 HCV infection and cirrhosis and had previously been treated with SOF and ribavirin. This patient had the NS5A RAS Y93H at baseline and at relapse and had treatment-emergent Q80R which does not confer in vitro resistance to VOX.

The studies enrolled 197 treatment-naive patients; 3 patients discontinued treatment due to adverse events. There were 135 patients infected with genotype 1 HCV and 62 patients infected with non-genotype 1 HCV, and 48% had cirrhosis (Table 4). At baseline, 50% had RASs in NS3, NS5A, and/or NS5B using a 15% assay cutoff. For the treatment-naive patients, the SVR12 rate among those without cirrhosis who

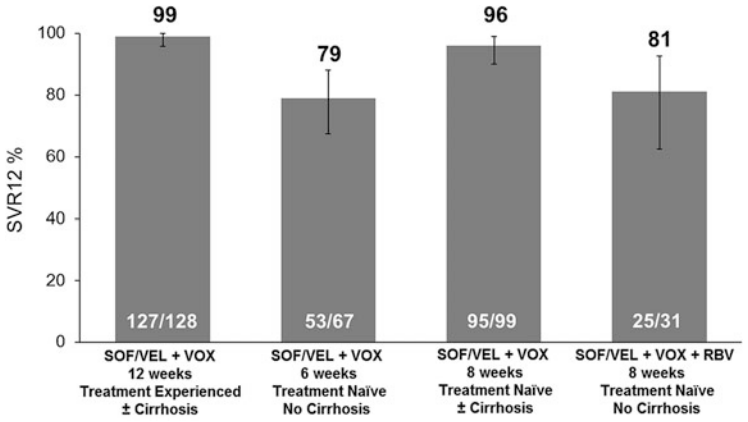

Fig. 2 SVR12 rates by treatment and duration in study GS-US-367-1168 and study GS-US-367-1169

received 6 weeks of SOF/VEL plus VOX was 79% (53 of 67 with 14 relapsers; Fig. 2). Overall, 96% (95 of 99) of patients who received 8 weeks of SOF/VEL plus VOX achieved SVR12, 100% (36 of 36 patients) of those without cirrhosis and 94% of those with cirrhosis (59 of 63 patients). Of the four patients who relapsed in this group, all had cirrhosis, two had genotype 1, one had genotype 3, and one had genotype 4. In the group of patients with genotype 1 infection and cirrhosis who received SOF/VEL plus VOX plus RBV for 8 weeks, the SVR12 rate was 81% (25 of 31patients with 6 relapsers). Of the 24 treatment-naive patients who relapsed, none had treatment-emergent RASs at the time of virologic failure using a 15% assay cutoff.

SOF/VEL plus VOX, with and without RBV, was generally safe and well tolerated. Across treatment groups, most patients had at least one adverse event (68%, 219 of 325 patients). The majority of adverse events were Grade 1 or Grade 2 in severity. The most common adverse events were headache (23%, 75 patients), nausea (18%, 58 patients), fatigue (18%, 58 patients), and diarrhea (15%, 50 patients). Patients in the ribavirin-containing group had higher rates of fatigue (32%, 10 of 31 patients), anemia (23%, 7 of 31 patients), and decreased hemoglobin (13%, 3 of 31 patients) than patients in the other groups.

These two 12-week Phase 2 multicenter studies of the three-DAA regimen containing SOF, VEL, and VOX led to high SVR12 rates across HCV genotypes in treatment-experienced patients with and without cirrhosis, including those with DAA experience with NS5A and/or NS5B inhibitors. Among patients who were treatment naive, 8 weeks of SOF/VEL plus VOX was highly effective in patients with HCV genotypes 1–6 with and without cirrhosis. Together, these data supported the 12- and 8-week durations of SOF/VEL/VOX treatment in the DAA-experienced and DAA-naive populations, respectively, in the Phase 3 program.

4.3 Study GS-US-367-1871 (TRILOGY-3)

Study GS-US-367-1871 (TRILOGY-3) was a Phase 2, open-label study conducted at one site in Texas [8]. This study enrolled patients with chronic HCV genotype 1 infection who were previously treated with a DAA and randomized them to receive SOF/VEL/VOX with or without ribavirin stratified by the presence of cirrhosis and prior exposure to an NS5A inhibitor. This study enrolled patients between August and October 2015 beginning after the multicenter Phase 2 studies GS-US-367-1168 and GS-US-367-1169. In advance of the start of this study, the results from the Phase 1 bioavailability study were available which demonstrated that the pharmacokinetics of the SOF/VEL/VOX fixed-dose combination (400/100/100 mg) tablets was similar to that of the coadministered SOF/VEL (400/100 mg) and VOX single-agent (100-mg) tablets, and GS-US-367-1871 was the first clinical study to use the SOF/VEL/VOX fixed-dose combination for treatment of patients infected with chronic HCV.

Of the 49 patients enrolled, 24 received SOF/VEL/VOX without ribavirin, and 25 received SOF/VEL/VOX with ribavirin. All patients completed SOF/VEL/VOX; ribavirin dosing was discontinued by three patients and interrupted or modified by three patients. Table 5 shows the demographic and baseline characteristics by study treatment. Overall, 51% of patients had cirrhosis, 41% had failed prior treatment with an NS5A inhibitor, and 73% had RASs in NS3, NS5A, and/or NS5B. Of the patients who received SOF/VEL/VOX without and with ribavirin, 100% and 96% achieved SVR12, respectively (Table 6). The one patient who relapsed in the SOF/VEL/VOX plus ribavirin treatment group had genotype 1a infection, cirrhosis, previously failed LDV/SOF treatment and treatment-emergent NS3 (V36M, Q41R, D168G) and NS5A (M28T, Q30R), and RASs detected at relapse using a 15% assay cutoff.

Treatment-emergent adverse events were reported by 46% of patients receiving SOF/VEL/VOX and 60% of patients receiving SOF/VEL/VOX plus ribavirin. The most common adverse events were diarrhea (13%) in patients receiving SOF/VEL/VOX. Patients receiving SOF/VEL/VOX plus ribavirin had more ribavirin-associated toxicities: the most common adverse events were fatigue (36%) and anemia (16%), and the most common laboratory abnormality was decreased hemoglobin (16%).

In TRILOGY-3, a small, single-site Phase 2 study, 12 weeks of treatment with a fixed-dose combination of SOF/VEL/VOX was effective and well tolerated among patients with genotype 1 infection who had previously failed a DAA-based regimen. The addition of ribavirin did not improve efficacy but did contribute to the safety profile. Based on these results, ribavirin was not further assessed in the SOF/VEL/VOX Phase 3 program.

Table 5 Demographic and baseline characteristics in study GS-US-367-1871 (TRILOGY-3)

	SOF/VEL/VOX 12 weeks ($N = 24$)	SOF/VEL/VOX + RBV 12 weeks ($N = 25$)
Mean age, years (range)	54 (18–71)	54 (22–75)
Patient sex, *n* (%)		
Male, *n* (%)	16 (67)	16 (64)
Female, *n* (%)	8 (33)	9 (36)
Race, *n* (%)		
White	17 (71)	22 (88)
Black	7 (29)	3 (12)
Mean BMI, kg/m^2 (range)	32 (21–55)	30 (20–50)
Mean HCV RNA, $\log_{10}$ IU/mL (SD)	6 (0.42)	6 (0.46)
HCV genotype, *n* (%)		
1a	21 (88)	22 (88)
1b	3 (13)	3 (12)
IL28B genotype, *n* (%)		
CC	2 (8)	5 (20)
CT	10 (42)	13 (52)
TT	12 (50)	7 (28)
Cirrhosis, *n* (%)	11 (46)	14 (56)
Prior DAA experience, *n* (%)		
NS5A ± DAA (s)	10 (42)	10 (40)
NS5A alone	3 (13)	0
NS5A + NS5B	1 (4)	6 (24)
NS5A + NS5B + NS3	6 (25)	4 (16)
Non-NS5A ± DAA (s)	14 (58)	15 (60)
NS5B alone	5 (21)	3 (12)
NS3 alone	6 (25)	9 (36)
NS5B + NS3	3 (13)	3 (12)

Table 6 Virologic outcomes in study GS-US-367-1871 (TRILOGY-3)

	SOF/VEL/VOX 12 weeks ($N = 24$)	SOF/VEL/VOX + RBV 12 weeks ($N = 25$)
SVR12, *n* (%)	24 (100)	24 (96)
95% CI	86–100	80–100
Virologic failure, *n* (%)		
Breakthrough	0	0
Relapse	0	1 (4)

5 Phase 3 Studies

The efficacy of SOF/VEL/VOX was evaluated in four Phase 3 registrational studies in patients with HCV infection without cirrhosis or with compensated cirrhosis; two studies investigated 12 weeks of treatment in DAA-experienced patients, and two studies investigated 8 weeks of treatment in DAA-naive patients (Fig. 3). These studies enrolled patients between November 2015 and April 2016 at 117 sites in the United States, Canada, the United Kingdom, France, Germany, Australia, and New Zealand.

5.1 Efficacy in DAA-Experienced Patients

The primary goal of the clinical development program was to demonstrate that SOF/VEL/VOX was a highly effective and safe salvage therapy for the most difficult-to-cure patients. The Phase 3 program was conducted to specifically assess the efficacy of SOF/VEL/VOX in DAA-experienced patients for a treatment duration of 12 weeks as supported by the Phase 2 studies, GS-US-367-1168, GS-US-367-1169, and TRILOGY-3. Initially, a single study was proposed which would enroll all DAA-experienced patients to receive SOF/VEL/VOX or blinded placebo for 12 weeks. In response to a request from the US Food and Drug Administration, the DAA-experienced patient population was separated into two separate studies, one with the original placebo-controlled design for NS5A inhibitor-experienced patients (POLARIS-1) and another with open-label SOF/VEL for 12 weeks as an

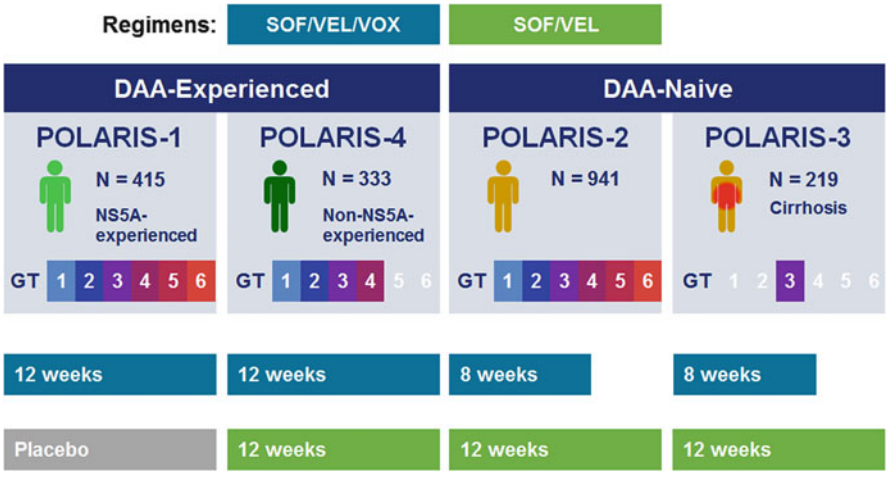

Fig. 3 Design of SOF/VEL/VOX Phase 3 studies (GS-US-367-1171 [POLARIS-1], GS-US-367-1172 [POLARIS-2], GS-US-367-1173 [POLARIS-3], and GS-US-367-1170 [POLARIS-4])

active comparator for DAA-experienced patients who have not received an NS5A inhibitor (POLARIS-4).

5.1.1 GS-US-367-1171 (POLARIS-1)

Study GS-US-367-1171 (POLARIS-1) was a Phase 3, randomized, double-blind, placebo-controlled study which assessed the antiviral efficacy, safety, and tolerability of SOF/VEL/VOX compared with placebo for 12 weeks in NS5A inhibitor-experienced patients with chronic HCV infection [9]. The placebo control was chosen for this study as there was no available approved treatment for NS5A inhibitor-experienced patients. Additionally, this design allowed for an assessment of the safety of SOF/VEL/VOX as compared with the patients who received placebo. Patients with genotype 1 HCV infection were randomized 1:1 to receive SOF/VEL/VOX or placebo for 12 weeks, stratified according to cirrhosis status. Patients with other HCV genotypes were not randomized and were enrolled to receive SOF/VEL/VOX for 12 weeks. The primary efficacy end point of the study was the SVR12 rate in the SOF/VEL/VOX 12-week group was compared with a pre-specified SVR performance goal of 85% using a two-sided exact one-sample binomial test at the 0.05 significance level.

Overall, 415 patients were enrolled and began treatment with SOF/VEL/VOX (263 patients) or placebo (152 patients). Five patients prematurely discontinued study treatment, two in the SOF/VEL/VOX group (one due to an adverse event and one was lost to follow-up) and three patients in the placebo group due to AEs. The demographics and baseline characteristics of the patients treated with SOF/VEL/VOX for 12 weeks are shown in Table 7. The majority of the patients had genotype 1 HCV infection (57%, 150 patients) or genotype 3 HCV infection (30%, 78 patients), and 46% (121 patients) had cirrhosis. Most patients (93%) previously received an NS5A inhibitor in combination with another DAA. The most common NS5A inhibitors used in previous unsuccessful treatments were ledipasvir (55%), daclatasvir (23%), and ombitasvir (13%). Among the 248 patients with available baseline sequencing, 205 (83%) had NS5A and/or NS3 RASs.

In POLARIS-1, 96% (253 of 263) of patients achieved SVR12, and this rate met the primary efficacy end point of being significantly superior to the pre-specified performance goal of 85% ($p < 0.001$). As shown in Table 8, treatment with SOF/VEL/VOX for 12 weeks in NS5A inhibitor-experienced patients demonstrated consistently high SVR12 rates across genotypes and regardless of cirrhosis status. Seven patients had virologic failure: six patients relapsed, and one patient had on-treatment virologic failure with PK data consistent with nonadherence. All six patients who relapsed had cirrhosis: one patient had genotype 1a HCV infection, four patients had genotype 3 HCV infection, and one patient had genotype 4 HCV infection. Baseline RASs did not impact treatment outcome: the SVR12 rate in patients with baseline RASs was 97% and in patients without baseline RASs was 98%. Of the six patients who relapsed, only one patient had treatment-emergent resistance (the patient with genotype 4 infection developed Y93H).

Table 7 Demographic and baseline characteristics in GS-US-367-1171 (POLARIS-1) and GS-US-367-1170 (POLARIS-4)

	POLARIS-1	POLARIS-4	
	SOF/VEL/VOX 12 weeks ($N = 263$)	SOF/VEL/VOX 12 weeks ($N = 182$)	SOF/VEL 12 weeks ($N = 151$)
Mean age, years (range)	58 (27–84)	57 (24–85)	57 (24–80)
Patient sex, *n* (%)			
Male	200 (76)	143 (79)	114 (75)
Female	63 (24)	39 (21)	37 (25)
Race, *n* (%)			
White	211 (80)	160 (88)	131 (87)
Black or African American	38 (14)	16 (9)	13 (9)
Asian	8 (3)	2 (1)	4 (3)
Other	1 (<1)	2 (1)	1 (<1)
Native Hawaiian or Pacific Islander	3 (1)	0	2 (1)
American Indian or Alaska native	1 (<1)	2 (1)	0
Not disclosed	1 (<1)	0	0
Mean BMI, kg/m^2 (range)	29 (18–67)	29 (18–45)	29 (18–53)
Mean HCV RNA, $\log_{10}$ IU/mL (SD)	6 (0.68)	6 (0.56)	6 (0.66)
HCV genotype, *n* (%)			
Genotype 1	150 (57)	78 (43)	66 (44)
1a	101 (38)	54 (30)	44 (29)
1b	45 (17)	24 (13)	22 (15)
1 other	4 (2)	0	0
Genotype 2	5 (2)	31 (17)	33 (22)
Genotype 3	78 (30)	54 (30)	52 (34)
Genotype 4	22 (8)	19 (10)	0
Genotype 5	1 (<1)	0	0
Genotype 6	6 (2)	0	0
Unknown	1 (<1)	0	0
IL28B genotype, *n* (%)			
CC	47 (18)	33 (18)	29 (19)
CT	165 (63)	107 (59)	95 (63)
TT	51 (19)	42 (23)	27 (18)
Cirrhosis, *n* (%)	121 (46)	84 (46)	69 (46)
Prior DAA experience, *n* (%)			
NS5A + NS3 ± NS5B	9 (32)	0	0
NS5A + NS5B	161 (61)	0	0
NS5A	18 (7)	0	0
NS5B + NS3	0	46 (25)	38 (25)
NS5B	1 (<1)	134 (74)	109 (72)
NS3	0	0	1 (<1)
None	0	0	1 (<1)

Table 8 SVR12 of DAA-experienced patients in GS-US-367-1171 (POLARIS-1) and GS-US-367-1170 (POLARIS-4)

	GS-US-367-1171 (POLARIS-1)	GS-US-367-1170 (POLARIS-4)	
	SOF/VEL/VOX 12 weeks ($N = 263$)	SOF/VEL/VOX 12 weeks ($N = 182$)	SOF/VEL 12 weeks ($N = 151$)
Overall, *n/N* (%)	253/263 (96)	178/182 (98)	136/151 (90)
95% CI	93 to 98	95% to 99	84 to 94
HCV genotype, *n/N* (%)			
Genotype 1	146/150 (97)	76/78 (97)	60/66 (91)
1a	97/101 (96)	53/54 (98)	39/44 (89)
1b	45/45 (100)	23/24 (96)	21/22 (96)
1 other	4/4 (100)	–	–
Genotype 2	5/5 (100)	31/31 (100)	32/33 (97)
Genotype 3	74/78 (95)	52/54 (96)	44/52 (85)
Genotype 4	20/22 (91)	19/19 (100)	–
Genotype 5	1/1 (100)	–	–
Genotype 6	6/6 (100)	–	–
Unknown	1/1 (100)	–	–
Cirrhosis, *n/N* (%)			
Yes	113/121 (93)	81/84 (96)	59/69 (86)
No	140/142 (99)	96/98 (98)	77/82 (94)

Of the 152 initially randomized to receive placebo in the primary study of POLARIS-1, 147 enrolled in a subsequent substudy to receive 12 weeks of open-label SOF/VEL/VOX [10]. All 147 patients completed treatment, and 97% (143 of 147 patients) achieved SVR12. Four patients experienced virologic relapse; all had HCV genotype 1a, one had cirrhosis, and two had treatment-emergent RASs.

5.1.2 GS-US-367-1170 (POLARIS-4)

Study GS-US-367-1170 (POLARIS-4) was a Phase 3, randomized, open-label study which assessed the antiviral efficacy, safety, and tolerability of 12 weeks of SOF/VEL/VOX and 12 weeks of SOF/VEL in DAA-experienced patients with chronic HCV infection with or without cirrhosis who had not previously received an inhibitor of the HCV NS5A protein, (with the exception that those who had received only a PI with pegylated interferon and ribavirin were not included, since these patients had approved retreatment options) [9]. The use of SOF/VEL/VOX and SOF/VEL within the same study allowed an assessment of the contribution of VOX to efficacy as well as to the safety of the treatment regimen. Patients with genotype 1, 2, or 3 HCV infection were randomized 1:1 to receive SOF/VEL/VOX or SOF/VEL once daily for 12 weeks, stratified according to HCV genotype and cirrhosis status. Patients infected with other HCV genotypes with or without

cirrhosis were enrolled in the SOF/VEL/VOX 12-week group. In the primary efficacy analysis, the SVR12 rate in the SOF/VEL/VOX 12-week and SOF/VEL 12-week groups was compared with a pre-specified SVR performance goal of 85% using a two-sided exact one-sample binomial test at the 0.025 significance level.

A total of 333 patients were enrolled in POLARIS-4, 182 in the SOF/VEL/VOX group and 151 in the SOF/VEL group. Two patients did not complete SOF/VEL treatment, one due to an adverse event and another due to a lack of efficacy. The demographics and baseline characteristics of the patients treated with SOF/VEL/VOX for 12 weeks are shown in Table 7. Similar to POLARIS-1, the majority of the patients enrolled in POLARIS-4 had genotype 1 HCV infection (43%, 144 patients) or genotype 3 HCV infection (32%, 106 patients), and 46% (153 patients) had cirrhosis. Most patients (72%) previously received an NS5A inhibitor without another DAA, and 85% of patients had received sofosbuvir as a part of previous unsuccessful treatment. At baseline sequencing, 49% of patients had NS5A and/or NS3 RASs.

In POLARIS-4, treatment with SOF/VEL/VOX for 12 weeks resulted in an SVR12 rate of 98%, which was statistically superior to the performance goal of 85% at the pre-specified 0.025 significance level ($p < 0.001$), meeting the primary efficacy end point. Treatment with SOF/VEL for 12 weeks resulted in an SVR12 rate of 90%, which was not statistically superior to the performance goal of 85% at the pre-specified 0.025 significance level ($p = 0.092$). Although not pre-specified in the POLARIS-4 statistical analysis plan, an ad hoc evaluation of the superiority of SOF/VEL/VOX treatment for 12 weeks compared with SOF/VEL treatment for 12 weeks was performed in patients with genotypes 1, 2, or 3 HCV infection. Treatment with SOF/VEL/VOX for 12 weeks was statistically superior to treatment with SOF/VEL for 12 weeks ($p = 0.005$).

As shown in Table 8, patient subgroups by genotype and by cirrhosis status, SOF/VEL/VOX for 12 weeks led to higher SVR12 rates compared with SOF/VEL for 12 weeks, demonstrating the contribution of VOX to the regimen. There was only one patient with virologic failure in the SOF/VEL/VOX group who had genotype 1a HCV infection and cirrhosis and was previously treated with SOF plus simeprevir who had no RASs at the time of relapse. There were 15 patients experienced virologic failure in the SOF/VEL group. There was one patient with genotype 2 HCV infection without cirrhosis who experienced virologic breakthrough with treatment-emergent resistance with the infrequently seen SOF signature mutation S282T in addition to Y93H. Of the 14 patients who relapsed following SOF/VEL treatment for 12 weeks, 8 patients had genotype 3 HCV infection, and 7 of these patients also had cirrhosis. Six patients who relapsed had genotype 1 HCV infection: three patients with genotype 1a with cirrhosis, two patients with genotype 1a without cirrhosis, and one patient with genotype 1b without cirrhosis who completed only 56 days of study treatment (discontinued treatment due to headache). Ten of these 14 patients with relapse had treatment-emergent RASs, most of which were in the NS5A gene at amino acid position 93. In both treatment groups, the presence of baseline RASs did not impact treatment outcome: in the SOF/VEL/VOX group, the SVR12 rates in patients with and without baseline RASs were 100 and 99%,

respectively; in the SOF/VEL group, the SVR12 rates in patients with and without baseline RASs were 90 and 89%, respectively.

5.2 Efficacy in DAA-Naive Patients

The second goal of the SOF/VEL/VOX clinical development program was to assess whether the addition of a third potent DAA to the regimen would allow shortening treatment duration for the relatively easy-to-cure DAA-naive patients. To this end, the Phase 3 program included an evaluation in DAA-naive patients of the efficacy of SOF/VEL/VOX for 8 weeks, as supported by the Phase 2 studies, LEPTON, GS-US-367-1168, and GS-US-367-1169, compared to SOF/VEL for 12 weeks. Initially, a single study was initially proposed which would enroll all DAA-naive patients to receive SOF/VEL/VOX for 8 weeks or SOF/VEL for 12 weeks (POLARIS-2). In response to a request from the US Food and Drug Administration, a separate study with a similar design was conducted in patients with genotype 3 HCV infection and cirrhosis (POLARIS-2), and this population was removed from POLARIS-3. For both POLARIS-2 and POLARIS-3, SOF/VEL for 12 weeks was chosen as the comparator for this study based on the data from the Phase 3 studies ASTRAL-1, ASTRAL-2, and ASTRAL-3 (see [2]) and because it was anticipated to be a standard of care regimen for DAA-naive patients with genotype 1–6 HCV infection during the development of SOF/VEL/VOX, including those without cirrhosis or with compensated cirrhosis. In addition, the choice of comparator allowed for the assessment of the safety profile of VOX in the SOF/VEL/VOX regimen.

5.2.1 GS-US-367-1172 (POLARIS-2)

Study GS-US-367-1172 (POLARIS-2) was a Phase 3, randomized, open-label study which assessed the antiviral efficacy, safety, and tolerability of 8 weeks of SOF/VEL/VOX compared with 12 weeks of SOF/VEL in DAA-naive patients with chronic HCV infection [11]. Patients with genotype 1, 2, or 4 HCV infection with or without cirrhosis or genotype 3 HCV infection without cirrhosis were randomized 1:1 to receive SOF/VEL/VOX for 8 weeks or SOF/VEL for 12 weeks (patients with genotype 3 HCV infection and cirrhosis were enrolled in POLARIS-3). Patients with other genotypes with or without cirrhosis were enrolled into the SOF/VEL/VOX 8-week group. In POLARIS-2, the primary efficacy analysis assessed the noninferiority of the rate of SVR among patients receiving SOF/VEL/VOX to the rate among patients receiving SOF/VEL using a noninferiority margin of 5%. A two-sided 95% confidence interval was constructed for the difference in the rates of SVR between the two treatment groups using stratum-adjusted Mantel–Haenszel proportions. Noninferiority was established if the lower bound was greater than −5%.

A total of 941 patients were enrolled and treated in POLARIS-2, 501 in the SOF/VEL/VOX group and 440 in the SOF/VEL group. One patient did not complete SOF/VEL/VOX treatment due to pregnancy, and three patients did not complete SOF/VEL treatment, two due to adverse events and one was lost to follow-up. The demographics and baseline characteristics of the patients enrolled in POLARIS-2 are shown in Table 9. The majority of patients had genotype 1 (49%) or genotype 3 (19%) HCV infection; 12% of patients had genotype 2, 13% had genotype 4, 2% had genotype 5, and 4% had genotype 6. Overall, 19% of patients had cirrhosis. A total of 218 patients (23%) had prior treatment with an interferon-based regimen; the majority of these patients (80%; 174 of 218 patients) had failed prior treatment with pegylated interferon plus ribavirin. At baseline sequencing, 50% of patients had NS5A and/or NS3 RASs.

In the POLARIS-2 trial, the SVR12 rate was 95% (95% CI 93–97%) among patients receiving 8 weeks of SOF/VEL/VOX and 98% (95% CI 96–99%) among those receiving 12 weeks of SOF/VEL (Table 10). The SVR12 rate for the SOF/VEL/VOX 8-week group did not demonstrate noninferiority to the SVR12 rate for the SOF/VEL 12-week group. The strata-adjusted difference (95% CI) in the proportions was −3% (−6% to −<1%), the lower bound of which is not greater than the pre-specified noninferiority margin of −5%.

The lower SVR12 rate observed in the SOF/VEL/VOX 8-week group compared with the SOF/VEL 12-week group was primarily due to a lower SVR rate among patients with genotype 1a HCV infection, particularly among those enrolled at US sites. Overall, the SVR12 rate for patients with genotype 1a infection who were treated with SOF/VEL/VOX for 8 weeks was 92% (155 of 169); among those in the United States, the SVR12 rate was 89% (95 of 107), and among those outside the United States, the SVR12 rate was 97% (60 of 62). Of the 21 patients in the SOF/VEL/VOX 8-week group who relapsed, 14 had genotype 1a HCV infection. The other seven patients with virologic relapse included two patients with genotype 1b, one of whom had cirrhosis; two patients with genotype 2 HCV infection without cirrhosis; two patients with genotype 4 infection, one of whom had cirrhosis; and one patient with genotype 5 infection without cirrhosis. Although the SVR12 rate was lower among patients receiving SOF/VEL/VOX for 8 weeks with cirrhosis (91%) compared with those without cirrhosis (96%), most of the patients with cirrhosis who relapsed had genotype 1a HCV infection (5 of 7 patients). The SVR12 rate was 94% for patients with baseline NS5A and/or NS3 RASs and 98% for patients without baseline NS5A and/or NS3 RASs. For patients with HCV genotype 1a, the rates of SVR in patients with and without baseline RASs were 89% and 95%, respectively. The Q80K RAS was the most commonly observed NS3 variant; although it confers no change to VOX susceptibility in vitro, the SVR12 rate was lower for genotype 1a patients with baseline Q80K compared to those without (88 and 94%, respectively). Of the 21 patients with virologic relapse at posttreatment week 12, one patient had treatment-emergent resistance (NS5A RASs Q30R and L31M).

In the SOF/VEL 12-week group, three patients had virologic relapse, one patient with genotype 1a with cirrhosis, one patient with genotype 1b without cirrhosis, and

Table 9 Demographic and baseline characteristics in GS-US-367-1172 (POLARIS-2) and GS-US-367-1173 (POLARIS-3)

	POLARIS-2		POLARIS-3	
	SOF/VEL/VOX 8 weeks ($N = 501$)	SOF/VEL 12 weeks ($N = 440$)	SOF/VEL/VOX 8 weeks ($N = 110$)	SOF/VEL 12 weeks ($N = 109$)
Mean age, years (range)	53 (18–78)	52 (19–82)	54 (25–75)	55 (8.4)
Patient sex, *n* (%)				
Male	255 (51)	237 (54)	74 (67)	83 (76)
Female	246 (49)	203 (46)	36 (33)	26 (24)
Race, *n* (%)				
White	391 (78)	365 (83)	100 (91)	97 (89)
Black or African American	48 (10)	47 (11)	0	1 (<1)
Asian	51 (10)	22 (5)	8 (7)	9 (8)
Other	5 (1)	2 (<1)	1 (<1)	0
Native Hawaiian or Pacific Islander	3 (<1)	2 (<1)	0	1 (<1)
American Indian or Alaska native	3 (<1)	2 (<1)	1 (<1)	1 (<1)
Mean BMI, kg/m^2 (range)	27 (17–57)	27 (18–54)	28 (20–50)	27 (18–46)
Mean HCV RNA, $\log_{10}$ IU/mL (SD)	6 (<1)	6 (<1)	6 (<1)	6 (<1)
HCV genotype, *n* (%)				
Genotype 1	233 (47)	232 (53)	0	0
1a	169 (34)	172 (39)	0	0
1b	63 (13)	59 (13)	0	0
1 other	1 (<1)	1 (<1)	0	0
Genotype 2	63 (13)	53 (12)	0	0
Genotype 3	92 (18)	89 (20)	110 (100)	109 (100)
Genotype 4	63 (13)	57 (13)	0	0
Genotype 5	18 (4)	0	0	0
Genotype 6	30 (6)	9 (2)	0	0
Unknown	2 (<1)	0	0	0
IL28B genotype, *n* (%)				
CC	166 (33)	136 (31)	41 (37)	52 (48)
CT	253 (51)	245 (56)	57 (52)	44 (40)
TT	82 (16)	59 (13)	12 (11)	13 (12)
Cirrhosis, *n* (%)	90 (18)	84 (19)	110 (100)	109 (100)
Prior treatment experience, *n* (%)				
Treatment naive	383 (76)	340 (77)	75 (68)	77 (71)
Treatment experienced	118 (24)	100 (23)	35 (32)	32 (29)

Table 10 SVR12 in GS-US-367-1172 (POLARIS-2) and GS-US-367-1173 (POLARIS-3)

	GS-US-367-1172 (POLARIS-2)		GS-US-367-1173 (POLARIS-3)	
	SOF/VEL/VOX 8 weeks ($N = 501$)	SOF/VEL 12 weeks ($N = 440$)	SOF/VEL/VOX 8 weeks ($N = 110$)	SOF/VEL 12 weeks ($N = 109$)
Overall *n/N* (%)	477/501 (95)	432/440 (98)	106/110 (96)	105/109 (96)
95% CI	93–97	96–99	91–99	91–99
HCV genotype, *n/N* (%)				
Genotype 1	217/233 (93)	228/232 (98)	–	–
1a	155/169 (92)	170/172 (99)	–	–
1b	61/63 (97)	57/59 (97)	–	–
1 other	1/1 (100)	1/1 (100)	–	–
Genotype 2	61/63 (97)	53/53 (100)	–	–
Genotype 3	91/92 (99)	86/89 (97)	106/110 (96)	105/109 (96)
Genotype 4	59/63 (94)	56/57 (98)	–	–
Genotype 5	17/18 (94)	–	–	–
Genotype 6	30/30 (100)	9/9 (100)	–	–
Unknown	2/2 (100)	–	–	–
Cirrhosis, *n/N* (%)				
Yes	82/90 (91)	83/84 (99)	106/110 (96)	105/109 (96)
No	395/411 (96)	349/356 (98)	–	–

one patient with genotype 4a HCV infection without cirrhosis. The patient with genotype 1a who relapsed developed treatment-emergent Y93N. The presence of baseline RASs did not impact the SVR12 rate for the SOF/VEL 12-week group; 100% (217 of 218 patients) of the patients with RASs and 99% (206 of 208 patients) of the patients without RASs achieved SVR12.

5.2.2 GS-US-367-1173 (POLARIS-3)

Study GS-US-367-1173 (POLARIS-3) was a Phase 3, randomized, open-label study which assessed the antiviral efficacy, safety, and tolerability of 8 weeks of SOF/VEL/VOX compared with 12 weeks of SOF/VEL in DAA-naive, cirrhotic patients with chronic genotype 3 HCV infection [11]. Patients were randomized 1:1 to receive SOF/VEL/VOX once daily for 8 weeks or SOF/VEL once daily for 12 weeks, stratified by treatment experience. In POLARIS-3, the primary efficacy analysis assessed first the SVR12 rate among patients in the SOF/VEL/VOX group against a performance goal of 83% using a two-sided exact one-sample binomial test at the 0.05 significance level. If this group met this criterion, the SVR12 rate in the SOF/VEL group also would be assessed against the performance goal of 83% at the 0.05 significance level. The performance goal of 83% was based on the prior results

of SOF/VEL for 12 weeks in this patient population in the ASTRAL-3 trial (SVR12 rate 91%; 95% CI 83–96%, see [2]).

A total of 219 patients were enrolled and treated in POLARIS-3, 110 in the SOF/VEL/VOX group and 109 in the SOF/VEL group. Two patients did not complete SOF/VEL treatment, one due to an adverse event and one due to a lack of efficacy. The demographics and baseline characteristics of the patients enrolled in POLARIS-3 are shown in Table 9. Per protocol, all patients had genotype 3 HCV infection and cirrhosis. Overall, 31% (67 of 219 patients) of patients had prior treatment with an interferon-based regimen, and the majority of these patients (91% 61 of 67 patients) had failed prior treatment with pegylated interferon plus ribavirin. Baseline NS5A and/or NS4 RASs were observed in 46 patients (21%).

In POLARIS-3, the SVR12 rate was 96% of patients (106 of 110) in the SOF/VEL/VOX 8-week group and 96% of patients (105 of 109) in the SOF/VEL 12-week group (Table 10). The SVR12 rate for each treatment group was statistically superior to the pre-specified performance goal of 83% ($p < 0.001$ for both groups). Two patients in the SOF/VEL/VOX group had a virologic relapse, both of whom were treatment experienced and had drug concentrations that were low for at least one study visit, suggesting that the patient was not fully adherent to study dosing: One patient who did not achieve on-treatment HCV RNA suppression had a virologic rebound by week 8 of treatment, and one patient had virologic relapse. Baseline RASs had no impact on virologic outcome in the SOF/VEL/VOX 8-week or SOF/VEL 12-week group; all patients with baseline NS3 and/or NS5A RASs achieved SVR12. The two patients who relapsed following treatment with SOF/VEL/VOX for 8 weeks did not have treatment-emergent RASs, in contrast to the two virologic failures in the SOF/VEL 12-week group, both of whom developed treatment-emergent Y93H.

5.3 Safety of SOF/VEL/VOX

The Integrated Phase 3 Safety Population provided the largest dataset to support the safety profile of SOF/VEL/VOX. It was comprised of 1908 patients enrolled in the four Phase 3 clinical studies, including 445 DAA-experienced patients who received SOF/VEL/VOX for 12 weeks in POLARIS-1 and POLARIS-4; 611 DAA-naive patients who received SOF/VEL/VOX for 8 weeks regimen in POLARIS-2 and POLARIS-3; 700 patients who received SOF/VEL for 12 weeks in POLARIS-2, POLARIS-3, and POLARIS-4; and 152 patients who received placebo for 12 weeks in POLARIS-1.

In general, the adverse event profile was similar between patients receiving SOF/VEL/VOX for 12 or 8 weeks, SOF/VEL, and placebo, and rates were low for patients with Grade 3 or 4 adverse events, serious adverse events, and adverse events leading to discontinuation with no trends observed across the treatment groups (Table 11). The comparable incidence of most adverse events among the SOF/VEL/VOX groups versus the placebo 12-week group suggests relatively high background rates of these adverse events in patients with HCV infection.

Table 11 Summary of adverse events in the SOF/VEL/VOX Integrated Phase 3 Safety Population

Patients, *n* (%)	SOF/VEL/VOX 8 weeks ($N = 611$)	SOF/VEL/VOX 12 weeks ($N = 445$)	SOF/VEL 12 weeks ($N = 700$)	Placebo 12 weeks ($N = 152$)
Patients experiencing				
Any adverse event	444 (73)	346 (78)	495 (71)	107 (70)
Grade 3 or above adverse event	14 (2)	7 (2)	12 (2)	4 (3)
Serious adverse event	17 (3)	9 (2)	14 (2)	7 (5)
Treatment-related serious adverse event	0	0	0	0
Adverse event leading to premature discontinuation of the study drug	0	1 (<1)	4 (<1)	3 (2)
Death	1 (<1)	1 (<1)	0	0
Most frequent adverse events (> 10%)				
Headache	161 (26)	116 (26)	174 (25)	26 (17)
Fatigue	134 (22)	99 (22)	164 (23)	30 (20)
Diarrhea	105 (17)	83 (19)	44 (6)	19 (13)
Nausea	103 (17)	59 (13)	62 (9)	12 (8)

All treatment groups had the same frequently occurring adverse events (>10% of patients), headache, fatigue, diarrhea, and nausea. Headache and fatigue were reported by 25 and 22% of patients overall, respectively, with similar frequencies reported in each treatment group. Consistent with the known effects of some protease inhibitors, patients receiving SOF/VEL/VOX had a higher incidence of gastrointestinal adverse events compared with patients receiving SOF/VEL. The diarrhea and nausea reported in patients receiving SOF/VEL/VOX were mostly mild and not treatment limiting with no patient discontinuing or interrupting treatment due to diarrhea or nausea. The duration of SOF/VEL/VOX treatment did not significantly impact the rates.

Most adverse events across all treatment groups were Grade 1 or 2 in severity. The incidence of Grade 3 or above adverse events in the SOF/VEL/VOX groups was low (2%) and similar to the incidence in the SOF/VEL group (2%) and placebo group (3%). The incidence was similar whether SOF/VEL/VOX treatment occurred for 8 (2%) or 12 (2%) weeks.

A total of two deaths were reported in the Integrated Phase 3 Safety Population. There was one treatment-emergent death in the SOF/VEL/VOX 12-week group (patient died 2 days after completion of study treatment from an illicit drug overdose) and one nontreatment-emergent death in the SOF/VEL/VOX 8-week group (patient had a medical history of hypertension and died 78 days after completion of study treatment from "hypertension" per the coroner's report). Both deaths were considered unrelated to study drug by the investigator.

Few patients (2%, 47 of 1908) in the Integrated Phase 3 Safety Population had serious adverse events. The highest rate of serious adverse events was reported in the

placebo 12-week group (5%). There were no serious adverse events in any treatment group that were considered related to study drug.

The incidence of adverse events leading to discontinuation of study drugs was low across all treatment groups (<1%). Only patient among those who received SOF/VEL/VOX for 8 or 12 weeks prematurely discontinued treatment due to an adverse event (a Grade 3 adverse event of angioedema considered unrelated to study drug and attributed by the investigator to ramipril initiated the day prior to the event).

In the Integrated Phase 3 Safety Population, graded laboratory abnormalities were observed more often in the placebo 12-week group (76%) and SOF/VEL/VOX 12-week group (69%) compared with the SOF/VEL/VOX 8-week group (58%) and SOF/VEL 12-week group (59%), most likely reflecting the higher percentage of patients with cirrhosis in the placebo 12-week and SOF/VEL/VOX 12-week groups. The higher rates of laboratory abnormalities in the patients receiving placebo were largely due to the higher rates of alanine aminotransferase (ALT) and aspartate aminotransferase abnormalities consistent with untreated HCV infection.

The higher incidence of graded laboratory abnormalities in the SOF/VEL/VOX 12-week group compared with the 8-week group was mostly due to more patients in the 12-week group with Grade 1 or 2 laboratory abnormalities (62%), notably in decreased platelets and increased total bilirubin, consistent with more patients with cirrhosis being enrolled in the 12-week duration regimen in the studies for DAA-experienced patients. For patients receiving SOF/VEL/VOX, there was a higher rate of Grade 1 hyperbilirubinemia compared with patients receiving SOF/VEL or placebo (Table 11). Similar to other protease inhibitors, VOX is an inhibitor of OATP1B1 and OATP1B3, which resulted in an increase in Grade 1 total bilirubin in patients receiving SOF/VEL/VOX. This increase is observed more often in patients with cirrhosis (10%) than in patients without cirrhosis (4%). There were no adverse events of jaundice. There was no pattern of VOX associated with ALT elevation (Table 12). Grade 1 or 2 ALT elevations occurred early in SOF/VEL/VOX treatment and were consistent with expected fluctuations prior to viral suppression. Of the 1,056 patients receiving SOF/VEL/VOX in the Integrated Phase 3 Safety Population, 1 patient had a Grade 3 elevation in ALT (<1%), and none had a Grade 4 elevation in ALT.

The rates of Grade 3 or 4 chemistry laboratory abnormalities in the SOF/VEL/VOX groups were similar to the SOF/VEL group and lower than the placebo 12-week group. Increased glucose, lipase, and creatine kinase were the most common Grade 3 or 4 chemistry laboratory abnormalities with SOF/VEL/VOX. Glucose elevations were observed primarily among patients with a history of diabetes or those with high glucose prior to study drug initiation. Elevations in lipase were generally isolated or intermittent; all were asymptomatic, and there were no adverse events of pancreatitis. Similarly, creatine kinase elevations were mostly transient and asymptomatic.

Table 12 Summary of alanine aminotransferase and total bilirubin laboratory abnormalities in the SOF/VEL/VOX Integrated Phase 3 Safety Population

Patients, *n* (%)	SOF/VEL/VOX 8 weeks ($N = 611$)	SOF/VEL/VOX 12 weeks ($N = 445$)	SOF/VEL 12 weeks ($N = 700$)	Placebo 12 weeks ($N = 152$)
Alanine aminotransferase				
Grade 1	5 (<1)	3 (<1)	4 (<1)	24 (16)
Grade 2	1 (<1)	4 (<1)	2 (<1)	5 (3)
Grade 3	0	1 (<1)	1 (<1)	2 (1)
Grade 4	0	0	0	1 (<1)
Total bilirubin				
Grade 1	34 (6)	30 (7)	15 (2)	65 (4)
Grade 2	6 (1)	12 (3)	4 (<1)	4 (3)
Grade 3	0	1 (<1)	1 (<1)	0
Grade 4	0	0	0	0

5.4 Summary of Phase 3 Data Supporting the Initial Registration of SOF/VEL/VOX

In POLARIS-1 and POLARIS-4, 445 DAA-experienced patients with and without compensated cirrhosis were treated with 12 weeks of SOF/VEL/VOX, and the overall SVR12 rate was 97%. Treatment was highly efficacious across HCV genotypes and regardless of prior DAA regimen, cirrhosis status, or the presence of baseline RASs. In POLARIS-2 and POLARIS-3, 611 DAA-naive patients with and without compensated cirrhosis were treated with 8 weeks of SOF/VEL/VOX, and the overall SVR12 rate was 95%. The SVR12 rate was lower in patients with genotype 1a HCV infection than in patients with other HCV genotypes. Treatment-emergent resistance following treatment with SOF/VEL/VOX was uncommon in both the DAA-experienced and DAA-naive populations consistent with the regimen having a high barrier to resistance. The regimen was well-tolerated with similar frequently occurring adverse events compared with SOF/VEL and placebo, with higher rates of mild diarrhea and nausea compared with SOF/VEL. There were no clinically meaningful laboratory abnormalities. Slight increases in total bilirubin were observed, consistent with VOX inhibition of OATP1B1 and OATP1B3.

These data supported the approval of SOF/VEL/VOX (Vosevi®) in the United States on July 18, 2017, as the first retreatment option for patients who have failed prior treatment with NS5A inhibitor and/or sofosbuvir [12]. Vosevi was also approved in the European Union shortly afterwards on July 28, 2017, where it is recommended for all HCV genotypes for 12 weeks in DAA-experienced patients with and without cirrhosis and DAA-naive patients with compensated cirrhosis and for 8 weeks in DAA-naive patients without cirrhosis [13].

6 Conclusion

The development of DAAs led to a transformation in the treatment of HCV. However, even with an anticipated 95% cure rate, there is a need for a treatment to address the small percentage of patients who are not cured with first-line therapy. The once-daily, single-tablet regimen of SOF/VEL/VOX for 12 weeks is a highly effective and safe treatment for DAA-experienced patients with chronic HCV infection and will provide this growing population with an option for retreatment and a high likelihood for cure, regardless of genotype, the presence of cirrhosis, RASs, or prior treatment regimen. For regions in which a shorter duration of initial HCV treatment is of particular interest, SOF/VEL/VOX for 8 weeks is also a safe and effective option. Vosevi was the fourth HCV treatment developed by Gilead to be approved in 4 years and completed the HCV portfolio providing safe and effective treatment options for nearly all patient populations.

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Compliance with Ethical Standards

Conflict of Interest Luisa M. Stamm and John G. McHutchison are employees of Gilead Sciences, Inc.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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Clinical Development of Viekira Pak to Mavyret

Daniel E. Cohen

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Abstract Viekira Pak (ombitasvir/paritaprevir/ritonavir and dasabuvir) was one of the first interferon-free direct-acting antiviral (DAA) regimens to be approved for the treatment of genotype 1 HCV infection. The research and development team at Abbott/AbbVie based their approach to HCV cure on the use of three DAAs to avoid emergence of resistance, anchored by a potent protease inhibitor and NS5A inhibitor. Clinical trials were designed to answer multiple questions within a single study, in order to advance the regimen as quickly as possible in a highly competitive environment. The global phase 2 and 3 development program allowed for rapid identification of optimal treatment regimens and durations for populations defined by HCV subtype, prior treatment experience, and the presence of specific

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comorbidities such as cirrhosis and orthotopic liver transplant. The clinical trial program also clarified the limitations of this first-generation DAA regimen, including activity that was limited to genotypes 1 and 4 and the need for ribavirin for some patients, which defined a target product profile for a next-generation regimen. This continued research and development activity ultimately led to the approval of the pangenotypic regimen glecaprevir/pibrentasvir (Mavyret).

Keywords Clinical development, Dasabuvir, Direct-acting antivirals, Glecaprevir, Hepatitis C, NS3 protease inhibitors, NS5A inhibitors, Ombitasvir, Paritaprevir, Pibrentasvir

1 Introduction

The advent of highly effective interferon-free curative regimens completely overturned the paradigm for treatment of hepatitis C virus (HCV) infection. Previous therapies had always included the inconvenience, toxicity, and poor tolerability of interferon injections. As a result, treatment was offered only to patients who met particular criteria of disease severity and overall health, who were willing to subject themselves to up to a year of difficult treatment for a chance of success significantly less than 100%. With the availability of in vitro systems like the replicon system that could assess the activity of compounds against HCV, medicinal chemistry rapidly began identifying compounds targeted against promising viral targets, including the NS3/4A protease enzyme, NS5A protein, and NS5B polymerase. However, it would require clinical trials of combinations of direct-acting antiviral agents (DAAs) to confirm that sustained virologic response (prolonged absence of detectable circulating HCV RNA months after the end of treatment) could be achieved without interferon. Once this fact had been demonstrated, innovators raced to identify the optimal combinations and shortest treatment durations that could offer the greatest number of patients a chance for cure. The field advanced rapidly, with breakthrough leapfrogging breakthrough resulting in incremental improvements in efficacy, safety, simplicity, and activity against a range of genotypes and resistant variants. The Abbott/AbbVie development of the protease inhibitor-based regimens ombitasvir/paritaprevir/ritonavir plus dasabuvir (Viekira, Viekirax plus Exviera) and glecaprevir/pibrentasvir (Mavyret, Maviret) epitomizes the rapid pace of science in this field.

2 Hepatitis C Therapy in 2003–2008

Abbott first initiated discovery activities aimed at identifying direct-acting inhibitors of HCV in the late 1990s, including programs directed at all three major targets (protease, NS5A protein, polymerase) [1, 2]. Although the tools to screen compounds for anti-HCV activity in vitro had recently become available, the strategy for developing and deploying these promising new tools had not yet been elucidated.

Since the only therapies that had demonstrated SVR in the clinic included interferons, a logical first step was to combine these novel compounds with interferon, specifically pegylated interferon alfa 2a or 2b (peginterferon). This strategy had the advantage that a potential increase in efficacy could be demonstrated in a relatively simple placebo-controlled trial, with all subjects receiving the standard background regimen of peginterferon and ribavirin. A meaningful improvement over standard of care in a phase 2 trial would justify large global phase 3 trials. Furthermore, even if no improvement in SVR rate could be demonstrated, the new regimen might still be better than the standard of care if the total duration of the burdensome peginterferon could be reduced. Indeed, addition to standard of care was the path to approval for the first approved DAAs: boceprevir (Schering/Merck), telaprevir (Vertex), simeprevir (Janssen), and sofosbuvir (Gilead; for patients with genotype 1 or 4[1]).

However, the add-on approach had drawbacks, the most obvious being that it did not eliminate interferon or ribavirin. Thus, although efficacy might be improved compared to the previous standard of care, safety and tolerability would be no better and might be significantly worse if the DAA caused additional side effects. Furthermore, an add-on approach was of little benefit to patients who were not candidates for interferon or ribavirin therapy (e.g., decompensated liver disease, autoimmune disease, severe cardiac or pulmonary disease) or those who wished to avoid the actual or perceived side effects of interferon. Finally, a path to regulatory approval that involved adding a new compound to peginterferon and ribavirin substantially limited the ability of a company like Abbott, a relatively late entrant to the HCV space, to differentiate its products from agents that were already in development. Early on, there was therefore a keen interest at Abbott in combining DAAs to obviate the need for interferon.

3 Sea Change: 2009–2010

3.1 The Perelson Paper

Early theoretical support for the feasibility of achieving SVR with a combination of DAAs alone was provided by a mathematical modeling exercise published by Alan S. Perelson and colleagues [3]. Perelson's simulation suggested that, in order to completely suppress viral replication with a combination of DAAs, the regimen had to be able to inhibit growth of more than three mutations. This result suggested that SVR might be achieved just with inhibition of viral replication but that it would

[1]In addition to its approval in combination with peginterferon and ribavirin for 12 weeks for genotype 1 or 4, sofosbuvir was approved in combination with ribavirin alone for genotypes 2 and 3 and for interferon-intolerant patients with genotype 1, the first approved interferon-free all-oral therapy for chronic hepatitis C.

require a sufficient number of agents with nonoverlapping viral targets to overcome the ability of the virus to escape by selection of mutations that conferred resistance to each individual agent.

3.2 INFORM-1

In 2009 Ed Gane took the podium at the Annual Meeting of the American Association for the Study of Liver Diseases (AASLD) in Boston and presented "First-in-Man Demonstration of Potent Antiviral Activity with a Nucleoside Polymerase (R7128) and Protease (R7227/ITMN-191) Inhibitor Combination in HCV: Safety, Pharmacokinetics, and Virologic Results from INFORM-1." This exploratory trial was sponsored by Roche/Pharmasset and demonstrated that two DAAs co-administered for 14 days could rapidly and profoundly suppress viral replication, as measured by serum HCV RNA, without interferon. Eight subjects who received the combination saw their HCV RNA levels reduced by a mean of 3.9 log10 IU/mL, including one subject whose HCV RNA level fell below the limit of quantification. Following the 14 days of DAA combination therapy in this trial, subjects were rolled onto standard therapy with peginterferon and ribavirin. Unfortunately, when the final results were ultimately published, there was no evidence that transiently lowering HCV RNA levels resulted in any improvement in the SVR rate compared to peginterferon and ribavirin alone [4]. Nevertheless, this presentation galvanized the room and caused people at Abbott to think more seriously about the possibility of an HCV cure without the need for interferon.

4 The Shift to Interferon-Free Therapy

4.1 Two Programs

Thus, the Abbott team was faced with a decision: whether to follow the same development path as others, i.e., adding a DAA to peginterferon and ribavirin to increase SVR rates and/or shorten treatment, or to pursue an interferon-sparing strategy, exploring DAA combinations intended to minimize or even dispense with interferon. The interferon-sparing DAA combination strategy was attractive. Abbott recognized that other pharmaceutical companies were already combining HCV protease inhibitors and nucleoside analog polymerase inhibitors with peginterferon in the clinic. Given their head start, it would be difficult for Abbott to bring a new entrant into this crowded competitive landscape, following the same path, and still be successful. Furthermore, Abbott had a robust internal discovery organization that could deliver assets in multiple classes, giving Abbott the ability to study combinations of internally owned compounds. Still, the all-oral DAA treatment strategy was completely unproven. There was as yet no clinical evidence that patients could be cured without interferon. Besides, even if an interferon-free DAA

combination worked in some patients, it was possible that some population of more difficult-to-treat patients would always require the added potency of interferon. Abbott therefore decided to pursue a dual strategy: an interferon-free approach to explore DAA combinations and an interferon-containing strategy that would seek to combine one or more DAAs with peginterferon. It was felt that these two paths would provide treatment to the vast majority of HCV-infected patients at need, at least those infected with genotype 1. As would soon become apparent, combination DAA therapy would prove to be so efficacious as to make peginterferon obsolete for the treatment of HCV.

4.2 Pilot and Co-Pilot

Having taken the decision to pursue an interferon-free DAA combination regimen, the Abbott team was embarking on uncharted waters. There were no data demonstrating that a combination of small molecules could achieve SVR. While the Perelson modeling was provocative, and the INFORM-1 results were exciting, they left a lot of questions unanswered. How long could a 2-DAA combination maintain viral suppression? What proportion of patients would see their virus break through, and when? How many would experience viral relapse, and how long after the end of therapy? How many of these patients would fail with resistance to one or both DAA classes? What duration of therapy would be sufficient to cure a meaningful proportion of patients? Was it even possible to cure HCV infection without interferon? DAA clinical research was moving rapidly, and the Abbott team felt it was important to start generating internal data quickly. They decided to combine those investigational DAAs that were ready for use in HCV-infected subjects to start answering those questions, even if those DAAs might not constitute the intended final marketed product.

By late 2010, Abbott had produced sufficient drug supply and had generated sufficient toxiciology coverage, to dose three DAAs in humans for up to 12 weeks: the protease inhibitor ABT-450 (later paritaprevir; discovered in collaboration with Enanta Pharmaceuticals) and two non-nucleoside polymerase inhibitors, ABT-072 and ABT-333 (dasabuvir).[2] Abbott's NS5A inhibitor, ABT-267 (ombitasvir), was still too early in development to be included in phase 2 trials, although the plan was still to initiate three-DAA combination trials as soon as possible, based on the Perelson modeling and in vitro data generated by Abbott's virologists demonstrating superior suppression of virus in cell culture. Phase 1 and phase 2a studies had defined an efficacious dose range for all three DAAs given as monotherapy, and

[2]ABT-072 and ABT-333 were closely related members of the same chemical series, with identical binding sites and similar antiviral activity. There was never any intention to combine these two drugs; rather the plan was to advance whichever one proved to have better properties. Although ABT-072 had a half-life that permitted once-daily dosing, due to formulation challenges, ABT-333 was ultimately selected.

drug-drug interaction studies had already confirmed the absence of meaningful pharmacokinetic interactions between ABT-450 (with ritonavir) and either ABT-072 or ABT-333. Accordingly the team settled on a combination of ABT-450, a 100 mg boosting dose of ritonavir, ABT-072, and ribavirin dosed according to body weight, all given for 12 weeks. This trial was to be conducted by four experienced hepatologists and was referred to as the PILOT study [5].

The PILOT study would be a trial balloon intended to answer fundamental questions about rates of breakthrough and timing of relapse following a short course of DAA therapy. The Abbott team did not realistically expect to cure more than a minority of the patients. Anna Lok's groundbreaking paper on the combination of daclatasvir and asunaprevir had not yet been published, but preliminary results presented at the 2010 AASLD meeting after the PILOT study had been started did not promise high SVR rates: 11 patients in that study received daclatasvir and asunaprevir alone for 24 weeks, and only 4 achieved SVR [6]. There was concern about the risk to participants in the PILOT study who might remain infected after study treatment, but in whom exposure to the DAA regimen might have selected viral variants with resistance to the protease inhibitor and non-nucleoside polymerase inhibitor classes. Indeed, an ongoing concern in studying DAAs was the risk of selecting long-lasting resistance that could eliminate an entire class of potential therapies in the future. With combination DAA regimens, there was the chance that multidrug resistance could render a patient incapable of being cured with DAAs at all. Accordingly, the PILOT study incorporated several safeguards to preserve additional chances for cure. Ribavirin was included in the regimen to maximize treatment response and hopefully forestall emergence of resistant variants. Enrollment in the trial would be small ($N = 11$) and limited to treatment-naïve patients without cirrhosis, for whom there was less urgency to treat and possibly a better chance of success. Finally, only patients with the favorable IL28B CC genotype[3] were to be enrolled, so that patients who failed would have a high likelihood of achieving SVR with a subsequent course of peginterferon and ribavirin should that prove necessary.

It turned out to be less necessary than expected. Not only were rapid declines in HCV RNA seen in all 11 subjects, but all subjects also had unquantifiable levels by the end of treatment, and over the next 24 weeks of follow-up only 1 subject relapsed. This SVR rate of 91% was unexpected[4] and constituted the first evidence

[3] A number of polymorphisms near the IL28B gene (also known as the interferon lambda gene) were found to be associated with the probability of achieving SVR following treatment with interferon. One of the most frequently studied was the IL28B single-nucleotide polymorphism rs12979860. For this polymorphism, patients homozygous for the C allele (CC) had the most favorable prognosis; those with a TT genotype had the worst prognosis, and heterozygotes (CT) had an intermediate prognosis [7]. Highly effective DAA regimens ultimately obviated the need for IL28B genotype testing.

[4] To everyone's greater surprise, a second subject relapsed at a follow-up visit 36 weeks after the end of treatment. This finding raised considerable discussion about the mechanism behind a delayed relapse, whether patients with the IL28B CC genotype might be uniquely prone to manifesting

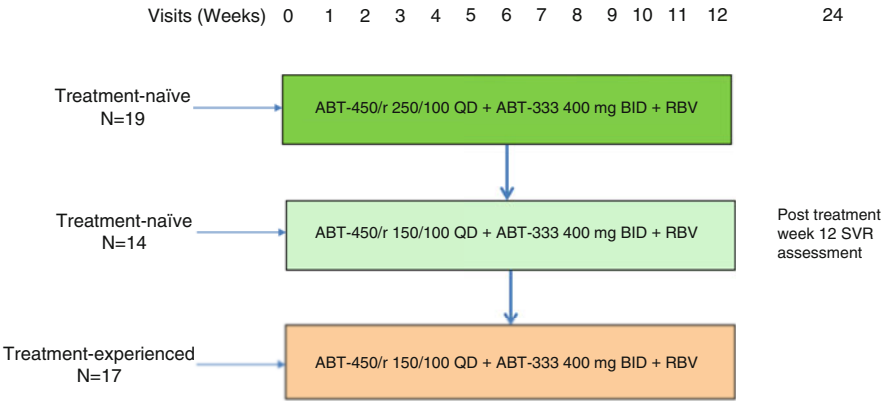

Fig. 1 Study design of CO-PILOT

that high cure rates would be achievable with a treatment duration less than 24 weeks. This result recalibrated the Abbott team's thinking about what was achievable with a short course of all-oral therapy.

Having concluded that high SVR rates were possible with DAA combination therapy, the team launched a phase 2 program to confirm these findings and investigate factors like prior treatment history and ABT-450 dosage, leading up to a planned phase 2b duration-ranging trial. Like the PILOT study, the CO-PILOT study (Fig. 1) also evaluated a two-DAA regimen with ribavirin for 12 weeks, with some differences. Due primarily to greater ease of formulation, a strategic decision had been made to advance ABT-333 instead of ABT-072, so ABT-333 replaced ABT-072 in this study. CO-PILOT included three sequentially enrolled treatment groups and evaluated a higher dose (250 mg daily) of ABT-450, the more potent DAA, as well as the performance of the regimen in subjects with the less favorable CT or TT IL28B alleles or with prior treatment experience. Among treatment-naïve subjects, there was no difference in efficacy between 150 mg of ABT-450 and 250 mg (no virologic failures occurred at either dose); however, one patient who received the higher dose experienced an episode of asymptomatic alanine aminotransferase elevation. IL28B genotype had no impact in the treatment-naïve population, but 9 of 14 treatment-experienced subjects failed either during or after treatment [8].

relapses at a later time point due to more robust immunologic control and whether SVR resulting from an interferon-free regimen might be less durable than that resulting from interferon. The latter question appears to have been conclusively answered in the negative.

5 The AVIATOR Study

The PILOT and CO-PILOT studies established 12 weeks as the baseline duration for treatment-naïve patients. In the meantime, the ABT-267 team had dramatically accelerated the development program, and ABT-267 was now available for combination trials. A number of questions remained to be answered before a regimen(s) could be advanced into phase 3 trials:

- What is the optimal drug combination? Are all three DAAs better than any two-DAA regimen?
- Is ribavirin necessary?
- What is the optimal treatment duration? Can treatment be shortened to less than 12 weeks if the regimen includes three DAAs? Or conversely, will 24 weeks show better efficacy than 12 weeks?
- What is the optimal ABT-450 dose? The transaminase elevation seen at the 250 mg dose made that dose unacceptable, but would doses of 100 mg or 200 mg have advantages over 150 mg?
- Finally, since CO-PILOT showed that prior peginterferon treatment can affect the response to an interferon-free regimen, would the optimal treatment be different for patients with prior treatment failure?

The team finally decided to address as many questions as possible in a single multiple-arm study. The phase 2b trial, to be known as the AVIATOR study, eventually included a treatment-naïve cohort and a null responder cohort (prior null responders were considered to be the most interferon nonresponsive, so it was assumed that results in this population could be extrapolated to patients with prior partial response or relapse) and multiple arms within each cohort [9]. The base case was the maximal regimen, i.e., three DAAs with ribavirin, which was assumed to be maximally efficacious for all patients. Changes to that regimen would be compared with the base case for safety and efficacy (Fig. 2).

In the naïve cohort, 12 weeks of treatment would be compared to durations of 8 and 24 weeks to establish an optimal duration. Regimens with fewer active components (two DAAs with ribavirin or three DAAs without ribavirin) would be compared to the three-DAA base case. A lower dose of ABT-450 (100 mg daily) would be compared with the 150 mg CO-PILOT dose in the 12- and 24-week treatment arms.

The null responder cohort would explore durations of 12 or 24 weeks, as well as ABT-450 doses of 100 and 150 mg daily. Since CO-PILOT already demonstrated that the efficacy of ABT-450, ABT-333, and ribavirin was inferior among treatment-experienced patients, this regimen was not assessed in null responders in AVIATOR; however, since ABT-267 had substantially greater potency than ABT-333, the study evaluated ABT-450 with ABT-267 and ribavirin in null responders.

Patients with cirrhosis were excluded from AVIATOR. Neither the safety nor the efficacy of these regimens had been established in cirrhotic patients. This population

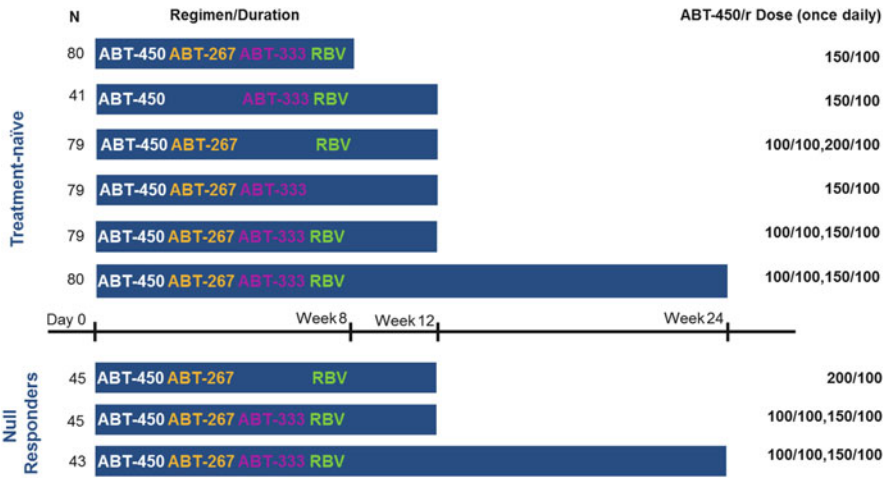

Fig. 2 Study design of AVIATOR

would be key to the success of any DAA regimen, since these patients were at the most urgent need of treatment, being at the highest risk of adverse outcomes. In addition, patients with cirrhosis responded most poorly to interferon-based therapy, so more efficacious treatment options were clearly needed. However, it was felt necessary to demonstrate safety and to define a dose-exposure relationship in patients with less advanced liver disease prior to administering the regimen to cirrhotic patients. Plasma levels of some components of the regimen were increased in individuals with cirrhosis compared to healthy volunteers, and accumulation of ABT-450 in the liver might pose a risk in these patients. In the interest of efficiency, the team therefore deferred assessment of the regimen in cirrhotic patients until phase 3, when the optimal regimen would be established for patients without cirrhosis.

The primary efficacy analysis for AVIATOR was comparison of the SVR rate among naïve patients treated for 8 versus 12 weeks. However, all the relevant comparisons were important in achieving the ultimate study objective, identifying optimal treatment regimens and durations. It would clearly not be possible to power such an ambitious trial to make statistically significant inferences about all the comparisons. Indeed, with only 20–40 subjects per arm, the trial would enroll a whopping 560 subjects. The team determined to make decisions based on directionality of differences in safety or efficacy among the various treatment arms, irrespective of statistical significance.

Beyond the study questions the trial was intended to answer, execution of a study of this scope and complexity would also answer numerous operational questions that would prove crucial in designing and executing a huge international phase 3 program. AVIATOR was a proving ground for Abbott's global regulatory, clinical operations, and site management and monitoring organizations. It provided the first opportunity to gain experience with a large number of hepatology clinical research

sites in North America, Europe, Australia, and New Zealand. Finally, AVIATOR helped to cement the crucial working relationships between Abbott's clinical development and virology teams and the experienced hepatologists and infectious disease experts who would provide insight and advice to inform the Abbott HCV program, through phase 3 and into the next generation.

AVIATOR opened in the fall of 2011 and included patients from the United States, Canada, France, Germany, Spain, the United Kingdom, Australia, and New Zealand. The trial enrolled with gratifying speed, reflecting the desire of patients and their treaters for alternatives to the standard of care and excitement at the prospect of short-course, highly effective therapy. A total of 571 subjects were enrolled between October 2011 and April 2012. Because of the short treatment durations and the propensity of treatment failures to occur early during treatment or follow-up (the vast majority of relapses occurred within 4 weeks after the end of treatment), some differences between treatment groups became obvious quite soon. The 8-week treatment group quickly began to demonstrate a higher relapse rate than those treated for 12 weeks. Likewise, it was quickly obvious that across the genotype 1 population as a whole, the regimen was more efficacious with ribavirin than without. Lastly, although final data would not be available until the last subjects in the 24-week treatment groups completed follow-up, it was obvious early on that treatment failures were extremely uncommon among patients treated with the three-DAA regimen with ribavirin for 12 weeks and that extending treatment duration to 24 weeks would provide no incremental benefit in this population.

The final topline efficacy and safety findings from AVIATOR are summarized in Table 1. The team concluded the following:

Table 1 Treatment groups and SVR rates in AVIATOR

Treatment group	Paritaprevir dose(s)	SVR rate (*n*/*N*, %)	Comment
Treatment-naïve patients			
3 DAAs + RBV for 8 weeks	150 mg	70/80 (88%)	Relapse in 9/56 GT1a, 1/24 GT1b
PTV/r + DSV + RBV for 12 weeks	150 mg	34/41 (83%)	Lower efficacy than was seen in CO-PILOT
PTV/r + OBV + RBV for 12 weeks	100 mg, 200 mg	70/79 (89%)	
3 DAAs for 12 weeks	150 mg	70/79 (89%)	9/52 GT1a failures, no GT1b failures
3 DAAs + RBV for 12 weeks	100 mg, 150 mg	76/79 (96%)	
3 DAAs + RBV for 24 weeks	100 mg, 150 mg	73/80 (91%)	
P/R null responders			
PTV/r + OBV + RBV for 12 weeks	200 mg	40/45 (89%)	
3 DAAs + RBV for 12 weeks	100 mg, 150 mg	42/45 (93%)	No relapses
3 DAAs + RBV for 24 weeks	100 mg, 150 mg	42/43 (95%)	

- For patients without cirrhosis, regardless of prior interferon treatment experience, the optimal treatment regimen comprised ABT-450/r, ABT-267, ABT-333, and ribavirin.
- The optimal treatment duration in this population was 12 weeks.
- There was no clear safety benefit to ABT-450 100 mg compared to 150 mg. Transaminase elevations were uncommon at both doses. While the two doses performed similarly in the treatment-naïve arms, 3 out of 46 null responders (6.5%) receiving the 100 mg dose experienced on-treatment virologic failure, compared to 1 out of 42 (2.4%) at the 150 mg dose.
- The regimen appeared to have greater activity against genotype 1b virus: three DAAs appeared to be equally efficacious without ribavirin in these patients, and the results suggested that 8 weeks of treatment might be sufficient.
- Among the few patients who failed, mutations conferring resistance to all three drug classes were frequently seen. However, emergence of resistance was less frequent in patients who failed after 8 weeks compared to 12 weeks.

6 The Phase 3 Program and Regulatory Approval

The results from AVIATOR and the remaining unanswered questions determined the configuration of the phase 3 program. It would confirm the efficacy and safety of the three-DAA regimen in treatment-naïve and treatment-experienced patients without cirrhosis, and the intriguing finding that ribavirin might not be needed in genotype 1b infection. It also included populations that were not studied in AVIATOR and in whom the activity, safety, and optimal regimen and duration were still unknown: patients with cirrhosis, HIV-1 coinfection, or prior liver transplantation. The planned phase 3 program would be the largest to date for an interferon-free regimen for hepatitis C. The pivotal phase 3 trials are summarized in Table 2.

Two double-blind placebo-controlled trials, the SAPPHIRE studies, confirmed the efficacy and safety of the three-DAA regimen with ribavirin in genotype 1-infected patients without cirrhosis. SAPPHIRE-I was conducted in treatment-naïve patients and SAPPHIRE-II in patients with prior peginterferon treatment. These trials demonstrated superiority to a historic control regimen of telaprevir with peginterferon and ribavirin [10–12].

The three PEARL studies elucidated the role of ribavirin, extending on the initial AVIATOR findings. PEARL-III was a large double-blind trial comparing the three-DAA regimen with ribavirin or with placebo in treatment-naïve patients with genotype 1b infection. A smaller phase 3 trial, PEARL-II, compared the regimen with or without ribavirin in open-label fashion in treatment-experienced patients with genotype 1b infection. While AVIATOR suggested that the regimen was less efficacious against genotype 1a without ribavirin, there was great interest in numerous quarters, including the US FDA, in understanding the magnitude of the loss, to

Table 2 Pivotal phase 3 trials

Study	Design	Findings
SAPPHIRE-I	Double blind ($N = 631$, 3:1) Three DAAs with ribavirin vs. placebo for 12 weeks in GT1 treatment-naïve patients without cirrhosis	SVR in 96% Adverse events greater than placebo included nausea, pruritus, insomnia, diarrhea, and asthenia
SAPPHIRE-II	Double blind ($N = 394$, 3:1) Three DAAs vs. placebo for 12 weeks in GT1 treatment-experienced patients without cirrhosis	SVR in 96% Adverse events greater than placebo included pruritus
PEARL-II	Single arm, open label ($N = 179$) Three DAAs for 12 weeks in GT1b treatment-experienced patients without cirrhosis	SVR in 95/95 (100%) of patients treated with three DAAs alone
PEARL-III	Double blind ($N = 419$, 1:1) Three DAAs + RBV vs. three DAAs + placebo for 12 weeks in GT1b treatment-naïve patients without cirrhosis	SVR in 209/209 (100%) of patients treated with three DAAs alone
PEARL-IV	Double blind ($N = 305$, 2:1) Three DAAs + RBV vs. three DAAs + placebo for 12 weeks in GT1a treatment-naïve patients without cirrhosis	SVR in 185/205 (90%) treated with three DAAs alone
TURQUOISE-II	Open label ($N = 380$, 1:1) Three DAAs + RBV for 12 vs. 24 weeks in GT1 patients with compensated cirrhosis	SVR rates of 92–100% with 12 or 24 weeks, except for prior null responders (see Table 3)

guide risk-benefit decisions in patients who might be unable to take ribavirin, and in identifying possible subgroups that might have a better response without ribavirin. For this reason AbbVie conducted PEARL-IV, a double-blind comparison of the three-DAA regimen with ribavirin or placebo in treatment-naïve patients with genotype 1a infection [13, 14].

Two duration-ranging trials, the TURQUOISE studies, assessed the optimal treatment duration (12 or 24 weeks) of the three-DAA regimen in the so-called special populations, i.e., patients with characteristics that might impact the safety or activity of a DAA regimen. TURQUOISE-I was a phase 2/3 trial in patients coinfected with HIV-1, and TURQUOISE-II was a phase 3 trial in patients with compensated cirrhosis. TURQUOISE-I was complicated by the fact that the three-DAA regimen could potentially interact with numerous antiretroviral medications, principally because it contained ritonavir, a potent inhibitor of cytochrome P450 3A. As a result, several drug-drug interaction studies preceded its implementation. At the end of the day, the safety and efficacy of the three-DAA regimen in this population were consistent with the results of the pivotal studies, confirming the growing consensus that HIV-coinfected patients were no longer a "special" population in the interferon-free DAA era [15–19].

TURQUOISE-II evaluated the regimen in patients with compensated cirrhosis, with treatment durations of 12 and 24 weeks. At 380 subjects enrolled, this was at the time the largest dedicated cirrhotic trial of HCV therapy. In contrast to the experience in patients without cirrhosis, the results of this trial suggested that some patients did benefit from extending treatment duration to 24 weeks: prior null responders to peginterferon with genotype 1a infection had an SVR rate of 80% following 12 weeks of treatment and 93% after 24 weeks (Table 3). There was little difference between 12 and 24 weeks among patients without prior null response, and the regimen was again highly efficacious in patients with genotype 1b infection [20].

The three-DAA regimen was investigated in patients with a prior orthotopic liver (or kidney) transplant. This trial was amended several times to include different patient populations and different treatment durations. As with the HIV-coinfected population, drug-drug interactions were a major concern in transplant recipients, because the most important immunosuppressants in this population (cyclosporine A, tacrolimus, sirolimus, everolimus) all had important interactions with the three-DAA regimen that either necessitated substantial dose reductions of the immunosuppressant and close monitoring of immunosuppressant blood levels or prevented their co-administration [21, 22].

The results of all these trials have been reported elsewhere. Generally, they confirmed the efficacy and safety seen in AVIATOR and established the optimal regimens for genotype 1a infection (three DAAs with ribavirin) and genotype 1b infection (three DAAs alone). Comparable safety and efficacy were seen in the HIV-1-coinfected patients, for whom the indicated treatment regimens would be the same as in the HCV-monoinfected patients.

Ombitasvir/paritaprevir/ritonavir and dasabuvir were submitted for marketing approval as a single regimen (Viekira Pak) in the United States and as two products (Viekirax and Exviera) in the EU, and by January 2015 they were approved in both regions. Ongoing research continued to further refine the optimal use of these regimens. Some of these studies and their key findings are summarized in Table 4.

Table 3 SVR_{12} rates in TURQUOISE-II

	12-week treatment ($N = 208$)	24-week treatment ($N = 172$)
Genotype 1a		
Treatment-naïve	59/64 (92.2%)	52/26 (92.9%)
Prior relapse	14/15 (93.3%)	13/13 (100%)
Prior partial responder	11/11 (100%)	10/10 (100%)
Prior null responder	40/50 (80.0%)	39/42 (92.9%)
Genotype 1b		
Treatment-naïve	22/22 (100%)	18/18 (100%)
Prior relapse	14/14 (100%)	10/10 (100%)
Prior partial responder	6/7 (85.7%)	3/3 (100%)
Prior null responder	25/25 (100%)	20/20 (100%)

Table 4 Additional Viekira studies

Study	Design	Findings
TURQUOISE-III [23]	Single arm, open label ($N = 60$) Three DAAs for 12 weeks in GT1b with compensated cirrhosis	SVR in 100%; regimen approved for use without RBV in genotype 1b-infected patients with compensated cirrhosis
TURQUOISE-CPB [24]	Open label ($N = 36$) Three DAAs with RBV for 12 or 24 weeks for GT1; OBV/PTV/r with RBV for 24 weeks for GT4	Efficacy; events consistent with decompensation in 54%; regimen not recommended or contraindicated in patients with decompensated liver disease (Child-Pugh B or C)
GARNET [25]	Single arm, open label ($N = 166$) Three DAAs for 8 weeks in GT1b without cirrhosis	SVR in 98%; regimen approved for use in treatment-naïve genotype 1b-infected patients with mild or moderate fibrosis (Metavir F0–F2)

6.1 2 DAAs

Of note, the 2-DAA fixed-dose combination of ombitasvir/paritaprevir/ritonavir was also developed on its own for two distinct indications. Since both ombitasvir and paritaprevir (but not dasabuvir) had in vitro activity against genotype 4 HCV, a separate 2-DAA development program was undertaken both globally and in Egypt specifically, which demonstrated the safety and efficacy of this regimen in combination with ribavirin in genotype 4 infection [26–29]. This product was approved globally for this indication, marketed in the United States as Technivie and in most of the rest of the world as Viekirax. Finally, because of somewhat higher drug exposures (especially for paritaprevir) in Japanese subjects and the predominance of genotype 1b infection in Japan, the 2-DAA regimen was approved in Japan for treatment of genotypes 1 and 2.

7 The Next-Generation Development Program: From ABT-493/ABT-530 to Mavyret

7.1 The Case for a Next Generation

The approval of Viekira Pak and Harvoni, which occurred within weeks of each other in the United States and Europe, marked the definitive end of the previous era of interferon-containing therapies for genotypes 1 and 4 and made highly effective curative therapy simple and convenient for the majority of these patients. These regimens were so effective and saw such rapid uptake that it was by no means clear that there was a major unmet need remaining to justify developing an improved next

generation of DAA therapy. Nevertheless, by the time Viekira Pak was approved, a discovery effort was well underway to identify new protease inhibitors and NS5A inhibitors that could address the two major needs not met by the first-generation assets: activity against resistant variants and across all six major genotypes.

7.2 Closing the Gaps

The discovery efforts were driven by the assumption that there would be little value in a next generation unless the compounds could fill the gaps in the Viekira profile. Besides limited genotypic coverage and susceptibility to resistance, those gaps included the need for ritonavir to enable once-daily administration and the need for ribavirin in a significant proportion of patients. Accordingly, it was essential for the next-generation protease inhibitor to both have robust pangenotypic activity and activity against the typical genotype 1-resistant variants with mutations at positions 155, 156, and 168, to have at least nanomolar potency against genotypes 1–6, and to have metabolic stability enabling once-daily dosing. The medicinal chemistry effort that led to the identification of ABT-493 (glecaprevir) is described in [30].

The first-generation NS5A inhibitor ombitasvir already had broad genotypic activity and a half-life allowing for once daily dosing. However, it shared the liability of all members of its class in that a number of single mutations would confer clinically significant resistance. A high priority in the NS5A discovery effort therefore centered around engineering a molecule that would retain activity against mutants selected by first-generation NS5A inhibitors, particularly mutations at position 93, which confer high-level resistance across the NS5A inhibitor class.

The clinical development program for glecaprevir/pibrentasvir was in some ways simpler than the first-generation effort. The universe of the possible had been outlined by the demonstration of short-course curative therapy with earlier DAA combination regimens, and the populations of interest with their respective challenges were well described. However, the bar for success was also considerably higher. It was no longer adequate to show improved efficacy compared to a historical interferon-containing control; instead, approval would require demonstrating efficacy comparable to the expected SVR rates of greater than 90% achieved with first-generation regimens in similar populations. In order to be competitive in the marketplace, the regimen needed to be simple and as short as possible in duration. The ultimate goal was a regimen that was uniformly efficacious and safe in the majority of patients, regardless of genotype, which could simplify treatment and enable patients to be successfully treated by healthcare providers who were not specialists. What follows is a high-level survey of a program that compressed hundreds of person-hours into a timeline of unprecedented speed.

7.3 *Learning from the First Generation*

The Mavyret clinical development program was conducted in a strikingly abbreviated time frame, with the first NDA submission occurring less than 3 years after the first patients were dosed in phase 2. The AbbVie team evaluated the process for submission of Viekira to identify areas for increased efficiency with the next-generation program. Under the breakthrough therapy designation, the team was able to interact frequently with regulatory agencies to understand agency expectations and to determine which study strategies would be acceptable as a basis for approval. This allowed the team to use innovative trial designs, with multiple treatment arms activated based on pre-specified safety and efficacy results from previous treatment groups.

SURVEYOR-II, a complex, staged phase 2–3 trial, was one of the most informative studies in the Mavyret program. This ambitious trial spanned 3 years and comprised four parts: a supportive/exploratory (phase 2) portion of the trial (parts 1 and 2), and a confirmatory/registrational (phase 3) portion (parts 3 and 4). In all the study included 22 separate dosing groups and was amended five times. This rolling study thus allowed numerous study questions to be answered and results from one set of analyses to inform the design and conduct of subsequent treatment groups, with the efficiency of a single protocol. It will be informative to examine the design of the four parts of this trial and to contrast it with the corresponding phase 2 AVIATOR trial from the Viekira program (Fig. 3).

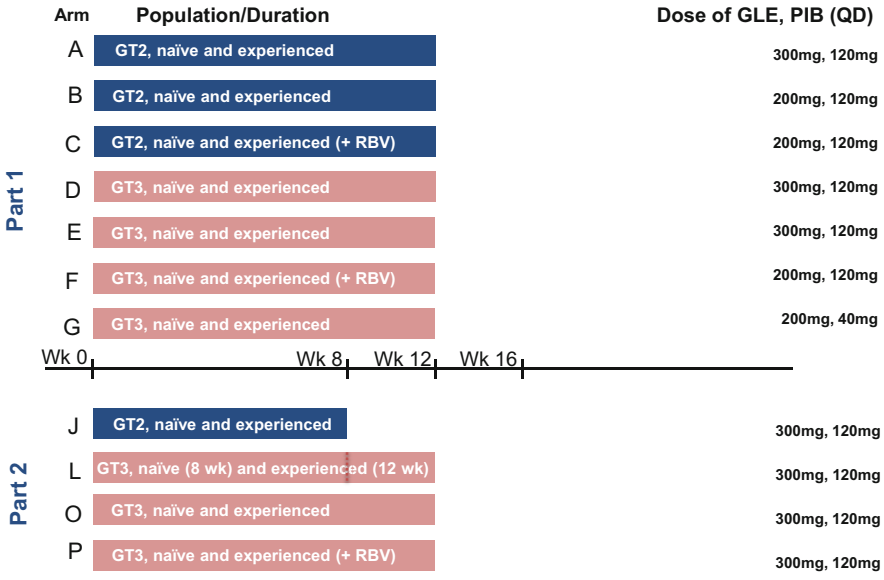

Fig. 3 Study design of SURVEYOR-II, parts 1 and 2

As with the development plan for Viekira, the primary objective was to determine how best to utilize active individual agents together in a regimen that would be simple to use across a range of patient types. In contrast to the Viekira program, however, the next-generation regimen needed excellent activity against multiple genotypes. The initial questions to be answered were fundamental and included dose ranging both agents and assessing the role of RBV. Treatment duration in the first arms (confined to patients without cirrhosis) was 12 weeks, but pharmacokinetic modeling suggested an 8-week duration might be sufficient for some genotypes, if not all.

By the time part 2 was finished, it was clear that, for genotypes 2 and 3, an 8-week course of treatment appeared to be as efficacious as 12 weeks, at least among treatment-naïve patients. Safety and efficacy in cirrhotic patients remained to be studied, as did activity in the less common genotypes 4–6. Since a single dosage of glecaprevir and of pibrentasvir had been identified across genotypes 1–3, the confirmatory, phase 3 portion of the study was able to utilize the final coformulated product, comprising three tablets containing a total of 300 mg of glecaprevir and 120 mg of pibrentasvir. Given the evident challenge posed by genotype 3-infected patients who had previous treatment failure, additional duration ranging was required to determine if an additional 4 weeks of treatment would improve efficacy. Finally, part 4 of the trial was dedicated to assessing efficacy in patients with the less common genotypes. In vitro systems allowed determination of EC_{50} values, which led the AbbVie team to hypothesize that the regimen would show activity against genotypes 4–6 comparable to that seen with genotypes 1 and 2; therefore, a single treatment arm with a duration of 8 weeks was expected to be sufficient for patients without cirrhosis (Fig. 4).

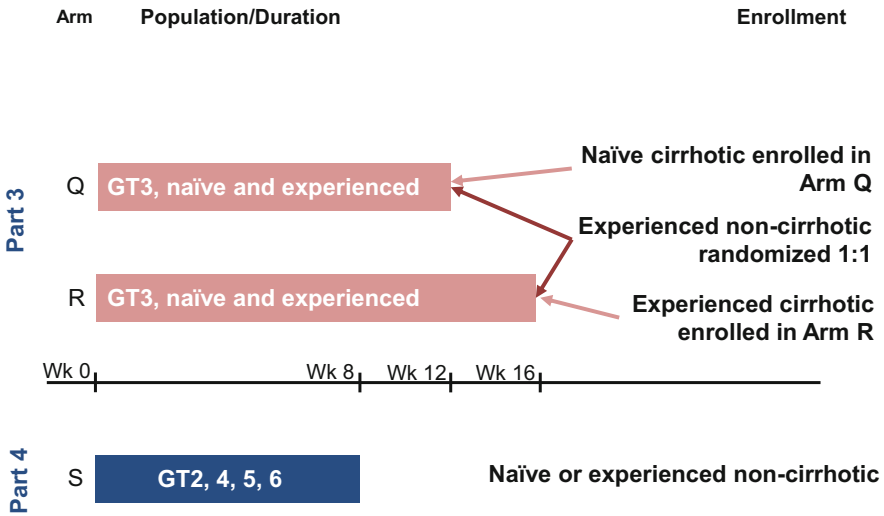

Fig. 4 Study design of SURVEYOR-II, parts 3 and 4

The AbbVie team was thus able to identify optimal study drug dosages, treatment duration, and regimen across multiple genotypes and in patients with and without cirrhosis, in the setting of a single ongoing trial. This strategy even allowed the newly developed final commercial formulation to be incorporated into the trial "on the fly" [22, 31, 32].

The rest of the phase 2–3 registrational program was both straightforward and comprehensive. The ENDURANCE studies 1–4 confirmed safety and efficacy in patients without cirrhosis infected with genotypes 1, 2, 3, and 4–6, respectively. The MAGELLAN-1 study explored the glecaprevir/pibrentasvir regimen in patients who had failed prior DAA regimens [33]. The highest priority subgroups of HCV-infected patients were again investigated in dedicated trials: patients with cirrhosis were assessed in EXPEDITION-1 (in addition to part 3 of SURVEYOR-2), patients with HIV-1 coinfection in EXPEDITION-2, patients with renal insufficiency in EXPEDITION-4, and patients with prior liver or kidney transplant in MAGELLAN-2 [17, 31, 34, 35]. The efficacy of the regimen was confirmed in all populations, except that the data from MAGELLAN-1 was not sufficient to confirm efficacy in patients with genotypic resistance to both NS5A and protease inhibitors. The approved Mavyret indications from the US Prescribing Information are summarized in the text box.

Treatment-naïve
GTs 1, 2, 3, 4, 5, or 6 subjects without cirrhosis for 8 weeks
GTs 1, 2, 3, 4, 5, or 6 with compensated cirrhosis (Child-Pugh A) for 12 weeks
Treatment-experience-PRS (defined as prior treatment experience with regimens containing interferon, pegylated interferon, ribavirin, and/or sofosbuvir but no prior treatment experience with an NS5A inhibitor or NS3/4A protease inhibitor)
GTs 1, 2, 4, 5, or 6 without cirrhosis for 8 weeks
GTs 1, 2, 4, 5, or 6 with compensated cirrhosis for 12 weeks
GT3 with or without cirrhosis for 16 weeks
Treatment-experienced with a regimen containing an HCV NS5A inhibitor or NS3/4A inhibitor (not both)
GT1 with or without compensated cirrhosis and any prior treatment regimen containing an NS5A inhibitor (no prior NS3/4A protease inhibitor) for 16 weeks
GT1 with or without compensated cirrhosis and any prior treatment regimen containing an NS3/4A protease inhibitor (no prior NS5A inhibitor) for 12 weeks

8 The Future of HCV Therapy

Further investigations are underway in an ongoing effort to characterize the optimal use of Mavyret in as many populations as possible, including pediatric patients and those in diverse geographies. While the standard of care for treatment of HCV infection may continue to evolve, currently available therapies like Mavyret appear to address a substantial portion of the infected population. Public health efforts are already turning from the technical questions of efficacy and safety, to issues of diagnosis and access to treatment. Safe and simple therapeutic options can expand the pool of treating healthcare providers beyond specialists, at least for a subset of the population. Ultimately, the long-term goal of elimination of chronic hepatitis C may no longer be simply a dream.

Compliance with Ethical Standards

Funding Daniel E Cohen did not receive any compensation for this chapter.

Conflict of Interest Daniel E Cohen is an employee of AbbVie.

Ethical Approval All clinical trials were approved by the independent ethics committee or institutional review board for each trial center and were conducted in accordance with the Good Clinical Practice Guidelines and the ethical principles of the Declaration of Helsinki.

Informed Consent All study participants (or their legal guardians) provided written informed consent prior to any study procedures being performed.

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Development of ZEPATIER®

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Abstract ZEPATIER® (MK-5172A; elbasvir and grazoprevir, Merck & Co., Inc.) is a fixed-dose combination treatment for individuals with chronic hepatitis C virus (HCV) infection. This novel direct-acting antiviral (DAA) regimen combines elbasvir, a selective inhibitor of the HCV nonstructural protein 5A, and grazoprevir, a reversible competitive inhibitor of the HCV nonstructural protein 3/4A protease. After extensive preclinical testing and evaluation of safety and pharmacokinetics (PK) in healthy volunteers, the efficacy of these agents was evaluated in a systematic and comprehensive clinical development program culminating in phase 3 clinical trials in a broad population of participants with HCV infection, including treatment-naive and treatment-experienced participants, those with chronic kidney disease or inherited blood disorders, and those receiving opioid agonist therapy. These studies led to the approval of the elbasvir/grazoprevir combination therapy for the treatment of people with HCV genotype 1 or genotype 4 infection in the United States, Europe, Canada, and many other countries worldwide.

Keywords Clinical trial, Elbasvir, Grazoprevir, ZEPATIER, Hepatitis C, Treatment

1 Overview

ZEPATIER® (MK-5172A; elbasvir and grazoprevir, Merck & Co., Inc.) is a fixed-dose combination of two novel direct-acting antivirals (DAAs) directed at well-validated targets within the hepatitis C virus (HCV): elbasvir (also known as MK-8742), a selective inhibitor of the HCV nonstructural protein 5A (NS5A) [1], and grazoprevir (also known as MK-5172), a novel, orally administered, reversible competitive inhibitor of the HCV nonstructural protein 3/4A (NS3/4A) protease [2].

Both of these novel agents were developed at Merck Research Laboratories through a concerted research effort focused on improving potency across a broad spectrum of HCV genotypes and maintaining potency against many of the viral variants with mutations that confer resistance to earlier-generation agents from these drug classes. After extensive preclinical testing [3–7] and evaluation of safety and pharmacokinetics in healthy volunteers, the efficacy of elbasvir and grazoprevir, both separately and as a fixed-dose combination therapy, was evaluated in a systematic and comprehensive clinical development program.

The objective of the ZEPATIER clinical development program was to develop a well-tolerated, convenient, and simple regimen that would be highly effective in clearing HCV infection and thereby reduce the burden of HCV-related morbidity and mortality across the spectrum of this disease. To meet this objective, the clinical development program evaluated "standard" segments of the HCV-infected population (such as treatment-naive, noncirrhotic individuals) as well as populations with a high unmet medical need (e.g., HCV-infected people with chronic kidney disease [CKD] grades 4/5 on hemodialysis; HCV-infected individuals receiving opioid agonist therapy). Figure 1 presents the entire spectrum of people with HCV infection and displays the diversity of study participants in whom the efficacy of elbasvir/grazoprevir, alone or in combination with other agents (ribavirin, sofosbuvir), was evaluated.

The following sections describe the clinical trials that supported the licensure of ZEPATIER. Concepts common to all studies are briefly addressed here. In the phase 2 and 3 clinical trials, the primary efficacy end point was sustained virologic response (HCV RNA levels below the assay lower limit of quantitation [LLoQ]) 12 weeks after completion of study medication (SVR12). Plasma HCV RNA levels were measured using the COBAS® AmpliPrep/COBAS® TaqMan® HCV test

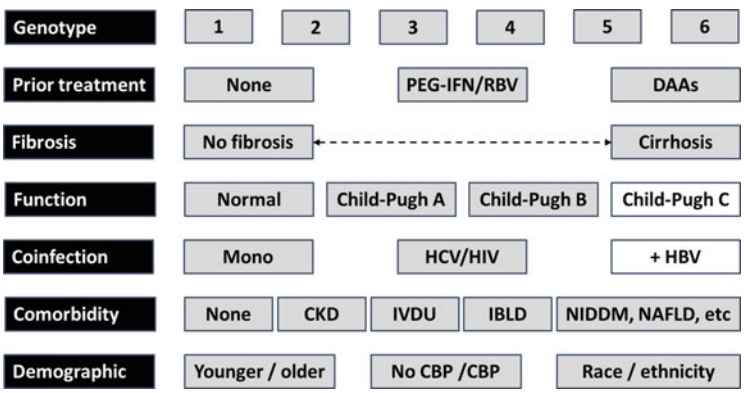

Fig. 1 Populations in which the efficacy of elbasvir/grazoprevir, alone or with other agents, has been evaluated. *CBP* child-bearing potential, *CKD* chronic kidney disease, *DAA* direct-acting antiviral agents, *HBV* hepatitis B virus, *HIV* human immunodeficiency, *IBLD* inherited blood disorders, *IVDU* intravenous drug user, *NAFLD* non-alcoholic fatty liver disease, *NIDDM* non-insulin-dependent diabetes mellitus, *PEG-IFN* pegylated interferon, *RBV* ribavirin

(version 2.0, Roche Molecular Diagnostics, Branchburg, NJ, USA) with a LLoQ of 25 IU/mL in the phase 2 studies and 15 IU/mL in the phase 3 studies. Determination of HCV genotyping was primarily conducted using VERSANT® HCV genotype assay (line probe assay [LiPA] 2.0) reverse hybridization technology (Innogenetics, Ghent, Belgium) or the Abbott RealTime HCV Genotype II polymerase chain reaction assay (Abbott Park, IL, USA). In all studies, relapse was defined as HCV RNA levels above the LLoQ after having previously achieved HCV RNA below the LLoQ at the end of therapy. Virologic breakthrough was defined as the presence of confirmed on-treatment detectable HCV RNA after a previous HCV RNA level below the LLoQ while on treatment. Elbasvir and grazoprevir were administered as separate, single-entity tablets in the phase 2 and early phase 3 studies and as a fixed-dose combination tablet in the later phase 3 studies. Ribavirin, when used, was administered using weight-based administration at doses of 800–1,400 mg/day. The participant populations varied across the different studies, but in general all participants had chronic HCV infection with a baseline viral load of greater than 10,000 IU/mL. Participants with cirrhosis were enrolled in several studies; however, in all studies addressed in this chapter, these participants had well-compensated liver disease, usually defined as one of the following: liver biopsy consistent with a METAVIR fibrosis score of F4 at any time prior to entry into the study, FibroScan® (Echosens, Waltham, MA) greater than 12.5 kPa within 12 months of study entry, or an aspartate aminotransferase (AST)-to-platelet ratio greater than 2.0 and FibroTest greater than 0.75 within 12 months of study entry. All studies excluded individuals with hepatitis B virus coinfection, evidence of decompensated liver disease (such as the presence or history of ascites, esophageal or gastric variceal bleeding, hepatic encephalopathy, or other signs of advanced liver disease), or evidence of hepatocellular carcinoma. Because people with HIV infection constitute a key segment of the HCV-infected population, some studies enrolled participants coinfected with human immunodeficiency virus (HIV). A summary of virologic outcomes from phase 2 studies is presented in Table 1 [8–15], and a summary of virologic outcomes from phase 3 studies is presented in Table 2 [16–26].

2 Phase 1 Trials

The elbasvir/grazoprevir clinical development program consisted of 58 phase 1 studies in a total of 1,234 healthy male and female volunteers, 139 participants infected with HCV, and 66 non-HCV-infected people with liver or kidney dysfunction who received elbasvir, grazoprevir, or both compounds simultaneously. Key intrinsic and extrinsic factors were evaluated in these populations, and thorough QTc studies were also conducted to rigorously assess the effect of elbasvir and grazoprevir on the QTc interval.

Table 1 Phase 2 studies of elbasvir and grazoprevir

Study name/ ClinicalTrials.gov identifier	Participant population/HCV genotype	Treatment regimen[a]	Analysis population	Received ≥1 dose of study medication (*N*)	SVR12, *n* (%)	Virologic failure, *n*	Nonvirologic failure,[b] *n*	Reinfection, *n*
PN003 [8]/ NCT01353911	Treatment-naive, noncirrhotic/ GT1	GZR (100 mg) + PEG-IFN/RBV	All	66	59 (89%)	5	2	0
		GZR (200 mg) + PEG-IFN/RBV	All	68	62 (91%)	1	4	1
		GZR (400 mg) + PEG-IFN/RBV	All	67	61 (91%)	0	6	0
		GZR (800 mg) + PEG-IFN/RBV	All	65	56 (86%)	0	9	0
		BOC + PEG-IFN/ RBV	All	66	40 (61%)	12	14	0
PN038 [9]/ NCT01710501	Treatment-naive, noncirrhotic/ GT1	GZR (25 mg) + PEG-IFN/RBV	All	24	13 (54.2%)	11	0	0
		GZR (50 mg) + PEG-IFN/RBV	All	25	21 (84.0%)	4	0	0
		GZR (100 mg) + PEG-IFN/RBV	All	26	23 (88.5%)	3	0	0

(continued)

Table 1 (continued)

Study name/ ClinicalTrials.gov identifier	Participant population/HCV genotype	Treatment regimen[a]	Analysis population	Received ≥1 dose of study medication (*N*)	SVR12, *n* (%)	Virologic failure, *n*	Nonvirologic failure,[b] *n*	Reinfection, *n*
C-WORTHY [10, 11]/ NCT1717326	Treatment-naïve, noncirrhotic/ GT1	EBR + GZR + RBV for 8 weeks	GT1a, HCV monoinfected	30	24 (80%)	5	1	0
		EBR + GZR + RBV for 12 weeks	GT1a + GT1b, HCV monoinfected	85	79 (93%)	3	3	0
		EBR + GZR for 12 weeks	GT1b, HCV monoinfected	44	43 (98%)	1	0	0
		EBR + GZR + RBV for 12 weeks	GT1a + GT1b, HCV/HIV coinfected	29	28 (97%)	1	0	0
		EBR + GZR for 12 weeks	GT1a + GT1b, HCV/HIV coinfected	30	26 (87%)	2	2	0
	Cirrhotic, treatment-naive, and prior PEG-IFN null responders	EBR + GZR + RBV for 12 weeks	Treatment-naive, cirrhotic	31	28 (90%)	3	0	0
		EBR + GZR for 12 weeks		29	28 (97%)	1	0	0
		EBR + GZR + RBV for 18 weeks		32	31 (97%)	0	1	0
		EBR + GZR for 18 weeks		31	29 (94%)	2	0	0
		EBR + GZR + RBV for 12 weeks	Cirrhotic and noncirrhotic, PEG-IFN null responders	32	30 (94%)	0	2	0
		EBR + GZR for 12 weeks		33	30 (91%)	3	0	0

		EBR + GZR + RBV for 18 weeks		33	33 (100%)	0	0	0
		EBR + GZR for 18 weeks		32	31 (97%)	1	0	0
C-SCAPE [12]/ NCT01932762	Treatment-naive, noncirrhotic/ GT2, 4, 5, 6	EBR + GZR + RBV for 12 weeks	GT2	30	24 (80%)	4	2	0
		GZR + RBV for 12 weeks	GT2	26	19 (73%)	7	0	0
		EBR + GZR + RBV for 12 weeks	GT4	10	10 (100%)	0	0	0
		EBR + GZR for 12 weeks	GT4	10	9 (90%)	0	1	0
		EBR + GZR + RBV for 12 weeks	GT5	4	4 (100%)	0	0	0
		EBR + GZR for 12 weeks	GT5	4	1 (25%)	3	0	0
		EBR + GZR + RBV for 12 weeks	GT6	4	3 (75%)	1	0	0
		EBR + GZR for 12 weeks	GT6	4	3 (75%)	1	0	0
C-SALVAGE [13, 14]/ NCT2105454	Cirrhotic or noncirrhotic; prior failure to PEG-IFN + first-generation protease inhibitor/ GT1	EBR + GZR + RBV for 12 weeks	All	79	76 (96.2%)	3	0	0
C-SWIFT [15]/ NCT02133131	Treatment-naive/GT1, 3	EBR + GZR + SOF for 4 weeks	GT1, noncirrhotic	31	10 (32.0%)	20	1	0
		EBR + GZR + SOF for 6 weeks	GT1, noncirrhotic	30	26 (86.7%)	4	0	0
			GT1, cirrhotic	20	16 (80.0%)	4	0	0

(continued)

Table 1 (continued)

Study name/ ClinicalTrials.gov identifier	Participant population/HCV genotype	Treatment regimen[a]	Analysis population	Received ≥1 dose of study medication (*N*)	SVR12, *n* (%)	Virologic failure, *n*	Nonvirologic failure,[b] *n*	Reinfection, *n*
		EBR + GZR + SOF for 8 weeks	GT1, cirrhotic	21	17 (81.0%)	2	1	1
		EBR + GZR + SOF for 8 weeks	GT3, noncirrhotic	15	14 (93.0%)	1	0	0
		EBR + GZR + SOF for 12 weeks	GT3, noncirrhotic	14	14 (100%)	0	0	0
		EBR + GZR + SOF for 12 weeks	GT3, cirrhotic	12	10 (83.3%)	1	1	0

BOC boceprevir, *EBR* elbasvir, *GT* genotype, *GZR* grazoprevir, *HCV* hepatitis C virus, *HIV* human immunodeficiency virus, *PEG-IFN* pegylated interferon, *RBV* ribavirin, *SOF* sofosbuvir, *SVR12* sustained virologic response 12 weeks after completion of study medication

[a]EBR was administered at a dose of 50 mg/day and GZR at a dose of 100 mg/day unless otherwise stated

[b]Nonvirologic failure category includes participants who discontinued treatment because of an adverse event

Table 2 Phase 3 studies of elbasvir and grazoprevir

Study name/ ClinicalTrials.gov identifier	Participant population/HCV genotype	Treatment regimen[a]	Analysis population	Received ≥1 dose of study medication, *N*	SVR12, *n* (%)	Virologic failure, *n*	Nonvirologic failure,[b] *n*	Reinfection, *n*
C-EDGE treatment-naive [16]/ NCT02105467	Cirrhotic or noncirrhotic, treatment-naive/ GT1, 4, 6	EBR/GZR for 12 weeks (ITG)	All	316	299 (95%)	13	4	0
			GT1a	157	144 (92%)	10	3	0
			GT1b	131	129 (99%)	1	1	0
			GT4	18	18 (100%)	0	0	0
			GT6	10	8 (80%)	2	0	0
C-EDGE treatment-experienced [17]/ NCT02105701	Cirrhotic or noncirrhotic, prior PEG-IFN/ RBV failures/ GT1, 4, 6	EBR/GZR for 12 weeks	All	105	97 (92.4%)	6	2	0
		EBR/GZR + RBV for 12 weeks	All	104	98 (94.2%)	6	0	0
		EBR/GZR for 16 weeks	All	105	97 (92.4%)	7	1	0
		EBR/GZR + RBV for 16 weeks	All	106	104 (98.1%)	0	2	0
C-SURFER [18, 19]/ NCT02092350	Treatment-naive or treatment-experienced, cirrhotic or noncirrhotic, CKD stage 4-5/ GT1	EBR/GZR for 12 weeks (ITG)		122	115 (94.3%)	1	6	–
C-EDGE CO-INFECTION [20]/ NCT02105662	Treatment-naive, cirrhotic or noncirrhotic, HIV coinfection/ GT1, 4, 6	EBR/GZR for 12 weeks	All	218	210 (96.3%)	5	1	2
			GT1a	144	139 (96.5%)	4	0	1
			GT1b	44	42 (95.5%)	0	1	1
			GT4	28	27 (96.4%)	1	0	0

(continued)

Table 2 (continued)

Study name/ ClinicalTrials.gov identifier	Participant population/HCV genotype	Treatment regimen[a]	Analysis population	Received ≥1 dose of study medication, *N*	SVR12, *n* (%)	Virologic failure, *n*	Nonvirologic failure,[b] *n*	Reinfection, *n*
C-EDGE CO-STAR [21]/NCT02105688	Treatment-naive; PWID on OAT/GT1, 4, 6	EBR/GZR for 12 weeks (ITG)	All	201	184 (91.5%)	7	5	5
			GT1a	154	144 (93.5%)	4	3	3
			GT1b	30	28 (93.3)	1	1	0
			GT4	12	11 (91.7%)	0	1	0
			GT6	5	1 (20.0%)	2	0	2
		EBR/GZR for 12 weeks (DTG)	All	95	85 (89.5%)	3	7	0
C-EDGE IBLD [22]/ NCT02252016	Treatment-naive or treatment-experienced; cirrhotic or noncirrhotic; sickle cell disease, β-thalassemia, hemophilia A/B, or von Willebrand/GT1, 4, 6	EBR/GZR for 12 weeks (ITG)	All	107	100 (93.5%)	6	1	0
			GT1a	47	43 (91.5%)	4	0	0
			GT1b	46	44 (95.7%)	1	1	0
			GT4	12	11 (91.7%)	1	0	0

C-EDGE Head-2-head [23]/NCT02358044	Cirrhotic or noncirrhotic, treatment-naive and treatment-experienced/ GT1, 4	EBR/GZR for 12 weeks	All	129	128 (99.2%)	0	1	0
		SOF + PEG-IFN/ RBV for 12 weeks	All	126	114 (90.5%)	11	1	0
C-CORAL [24]/ NCT02251990	Treatment-naive, HIV-negative, cirrhotic or noncirrhotic/ GT1, 4, 6	EBR/GZR for 12 weeks (ITG)	All	365	344 (94.2%)	20	1	0
		EBR/GZR for 12 weeks (DTG)	All	121	115 (95.0%)	5	1	0
		EBR/GZR for 12 weeks (ITG and DTG)	GT1a	37	34 (91.9%)	3	0	0
			GT1b	389	382 (98.2%)	5	2	0
			GT4	3	3 (100%)	0	0	0
			GT6	51	34 (66.7%)	17	0	0
Japan phase 2/3 study [25]/NCT02203149	Japanese pts., treatment-naive or treatment-experienced, cirrhotic or noncirrhotic	Part 1 EBR/GZR (50 mg) for 12 weeks	All	31	31 (100%)	0	0	0
		Part 1 EBR/GZR (100 mg) for 12 weeks	All	31	30 (96.8%)	1	0	0
		Part 2 EBR/GZR (100 mg) for 12 weeks (ITG)	Noncirrhotic	227	219 (96.5%)	5	3	0
		Part 2 EBR/GZR (100 mg) for 12 weeks (ITG)	Cirrhotic	35	34 (97.1%)1	1	0	0
C-ISLE [26]/ NCT02601573	Cirrhotic, treatment-naive and prior PEG-IFN/RBV relapse pts./GT3	EBR/GZR + SOF + RBV for 8 weeks	Treatment-naive, GT3, cirrhotic	23	21 (91%)	2	0	0
		EBR/GZR + SOF for 12 weeks		24	23 (96%)	0	1	0

(continued)

Table 2 (continued)

Study name/ ClinicalTrials.gov identifier	Participant population/HCV genotype	Treatment regimen[a]	Analysis population	Received ≥1 dose of study medication, *N*	SVR12, *n* (%)	Virologic failure, *n*	Nonvirologic failure,[b] *n*	Reinfection, *n*
		EBR/GZR + SOF for 12 weeks	Treatment-experienced, GT3, cirrhotic	17	17 (100%)	0	0	0
		EBR/GZR + SOF + RBV for 12 weeks		18	17 (94%)	0	1	0
		EBR/GZR + SOF + RBV for 16 weeks		18	17 (94%)	0	1	0

CKD chronic kidney disease, *DTG* deferred-treatment group, *EBR* elbasvir, *GT* genotype, *GZR* grazoprevir, *HCV* hepatitis C virus, *HIV* human immunodeficiency virus, *ITG* immediate-treatment group, *OAT* opioid agonist therapy, *PEG-IFN* pegylated interferon, *PWID* people who inject drugs, *RBV* ribavirin, *SOF* sofosbuvir, *SVR12* sustained virologic response 12 weeks after completion of study medication

[a]EBR was administered at a dose of 50 mg/day and GZR at a dose of 100 mg/day unless otherwise stated

[b]Nonvirologic failure category includes participants who discontinued treatment due to an adverse event

2.1 Elbasvir Monotherapy Proof of Concept Study

Elbasvir was administered as monotherapy to individuals with HCV genotype (GT) 1 and GT3 infection at doses ranging from 5 to 100 mg once daily for 5 days [27]. Participants administered elbasvir had dose-dependent reductions in HCV RNA at all doses compared with those who received placebo. Observed mean maximal viral load reductions on day 5 of dosing exceeded 3 $\log_{10}$ IU/mL at doses of 5 mg or higher in participants with HCV GT1 infection and exceeded 2 $\log_{10}$ IU/mL at doses of 50 mg or higher in those with HCV GT3 infection.

2.2 Grazoprevir Monotherapy Proof of Concept Study

Grazoprevir was administered as a monotherapy to participants with HCV GT1 and GT3 infection at doses ranging from 30 to 800 mg once daily for 7 days [27]. Observed mean maximum viral load reductions exceeding 3 $\log_{10}$ were achieved by day 7 at all doses in participants with HCV GT1 infection and at doses of 400 mg or higher in participants with GT3 infection. In participants with HCV GT1 infection, viral load reduction appeared to plateau at doses between 50 and 800 mg, whereas in those with GT3 infection, a dose-dependent reduction in viral load was observed at doses between 100 and 600 mg.

3 Phase 2 Trials

3.1 Phase 2 Dose-Ranging Trials of Grazoprevir in Combination with Pegylated Interferon and Ribavirin

MK-5172 Protocol 003 was a phase 2 randomized, double-blind, active-controlled, dose-ranging study (NCT01353911) in which treatment-naive participants with HCV GT1 infection were randomized to receive once-daily grazoprevir at doses of 100, 200, 400, or 800 mg or boceprevir (800 mg three times daily), each in combination with pegylated interferon (PEG-IFN) and ribavirin [8]. A high proportion (89–93%) of participants achieved SVR12 at all grazoprevir doses evaluated, with no clear dose-response relationship. However, elevations of alanine aminotransferase (ALT) and/or AST levels were observed late in the course of therapy (i.e., after treatment week 4) among a proportion of participants who received grazoprevir at doses of 200, 400, or 800 mg. Late ALT/AST increases above 2× upper limit of normal (ULN) were observed in up to 23% of participants, and increases above 5× ULN were observed in up to 9% of participants at grazoprevir doses of 400 mg or higher. Given that these events were not observed in participants receiving the 100-mg dose and that doses higher than 100 mg did not increase the proportion

who achieved SVR12, the 100-mg dose was selected for further evaluation in subsequent studies of participants with HCV infection. Further PK/safety analyses of the data from this study confirmed that the 100-mg dose had an adequate safety margin, with a population geometric mean (GM) area under the plasma concentration-time curve from time 0–24 h (AUC_{0-24}) that is greater than 14× below that associated with a predicted population rate of late transaminase elevations of 5%.

Because high SVR rates were observed at all doses evaluated in Protocol 003, a second phase 2 dose-ranging study, Protocol 038 (MK-5127 Protocol 038; NCT01710501), was conducted to further define the lower end of the grazoprevir dose–SVR relationship [9]. Protocol 038 was a phase 2, double-blind, dose-ranging study that randomized treatment-naive participants with HCV GT1 infection to receive grazoprevir doses of 25, 50, or 100 mg in combination with PEG-IFN/ribavirin for 12 weeks. This study confirmed the efficacy of the 100-mg dose and demonstrated a dose-response trend at lower doses: the proportion of participants who achieved SVR12 was numerically higher in the group receiving 100 mg (87% [95% confidence interval [CI], 69.3–96.2%]) compared with the group receiving 50 mg (75% [95% CI, 55.1–89.3%]), and efficacy was substantially lower in the group receiving 25 mg (48% [95% CI, 29.4–67.5]).

These results supported the selection of the 100-mg dose of grazoprevir for further evaluation. The choice of the 100-mg dose also offered an advantage in that factors that might result in a decrease in grazoprevir levels, such as drug–demographic, drug–disease, and drug–drug interactions, would be less likely to result in lower efficacy.

3.2 C-WORTHY: Elbasvir and Grazoprevir Among a Broad Population of HCV GT1–Infected Participants

C-WORTHY (MK-5172 Protocol 035; NCT1717326) was a phase 2 multicenter, randomized, parallel-group trial that evaluated grazoprevir plus elbasvir with or without ribavirin in patients with HCV GT1 and GT3 infection [10, 11]. The study was conducted in multiple parts. Part A evaluated elbasvir plus grazoprevir with or without ribavirin administered for 12 weeks in 65 treatment-naive, noncirrhotic participants with GT1 infection. A total of 52 participants were randomized in a 1:1 ratio to two treatment arms (A1 and A2) in which open-label grazoprevir at a dose of 100 mg once daily was administered concomitantly with double-blind elbasvir doses of either 20 or 50 mg once daily, plus twice-daily ribavirin [11]. A third arm (A3) including 13 participants with HCV GT1b infection received a regimen of 100 mg of grazoprevir once daily and 50 mg of elbasvir once daily (without ribavirin). All regimens were administered for 12 weeks, and all participants were followed for an additional 24 weeks after the end of treatment. SVR12 was achieved in more than 95% of participants receiving both the 20- and 50-mg doses of elbasvir, with no

apparent dose-response relationship. Because the safety profile was also similar at both dose levels and in vitro studies suggested that the elbasvir exposures associated with the 50-mg dose are more likely to suppress HCV variants containing common NS5A resistance-associated substitutions (RASs) than the 20-mg dose [4, 6], the 50-mg dose was selected for subsequent evaluation. The choice of the 50-mg dose of elbasvir also offered the advantage that factors that might result in decreases in elbasvir levels, such as drug–drug interactions, would be less likely to result in lower efficacy.

Although SVR12 was achieved by more than 95% of treatment-naive, noncirrhotic participants with HCV GT1 infection receiving elbasvir and grazoprevir plus ribavirin for 12 weeks in Part A, it is also well-recognized that the optimal duration of therapy may differ in the presence of disease factors associated with an unfavorable response (e.g., cirrhosis, prior treatment failure). Various alternative treatment durations were therefore explored in Parts B and C of C-WORTHY [10, 11]. The study populations evaluated in these latter parts of the study were divided into two broad categories encompassing easier-to-cure ($n = 279$) and harder-to-cure patient populations ($n = 253$).

Easier-to-cure patients included those with favorable disease factors, such as those who were treatment-naive and noncirrhotic [11]. In parts A and B of the C-WORTHY study, participants with HCV GT1a and GT1b infection and disease-favorable characteristics received elbasvir plus grazoprevir, with or without ribavirin, for 8 or 12 weeks. Participants with HIV coinfection were also enrolled in Part B of the study. In treatment-naive, noncirrhotic participants with HCV monoinfection, a 12-week regimen of 50 mg of elbasvir plus 100 mg of grazoprevir administered once daily without ribavirin resulted in SVR12 rates of 98% (43/44) in those with GT1a or GT1b infection. The addition of ribavirin did not increase the proportion of participants who achieved SVR12. In a similar patient population but including those with HIV coinfection, the same 12-week treatment regimens achieved SVR12 rates of 97% (28/29) and 87% (26/30) in participants receiving elbasvir and grazoprevir with or without ribavirin, respectively. The lower SVR12 rate in the ribavirin-free arm was owing to the fact that two patients were lost to follow-up or withdrawn from the trial who had had undetectable HCV RNA at their last visit. An SVR12 rate of 80% (24/30) was achieved in patients with HCV GT1a infection receiving an 8-week regimen of 100 mg of grazoprevir and 50 mg of elbasvir plus ribavirin. This suboptimal response rate was the result of a higher frequency of virologic relapse in the 8-week compared with the 12-week regimen. Conversely, an 8-week regimen of 100 mg of grazoprevir administered with 50 mg of elbasvir, with or without ribavirin, resulted in SVR12 rates of 93 and 94% in patients with HCV GT1b infection in Part C of C-WORTHY. The higher SVR12 rate among patients with HCV GT1b infection compared with those with GT1a infection is consistent with the greater decrease in HCV RNA levels seen in patients with HCV GT1b infection compared with those with GT1a infection following administration of elbasvir as monotherapy [27]. These results are also consistent with in vitro data demonstrating that elbasvir has greater potency against GT1b replicons and that several common mutations that confer resistance to NS5A inhibitors in a GT1a

backbone do not cause comparable half-maximal response (EC_{50}) shifts in replicons with a GT1b backbone [6].

Harder-to-cure patients enrolled in Part B of C-WORTHY included those with either cirrhosis, prior PEG-IFN/ribavirin null response, or both [10]. This part of the study included a 2 × 2 factorial evaluation separately for cirrhotic patients and prior PEG-IFN/ribavirin–null responders, which included ribavirin (yes, no) and treatment duration (12 weeks, 18 weeks) as variables. SVR12 was achieved by 97% (28/29) of treatment-naive participants with cirrhosis receiving elbasvir plus grazoprevir for 12 weeks, with no improvement in response when treatment duration was extended from 12 weeks to 18 weeks or by the inclusion of ribavirin. In prior PEG-IFN/ribavirin–null responders with or without cirrhosis, administration of elbasvir plus grazoprevir with or without ribavirin for 12 or 18 weeks resulted in SVR12 in more than 90% of participants. The highest efficacy (SVR12 of 100%; 33/33) was achieved in PEG-IFN/ribavirin–null responders receiving elbasvir plus grazoprevir with ribavirin for 18 weeks, although confidence intervals overlapped across the treatment arms, making it difficult to definitively ascertain the accuracy of the observed differences. To further refine these observations, the efficacy of 12- and 16-week regimens with or without ribavirin was evaluated among PEG-IFN/ribavirin treatment-experienced patients in the phase 3 Protocol 068 C-EDGE Treatment-Experienced study [17] discussed in more detail later in this chapter.

3.3 C-SCAPE: Elbasvir plus Grazoprevir, with or Without Ribavirin, Among Participants with HCV GT2, GT4, GT5, or GT6 Infection

The C-SCAPE study evaluated the efficacy and safety of elbasvir and grazoprevir, with or without ribavirin, in participants with HCV GT2, GT4, GT5, or GT6 infection (MK-5172 protocol 047; NCT 01932762) [12]. This part-randomized, open-label, parallel-group study of treatment-naive, noncirrhotic participants was conducted in two parts. In Part A, 30 treatment-naive, noncirrhotic participants with GT2 infection received elbasvir plus grazoprevir with ribavirin for 12 weeks. In Part B, a further 30 treatment-naive, noncirrhotic participants with GT2 infection received grazoprevir with ribavirin for 12 weeks; and participants with GT4, GT5, or GT6 infection were randomized to receive elbasvir plus grazoprevir with or without ribavirin for 12 weeks.

Among participants with GT2 infection, SVR12 rates were slightly higher in those receiving elbasvir plus grazoprevir with ribavirin compared with participants receiving grazoprevir plus ribavirin (80% [24/30] vs 73% [19/26]). GT2 virions contain naturally occurring variants that encode for either methionine or lysine residues at amino acid 31 of the NS5A protein. Among participants receiving elbasvir and grazoprevir plus ribavirin, SVR12 rates were higher in those with the 31L subtype compared with the 31M subtype (93% [13/14] vs 67% [10/15]), but

SVR12 rates were similarly low in participants with 31L and 31M subtypes receiving grazoprevir plus ribavirin (73% [8/11] vs 75% [9/12]). Thus, among participants with the 31M polymorphism, SVR rates were 67% (10/15) when elbasvir/grazoprevir plus ribavirin was administered and 75% (9/12) when grazoprevir plus ribavirin was administered, indicating that elbasvir offers little or no contribution to efficacy in these patients. This is consistent with in vitro studies showing that the potency of elbasvir is reduced by approximately 1,000-fold in replicons containing the 31M compared with the 31L substitution [7].

Treatment with elbasvir and grazoprevir for 12 weeks was highly effective in participants with HCV GT4 infection. Nine of ten participants achieved SVR12, no virologic failures occurred, and only one participant, who discontinued treatment for reasons unrelated to study medication, failed to achieve SVR12.

In participants with GT5 infection, elbasvir plus grazoprevir with ribavirin was more effective than the same regimen without ribavirin. Three of four participants with GT5 infection receiving elbasvir with grazoprevir had virologic failure (two relapsed and one had virologic breakthrough) compared with one of four participants receiving elbasvir plus grazoprevir with ribavirin (SVR12 was 25% vs 75%, respectively). Based on this small number of noncirrhotic participants with GT5 infection, the addition of ribavirin appears important in attaining high rates of SVR.

In contrast, elbasvir with grazoprevir alone was effective in treating HCV infection in noncirrhotic, treatment-naive participants with HCV GT6 infection. Of the four participants treated, three achieved SVR, and one had virologic breakthrough.

Data from this study were used to inform participant selection for the phase 3 clinical development program of elbasvir/grazoprevir. The data supported the inclusion of participants with HCV GT4 or GT6 infection in these studies, but elbasvir/grazoprevir with or without ribavirin was unsatisfactory for participants with HCV GT2 or GT5 infection. Although treatment with elbasvir plus grazoprevir showed efficacy in participants with the GT2 31L variant, it was decided that inclusion of participants with GT2 infection would not be pursued in the phase 3 program because of the subsequent requirement for baseline sequencing to select out those with the 31M variant. Similarly, the low rates of SVR seen in participants with GT5 infection receiving elbasvir with grazoprevir precluded their further inclusion in the phase 3 clinical program.

3.4 C-SALVAGE: Elbasvir/Grazoprevir with Ribavirin Among GT1–Infected Participants Who Failed Prior Treatment with Boceprevir, Telaprevir, or Simeprevir

C-SALVAGE (MK-5172 Protocol 048; NCT2105454) was an open-label, single-arm study of elbasvir/grazoprevir with ribavirin in participants with HCV GT1 infection who had failed a prior regimen of boceprevir, telaprevir, or simeprevir taken concomitantly with PEG-IFN/ribavirin [13, 14]. Of the 79 participants who

received study drug, 66 (84%) had a history of virologic failure on a regimen containing a first-generation NS3/4A protease inhibitor; and of the remaining 13 participants, 12 had discontinued prior treatment because of an adverse experience. At baseline, 34 (43.6%) participants harbored NS3 RASs and 8 harbored NS5A RASs. SVR12 was achieved by 76 of 79 (96.2%) participants overall, including 28 of 30 (93.3%) with HCV GT1a infection, 63 of 66 (95.5%) with prior virologic failure, and 32 of 34 (94.1%) of those with cirrhosis. With regard to the impact of baseline RASs, SVR12 was achieved by 43 of 43 (100%) without baseline RASs, 31 of 34 (91.2%) with baseline NS3 RASs, 6 of 8 (75.0%) with baseline NS5A RASs, and 4 of 6 (66.7%) with both baseline NS3 and NS5A RASs.

3.5 C-SWIFT: Short-Duration Treatment with Elbasvir plus Grazoprevir and Sofosbuvir Among GT1– or GT3–Infected Treatment-Naive Participants with or Without Cirrhosis

The objective of the C-SWIFT study (MK-5172 protocol 074; NCT02133131) was to identify the minimum effective treatment duration across multiple genotypes [15]. C-SWIFT was an open-label, single-center trial in treatment-naive participants with chronic HCV GT1 or GT3 infection. All participants received 50 mg of elbasvir and 100 mg of grazoprevir plus sofosbuvir 400 mg for 4–12 weeks; those with GT1 infection who failed therapy were eligible for re-treatment with elbasvir plus grazoprevir with sofosbuvir and ribavirin for 12 weeks.

Rates of SVR12 were 32% (10 of 31) and 87% (26 of 30) in noncirrhotic participants with HCV GT1 infection treated for 4 and 6 weeks, respectively, and 80% (16 of 20) and 81% (17 of 21) in cirrhotic participants with GT1 infection treated for 6 and 8 weeks, respectively. Genotyping of plasma samples taken at the time of virologic failure indicated that in one of the cirrhotic participants with HCV GT1 infection treated for 8 weeks, GT2 infection was detected at the time of virologic failure, and thus, this participant was reclassified as having a reinfection. Twenty-three HCV GT1–infected participants who experienced relapse following initial treatment with elbasvir plus grazoprevir with sofosbuvir were re-treated with elbasvir/grazoprevir plus sofosbuvir and ribavirin for 12 weeks; all achieved SVR12.

Among participants with GT3 infection, SVR12 rates were 93% (14 of 15) and 100% (14 of 14) with 8- and 12-week treatment regimens. The SVR12 rate in cirrhotic participants with GT3 infection was 83% (10 of 12) after 12 weeks of treatment.

4 Phase 3 Trials

4.1 C-EDGE Treatment-Naive: Elbasvir/Grazoprevir in Treatment-Naive Participants with HCV Infection, with or Without Cirrhosis

The C-EDGE Treatment-Naive study (MK-5172 protocol 060; NCT02105467) was a randomized, double-blind, placebo-controlled, parallel-group trial of elbasvir/grazoprevir in treatment-naive cirrhotic and noncirrhotic participants with chronic HCV GT1, GT4, or GT6 infections [16]. To assess safety, participants were randomized 3:1 in a double-blinded fashion to receive either elbasvir (50 mg)/grazoprevir (100 mg) (immediate-treatment group) or a matched placebo for 12 weeks; after completing 12 weeks of randomized treatment and an additional 4-week follow-up period, placebo recipients received open-label elbasvir (50 mg)/grazoprevir (100 mg) (deferred-treatment group) so that all randomized participants would receive active therapy during the study, regardless of their initial treatment group.

Of the 316 participants in the immediate-treatment group, 299 (95%) achieved SVR12. SVR12 rates were 92% (144 of 157) in those with HCV GT1a infection, 99% (129 of 131) in those with GT1b infection, 100% (18 of 18) in those with GT4 infection, and 80% (8 of 10) in those with GT6 infection. SVR12 was achieved in 97% (68 of 70) of cirrhotic and 94% (231 of 246) of noncirrhotic participants. Subgroup analyses did not identify meaningful effects of age, sex, race, ethnicity, or *IL28B* genotype on treatment outcome. SVR12 was achieved in 100% of participants with baseline HCV RNA levels of 800,000 IU/mL or less compared with 92% of patients with baseline HCV RNA levels of greater than 800,000 IU/mL.

Elbasvir/grazoprevir was generally well tolerated in this study. The safety profile was similar in the elbasvir/grazoprevir and placebo treatment groups and in cirrhotic and noncirrhotic participants receiving elbasvir/grazoprevir. During the immediate-treatment period, drug-related adverse events occurred in 114 (36.1%) and 41 (39.0%) participants in the active elbasvir/grazoprevir and placebo groups, respectively. Serious adverse events during treatment and the first 14 follow-up days were reported in nine (2.8%) and three (2.9%) patients in the active and placebo groups, respectively; none were considered drug-related.

During the immediate-treatment period, treatment was discontinued because of adverse events in three (0.9%) elbasvir/grazoprevir recipients (two participants with elevated aminotransferase levels and one with palpitations and anxiety on treatment day 4) and one (0.9%) placebo recipient (rash on treatment day 2). One cirrhotic and three noncirrhotic elbasvir/grazoprevir recipients (1.3%) developed late elevations of aminotransferase level more than 5× ULN, without an associated increase in bilirubin. Two of these four participants discontinued treatment because of these late aminotransferase elevations at treatment week 8 (one cirrhotic patient) and week 10 (one noncirrhotic patient), as stipulated by protocol. In both patients, aminotransferase elevations resolved rapidly after cessation of study therapy and SVR12 was achieved.

4.2 C-EDGE Treatment-Experienced: Elbasvir/Grazoprevir in Participants with HCV Infection Who Experienced Virologic Failure After Prior Treatment with Pegylated Interferon Alfa and Ribavirin

C-EDGE Treatment-Experienced (MK-5172 protocol 068; NCT02105701) was a randomized, parallel-group, multisite, open-label trial of elbasvir/grazoprevir administered once daily with or without ribavirin for 12 or 16 weeks in participants with HCV GT1, GT4, or GT6 infection who had experienced virologic failure after prior treatment with PR [17]. Participants coinfected with HIV were also eligible for enrollment. In total, 420 participants were randomized in a 1:1:1:1 ratio to treatment with elbasvir (50 mg)/grazoprevir (100 mg) once daily for 12 weeks with or without ribavirin or for 16 weeks with or without ribavirin. Randomization was stratified by the presence or absence of cirrhosis and by prior PEG-IFN/ribavirin treatment response (relapse, partial response, or null response). The investigators and participants were blinded to the assigned treatment duration during the period from randomization through treatment week 12.

SVR12 rates were 92.4% (97/105) in the 12-week elbasvir/grazoprevir arm, 94.2% (98/104) in the 12-week elbasvir/grazoprevir plus ribavirin arm, 92.4% (97/105) in the 16-week elbasvir/grazoprevir arm, and 98.1% (104/106) in the 16-week elbasvir/grazoprevir plus ribavirin arm. Pooling across treatment durations, the difference in SVR12 between the participants who received ribavirin and those who did not was 3.8%. Pooling arms with and without ribavirin, the difference in SVR12 rates between participants who received 16 weeks of treatment and those who received 12 weeks of treatment was 2.0%.

A per-protocol analysis, which focused on virologic failures, was conducted to evaluate the efficacy of elbasvir/grazoprevir among participant subgroups. Across arms, 207 of 218 (95.0%), 143 of 145 (98.6%), 32 of 36 (88.9%), and 5 of 6 (83.3%) participants with GT1a, GT1b, GT14, and GT16 infection, respectively, achieved SVR12. Overall, the SVR12 rates were 93.8% (135 of 144) in participants with cirrhosis and 96.6% (255 of 264) in those without cirrhosis. Across all treatment arms, SVR12 was achieved by 98% (202 of 207) of participants with a baseline viral load of 2,000,000 IU/mL or less and by 94% (188 of 201) of those with a baseline viral load greater than 2,000,000 IU/mL. Among those who received the 12-week regimen, SVR12 rates were highest in participants with HCV GT1b infection (34 of 34 [100%]), those with prior relapse after treatment with PEG-IFN/ribavirin (35 of 35 [100%]), or those with partial response (17 of 18 [94.4%]). Efficacy among those with GT1a infection (55 of 59 [93.2%]) and those with prior null response (45 of 49 [91.8%]) was lower. SVR12 rates were 100% for all participants who received elbasvir/grazoprevir with ribavirin for 16 weeks, including those with HCV GT1a infection and prior null response (20 of 20), participants with baseline NS3 RASs (37 of 37), and those with NS5A baseline RASs (6 of 6).

Across all treatment arms, drug-related adverse events were reported in 56% (235/420) of participants with higher rates in the ribavirin-containing compared with

no ribavirin arms (64–76% vs 39–44%). Serious adverse events occurred in 3.3% of patients, with similar frequencies across the four treatment arms. Discontinuations due to adverse events occurred in 1.7% of patients, most often in the treatment arm that received 16 weeks of treatment with elbasvir/grazoprevir plus ribavirin ($n = 5$). However, none of the discontinuations were attributed to the study drugs. Hemoglobin levels of 9.9 g/dL or less were reported in 31 of 210 (14.8%) participants in the ribavirin-containing arms and no participants (0 of 210) in the ribavirin-free treatment arms. Decreases in hemoglobin levels were managed by dose reductions of ribavirin, and no treatment discontinuations owing to anemia occurred. Four participants (1.0%) had late elevations of ALT/AST above 5× ULN, but these elevations were transient and did not require interruption or discontinuation of treatment with elbasvir/grazoprevir. All ALT elevations returned to baseline after study medication was discontinued, and all participants with an ALT elevation above 5× ULN achieved SVR.

4.3 C-SURFER: Elbasvir/Grazoprevir in HCV GT1–Infected Participants with Advanced Chronic Kidney Disease

C-SURFER was a randomized, parallel-group, multisite, placebo-controlled trial of elbasvir/grazoprevir, administered for 12 weeks without ribavirin in HCV GT1–infected participants with advanced chronic kidney disease (CKD) stages 4 and 5, including those receiving hemodialysis (MK-5172 protocol 052; NCT02092350) [18, 19]. Ribavirin was not included in the regimen, since it is contraindicated in people with advanced CKD. Cirrhotic, noncirrhotic, treatment-naive, and treatment-experienced adults were eligible for enrollment. CKD stage 4 was defined as an estimated glomerular filtration rate (eGFR) of 15–29 mL/min/1.73 m^2 and CKD stage 5 as an eGFR less than 15 mL/min/1.73 m^2, including dialysis dependence.

Overall, 224 participants were randomized in a 1:1 ratio to receive immediate or deferred treatment with elbasvir (50 mg)/grazoprevir (100 mg). In total, 179 (76.2%) participants were receiving maintenance hemodialysis (including those awaiting renal transplant or with a previous failed kidney transplant who were no longer on immunosuppressant therapy). A total of 111 participants were enrolled in the immediate-treatment group and received elbasvir/grazoprevir for 12 weeks. An additional 113 participants who were enrolled in the deferred-treatment group received placebo for 12 weeks, followed by a 4-week unblinding period, open-label elbasvir/grazoprevir for 12 weeks, and then an additional 24 weeks of follow-up after treatment with study medication was completed. Eleven participants (six on hemodialysis and five not on hemodialysis) were also enrolled in an open-label intensive PK arm and received elbasvir/grazoprevir for 12 weeks while undergoing intensive PK sampling. The deferred-treatment group was used to provide a comparator for safety data collected in the immediate-treatment group, given the substantial comorbidities seen in patients with stage 4–5 CKD. Randomization in this

study was stratified by the presence of diabetes (a predictor for serious cardiovascular adverse events that occur at an increased frequency among participants with CKD stages 4–5) and by dialysis dependence.

The primary analysis population was the modified full analysis set (mFAS) population, which excluded participants who failed to complete treatment due to death or early discontinuation for reasons unrelated to their response to the HCV treatment. This population was selected as the primary analysis population in C-SURFER because people with CKD stages 4–5 have a high incidence of major cardiovascular events that may lead to study discontinuation. Any bias that may have been incurred through considering non–drug and non–HCV-related discontinuations as treatment failures is therefore removed.

Of the 122 patients in the immediate-treatment group and intensive PK arms, 6 were excluded from the mFAS population for reasons other than virologic failure (death, lost to follow-up, noncompliance, participant withdrawal, and withdrawal by physician owing to violent behavior). All six participants had an HCV RNA level of less than 15 IU/mL at the time of discontinuation. Of the 116 remaining participants, 115 (99%) achieved SVR12. Relapse occurred in one noncirrhotic participant with HCV GT1b infection and CKD stage 5. High response rates were observed in all subgroups, including hemodialysis and nonhemodialysis, and participants with characteristics historically associated with poor response to HCV therapy. In particular, SVR12 was achieved in 100% (51/51) of African-American participants, 99% (86/87) of participants with the *IL28B* non-CC genotype, 98% of (40/41) participants with diabetes, and all 6 participants with cirrhosis.

Drug-related adverse events were reported in 38 (34.2%) participants in the immediate-treatment group and 39 (34.5%) of those in the deferred-treatment group during the placebo phase. Serious adverse events were also reported at similar frequencies in both treatment arms (14% vs 17%, respectively), most of which were consistent with the underlying comorbidities and complications within this population. Serious adverse events that occurred in more than one participant receiving elbasvir/grazoprevir in the immediate-treatment group were hypertension and pneumonia ($n = 2$ each), and none were considered to be drug-related. Treatment discontinuations due to an adverse event occurred in five patients in the deferred-treatment group and none in the immediate-treatment group. Increases in liver transaminase levels during treatment were more common in participants receiving deferred treatment than in those receiving immediate treatment. Increases in ALT and AST levels more than 2.5× baseline in the deferred-treatment group were reported in six (5.3%) and four (4.6%) participants, respectively, compared with one (0.8%) and zero participants in the immediate-treatment group.

Adverse events related to the renal system also occurred at similar frequencies in both treatment groups. During treatment, maintenance dialysis was initiated by two participants in the immediate-treatment group, and renal function in six participants (four in the immediate-treatment group, two in the deferred-treatment group) changed from 15 to 29 mL/min/1.73 m^2 at baseline to less than 15 mL/min/1.73 m^2 during the study.

4.4 C-EDGE CO-INFECTION: Elbasvir/Grazoprevir in Treatment-Naive, HCV-/HIV-Coinfected Participants with or Without Cirrhosis

The C-EDGE CO-INFECTION study (MK-5172 protocol 061; NCT02105662) was an open-label, multicenter study that evaluated the safety, tolerability, and efficacy of elbasvir (50 mg)/grazoprevir (100 mg) in treatment-naive, HIV-coinfected, and HCV GT1–, GT4–, and GT6–infected participants with or without cirrhosis [20]. A total of 218 participants were enrolled: all were coinfected with HIV-1 and were either naive to antiretroviral therapy or on stable antiretroviral therapy with tenofovir or abacavir and either emtricitabine or lamivudine plus raltegravir, dolutegravir, or rilpivirine for at least 8 weeks before enrollment. Antiretroviral therapy-naive patients had CD4 T-cell counts greater than 500 cells/μL and an HIV RNA viral load of less than 50,000 copies/mL; participants on stable antiretroviral therapy had CD4 T-cell counts greater than 200 cells/μL and undetectable HIV RNA (less than 20 copies/mL). Because of the potential for drug–drug interactions, boosted HIV-1 protease inhibitors or efavirenz are not recommended for use in combination with elbasvir/grazoprevir.

Overall, 210 of the 218 enrolled participants (96%) achieved SVR12. Five participants relapsed: all were noncirrhotic and included four with HCV GT1 infection and one with GT4 infection. Among this small number of relapsed participants, no clear association was observed between any individual patient characteristic and the propensity for relapse. Two additional participants who did not achieve SVR12 were infected with a different HCV genotype during follow-up (one with HCV GT1a and one with GT1b infection at enrollment and both with GT3 infection at follow-up week 12). In the primary analysis, these participants were classified as having relapsed, but sequencing data are consistent with reinfection after treatment. One participant did not achieve SVR12 for a nonvirologic reason.

Two participants who were receiving antiretroviral therapy had transient HIV viremia during the treatment period. Both participants subsequently achieved undetectable HIV RNA with additional compliance education and without a change in antiretroviral regimen. Throughout the trial, there were no notable changes in the CD4 T-cell count or percentage at treatment week 12 or follow-up week 12.

A total of 75 (34%) participants experienced drug-related adverse events, the most common of which were fatigue (13%), headache (12%), and nausea (9%). Six participants experienced serious adverse events, of which four occurred after dosing was complete (pneumonia and generalized seizure during treatment and erysipelas, acute psychosis, ulnar fracture, and spontaneous bacterial peritonitis during follow-up). None of the serious adverse events required discontinuation of study drug, and none were considered to be related to treatment. Two participants had late ALT/AST increases above 5× ULN (one at treatment week 6 and the other at treatment week 10) and both normalized without discontinuation of treatment.

4.5 C-EDGE CO-STAR: Elbasvir and Grazoprevir in HCV-Infected Participants Receiving Opioid Agonist Therapy

The aim of the CO-STAR (Hepatitis *C* Patients on *O*pioid *S*ubstitution *T*herapy *A*ntiviral *R*esponse) study (MK-5172 protocol 062; NCT02105688) was to assess the efficacy and safety of elbasvir (50 mg)/grazoprevir (100 mg) administered for 12 weeks in persons who inject drugs (PWID) who had HCV GT1, GT4, or GT6 infection and who were receiving opiate agonist therapy [21].

CO-STAR was a randomized, placebo-controlled, double-blind trial. Similar to the C-SURFER and C-EDGE Treatment-Naive studies, C-EDGE CO-STAR had an immediate-treatment arm in which participants received elbasvir/grazoprevir for 12 weeks and a deferred-treatment arm in which participants received placebo for 12 weeks followed by deferred active therapy with elbasvir/grazoprevir for 12 weeks. As in C-SURFER, the deferred-treatment group served as a comparator for safety data collected in the immediate-treatment group, given the substantial comorbidities seen in PWID. Following completion of treatment and a 24-week follow-up period, participants were eligible to enroll in a 3-year observational study to assess the durability of SVR, incidence of HCV reinfection, and drug use behaviors.

SVR12 was achieved by 91.5% (184/201) of participants in the immediate-treatment group and 89.5% (85/95) of those receiving deferred treatment with elbasvir/grazoprevir. Although SVR12 rates were similar in participants with HCV GT1a, GT1b, and GT4 infection in the immediate-treatment group (93.5% [144/154], 93.3% [28/30], and 91.7% [11/12], respectively), it was lower in the few participants with GT6 infection (20% [1/5]). Of the 17 patients who failed to achieve SVR12, 12 had viral recurrence and 5 had nonvirologic failure (discontinuation due to an adverse event [$n = 1$], an administrative reason [$n = 1$], or loss to follow-up [$n = 3$]). Seven of the 12 patients with viral recurrence had findings consistent with relapse (based on GT assessment, sequencing, and phylogenetic analysis), and 5 had signs consistent with probable reinfection. The SVR12 rate in the immediate-treatment group was 94.0% (189 of 201), when participants with probable reinfection were considered to have initially cleared the virus prior to reinfection.

Ongoing drug use during the study did not appear to impact adherence to study medication. Urine drug screen (UDS) was positive for at least one potential drug of abuse at each clinic visit (excluding methadone and buprenorphine) in more than 50% of participants in both the immediate-treatment and deferred-treatment groups, remaining relatively stable throughout treatment. During the same period, 96.5% of participants (192/199) in the immediate-treatment group and 100% of those in the deferred-treatment group during the placebo phase (97/97) were more than 95% adherent.

At follow-up week 24, recurrent viremia was reported in 18 participants (immediate-treatment group, $n = 14$; deferred-treatment group [elbasvir/grazoprevir], $n = 4$).

Five participants in the immediate-treatment group were considered to have probable reinfection, all with recurrent viremia at follow-up week 8, and one participant in the deferred-treatment group (during the active treatment phase) was considered to have probable reinfection with recurrent viremia at follow-up week 24. In four of six probable reinfections, the HCV GT detected at the time of recurrence differed from that present at baseline, and in all six participants, the virus present at recurrence was from a distinct lineage compared with the virus detected at baseline. Ultradeep sequencing of plasma samples taken at baseline failed to amplify when GT-dependent primers based on the virus present at recurrence were used, indicating that the virus present at recurrence was not present at baseline, that these participants acquired a new virus, and that they did not have a mixed infection at baseline. Of note, in three of the six cases, recurrent viremia was transient, with subsequent samples taken after recurrence having undetectable HCV RNA. Four of the participants with probable reinfection tested positive for opioids other than opiate agonist therapy. From the end of treatment through follow-up week 24, the incidence of reinfection was 4.6 reinfections (CI, 1.7–10.0) per 100 person-years (130.6 person-years of follow-up).

Drug-related adverse events were reported in 41.3% (83/201) of participants in the immediate-treatment group and in 34.0% (34/100) and 26.3% (25/95) of those in the deferred-treatment group during the placebo phase and active treatment phase, respectively. The frequency of serious adverse events (3.5% in the immediate-treatment group and 4% in the deferred-treatment group) and discontinuations due to adverse events (less than 1% in the immediate-treatment group and 1% in the deferred-treatment group) were also low in both arms. One serious adverse event in each treatment group was considered to be drug-related, and one participant in each treatment arm discontinued treatment owing to an adverse event.

Despite the general perception that PWID would not be able to adhere to HCV therapy, this study demonstrated high efficacy and safety coupled with excellent treatment adherence in PWIDs receiving stable opiate agonist therapy despite ongoing drug use among most participants. In particular, the potential impact of HCV reinfection following successful treatment is of considerable clinical and public health interest. High levels of HCV reinfection might undermine any benefit associated with initially successful treatment, from both individual and public health perspectives. Data from the CO-STAR study indicate that HCV reinfection in the early posttreatment period (to 24 weeks) does occur in PWIDs, with six cases of probable HCV reinfection in this study population. The observation that four of six participants with reinfections had positive results on opioid testing during posttreatment follow-up suggests that injection drug use was the probable source of reinfection.

4.6 C-EDGE IBLD: Elbasvir/Grazoprevir in Participants with HCV Infection and Inherited Blood Disorders

Before the introduction of screening of blood donors and blood-derived clotting factors, HCV infection was common among people with inherited blood disorders (IBLDs), including those with hemoglobinopathies such as sickle cell disease and β-thalassemia or clotting factor deficiencies such as hemophilia and von Willebrand disease. With improved medical care, patients with IBLDs are living longer but remain at risk for the significant morbidity and mortality associated with HCV infection.

The C-EDGE IBLD study (MK-5172 protocol 065; NCT02252016) was a randomized, double-blind, phase 3 study of elbasvir (50 mg)/grazoprevir (100 mg) in participants with HCV GT1, GT4, or GT6 infection and an IBLD [22]. The study design incorporated randomization to immediate treatment or deferred treatment, similar to the study design of the previously described phase 3 studies. Participants in the immediate-treatment group received elbasvir/grazoprevir for 12 weeks, and those in the deferred-treatment group received placebo for 12 weeks, followed by a 4-week follow-up period and then elbasvir/grazoprevir for 12 weeks. As in the C-SURFER and C-EDGE CO-STAR studies, the deferred-treatment group was used to provide a comparator for safety data collected in the immediate-treatment group, given the substantial comorbidities seen in patients with IBLDs.

In the immediate-treatment group, 100 of 107 participants (93.5%) achieved SVR12. Among those participants who failed to achieve SVR12, six experienced relapse and one was lost to follow-up. SVR12 rates were 91.5% (43/47), 95.7% (44/46), and 91.7% (11/12) in participants with HCV GT1a, GT1b, or GT4 infection and 100.0% (26 of 26) and 91.4% (74 of 81) in those with and without cirrhosis, respectively. High rates of SVR12 were also achieved regardless of IBLD comorbidities in participants with sickle cell disease (94.7%, 18/19), β-thalassemia (97.6%, 40/41), and hemophilia/von Willebrand disease (89.4%, 42/47).

The safety profile was similar in participants receiving elbasvir/grazoprevir in the immediate-treatment group compared with those receiving placebo in the deferred-treatment group. Drug-related adverse events were reported in 36 (33.6%) participants who received elbasvir/grazoprevir in the immediate-treatment group and 16 (30.8%) of those in the deferred-treatment group during the placebo phase. Three participants in the immediate-treatment group reported serious adverse events: one participant with β-thalassemia and erosive gastritis and hypophosphatemia, one with sickle cell disease with crisis, and one with hemophilia A and rectal hemorrhage (only the serious adverse event of erosive gastritis with hypophosphatemia was considered to be drug-related). Five serious adverse events were related to the underlying blood disorder: sickle cell disease with crisis and rectal hemorrhage in two participants receiving elbasvir/grazoprevir in the immediate-treatment group and sickle cell disease with crisis ($n = 2$) and anemia in three participants in the deferred-treatment group during the placebo phase. No discontinuations due to

adverse events occurred in the immediate-treatment group, and one participant in the deferred-treatment group had increased ALT/AST levels that met the protocol-specified criteria for treatment discontinuation.

4.7 C-EDGE Head-2-Head: Elbasvir/Grazoprevir Versus Sofosbuvir plus Pegylated Interferon and Ribavirin in Participants with HCV Infection

C-EDGE Head-2-Head (MK-5172 protocol 077; NCT02358044) was a randomized, open-label, phase 3 trial comparing the safety and efficacy of elbasvir/grazoprevir with sofosbuvir plus PEG-IFN/ribavirin in treatment-naive and treatment-experienced participants with HCV infection [23]. Two hundred fifty-seven participants with HCV GT1 or GT4 infection were randomized to receive 12 weeks of treatment with elbasvir (50 mg)/grazoprevir (100 mg) ($n = 129$) or sofosbuvir (400 mg) plus PEG-IFN/ribavirin ($n = 128$). The primary efficacy objective was SVR12, and the primary safety objective was the proportion of patients experiencing a tier 1 safety event (serious drug-related adverse event, any drug-related adverse event leading to treatment discontinuation, neutrophil count less than 0.75×10^9/L, hemoglobin level of less than 10 g/dL, or any safety event meeting the hepatic transaminase stopping criteria).

The majority of patients were noncirrhotic (83.1%), were treatment-naive (74.9%), and had HCV GT1b infection (82.0%). SVR12 rates were 99.2% (128/129) and 90.5% (114/126) in the elbasvir/grazoprevir and sofosbuvir plus PEG-IFN/ribavirin groups, respectively. The estimated adjusted difference in SVR12 was 8.8% (95% CI, 3.6–15.3%). Because the lower bound of the one-sided one-sample exact test was greater than −10% and greater than zero, both noninferiority and superiority of elbasvir/grazoprevir compared with sofosbuvir plus PEG-IFN/ribavirin were established. In subgroup analyses, all participants with HCV GT1a infection in both treatment arms achieved SVR12. However, SVR12 rates were higher in participants receiving elbasvir/grazoprevir compared with those receiving sofosbuvir plus PEG-IFN/ribavirin across multiple subgroup populations, including those with HCV GT1b infection (99% [104/105] vs 90% [94/104]), GT4 infection (100% [6/6] vs 60% [3/5]), and cirrhosis (100% [22/22] vs 76% [16/21]), and in prior PEG-IFN/ribavirin-null responders (100% [11/11] vs 50% [7/14]) and partial responders (100% [6/6] vs 88% [7/8]).

Overall, the frequency of tier 1 safety events was lower among patients receiving elbasvir/grazoprevir than those receiving sofosbuvir plus PEG-IFN/ribavirin (0.8% vs 27.8%, between-group difference, 27.0% [95% CI, −35.5% to −19.6%; $P < 0.001$]). Drug-related adverse events were reported in 90.5% (114/126) of participants receiving sofosbuvir plus PEG-IFN/ribavirin and 24.8% (32/129) of those receiving elbasvir/grazoprevir. Three serious drug-related adverse events occurred in participants receiving sofosbuvir plus PEG-IFN/ribavirin (perirectal abscess, anemia, and

heroin abuse); however, the only serious adverse event among participants receiving elbasvir/grazoprevir was a participant with periodontal abscess, which was not considered drug-related. One participant receiving sofosbuvir plus PEG-IFN/ribavirin discontinued treatment at treatment week 1 because of the drug-related adverse events of headache, nausea, myalgia, and decreased appetite. No late ALT/AST events were reported, and no participants discontinued study medication as a result of protocol-specified hepatic laboratory abnormalities.

4.8 C-CORAL: Elbasvir/Grazoprevir in HCV GT1–, GT4–, or GT6–Infected People from the Asia-Pacific Region and Russia

C-CORAL (MK-5172 protocol 067; NCT02251990) was a phase 3, randomized, placebo-controlled, double-blind study conducted in China, Australia, South Korea, Taiwan, Thailand, Vietnam, and Russia [24]. Similar to other phase 3 studies, the study design was again based on randomization of participants to immediate- and deferred-treatment arms, permitting a placebo-controlled comparison of safety events. Treatment-naive, HIV-negative, cirrhotic, and noncirrhotic participants with chronic HCV GT1, GT4, or GT6 infection were randomized 3:1 to receive elbasvir (50 mg)/grazoprevir (100 mg) for 12 weeks (immediate-treatment group) or placebo for 12 weeks followed by deferred treatment with elbasvir/grazoprevir for 12 weeks (deferred-treatment group).

In the immediate-treatment group, 94.2% (344/365) of participants receiving elbasvir/grazoprevir achieved SVR12, and, when combined with participants who received deferred active treatment, the overall SVR12 rate for the total study population was 94.4% (459/486). SVR12 rates were 98.2% (382/389) in participants with HCV GT1b infection and 91.9% (34/37) in those with GT1a infection but were lower at 66.7% (34/51) in those with GT6 infection. The reduced efficacy of elbasvir/grazoprevir in participants with GT6 infection was the main contributing factor to the lower response rates in countries that enrolled a high proportion of people with HCV GT6 infections, such as Vietnam (81.8% [27/33]) and Thailand (57.1% [12/21]). Notably, the population from Thailand included six participants with HCV GT6f infection, of whom only one achieved SVR12 (16.7%). Subgroup analyses revealed that SVR12 rates for the combined immediate-treatment and deferred-treatment populations were consistently high across most major participant subgroups. SVR12 was achieved by 93.3% (84/90) of participants with cirrhosis, 91.1% (205/225) of those with baseline viral load of greater than 2,000,000 IU/mL, and 92.9% (39/42) of those aged $\geq$65 years. Efficacy was also high in Russian (99.2% [117/118]), Taiwanese (97.6% [83/85]), Chinese (96.7% [146/151]), and South Korean (96.0% [48/50]) participants.

The incidence of drug-related adverse events was similar in the immediate-treatment group and during the placebo phase of deferred treatment (21.4% [78/365] vs 21.1% [26/123]), and drug-related adverse events were also reported by 10.7% (13/121) of participants receiving deferred active treatment. One participant in the immediate-treatment group and the placebo phase of deferred treatment discontinued treatment because of an adverse event. Serious adverse events were reported by five participants in the immediate-treatment group (suicide, contusion, Evans syndrome, lymphoma, and enteritis), by two participants in deferred-treatment group during the placebo phase (influenza and foot fracture), and three participants in the deferred-treatment group during the active treatment phase (ankle fracture, atrial fibrillation, and uterine hemorrhage). Only the serious adverse event of atrial fibrillation was considered drug-related. Late on-treatment ALT/AST elevations of more than 2.0 to 5.0× ULN were reported in 1.4% (5/363) of participants in the immediate-treatment group and in 2.5% (3/122) and 1.7% (2/121) of those in the deferred-treatment group during the placebo phase and active treatment phase, respectively. Late ALT/AST elevations above 5× ULN occurred in four participants (1.1%) in the immediate-treatment group and in three participants receiving elbasvir/grazoprevir in the deferred-treatment group (2.5%). Two of these participants discontinued therapy, and the remainder continued therapy and experienced a gradual reduction in ALT/AST while on treatment, with eventual normalization.

4.9 Japanese Phase 2/3 Study: Elbasvir/Grazoprevir in Japanese Participants with HCV GT1 Infection

Protocol 058 (NCT02203149) was a phase 2/3 trial of the safety and efficacy of elbasvir and grazoprevir in Japanese participants with HCV GT1 infection [25]. The study was conducted in two parts. In Part 1, noncirrhotic participants were randomized 1:1 to receive elbasvir (50 mg) in combination with grazoprevir (50 or 100 mg) once daily for 12 weeks. Participants randomized to receive 100 mg of grazoprevir received two 50-mg tablets once daily, and those randomized to receive 50 mg of grazoprevir received one 50-mg tablet once daily plus a matching placebo tablet. The objective of Part 1 of the study was to confirm that the 100-mg dose (the dose used in other regions) was the appropriate dose for Japanese patients.

The rates of virologic response in Part 1 were similar with the 50- and 100-mg doses of grazoprevir between treatment arms. In all patients, HCV RNA was undetectable by the end of treatment, and at follow-up week 4, all participants in both treatment arms had undetectable HCV RNA. One participant in the grazoprevir 100-mg arm relapsed at follow-up week 12, resulting in SVR12 rates of 100% (31/31) in the grazoprevir 50-mg arm and 96.8% (30/31) in the grazoprevir 100-mg arm. Overall tolerability was similar between the groups, and therefore based on these results, a dose of 100 mg of grazoprevir was selected for use in combination with EBR in Part 2 of the study.

In Part 2, noncirrhotic patients were randomized 3:1 to receive immediate or deferred treatment with elbasvir (50 mg) and grazoprevir (100 mg, as determined in Part 1) for 12 weeks; cirrhotic patients received open-label immediate treatment. SVR12 was achieved by 96.5% (219/227) of participants receiving elbasvir/grazoprevir in the immediate-treatment group. Eight participants failed to achieve SVR12: three discontinued because of nonvirologic failure (adverse event, $n = 2$; administrative reasons, $n = 1$) and five relapsed. In a supportive analysis that included treatment-naive participants (and excluded those who discontinued treatment for reasons unrelated to study medication), SVR12 was achieved by 98.6% (142/144) of participants. Subgroup analyses of participants who received elbasvir/grazoprevir in the immediate-treatment group indicated high efficacy across the most important populations. SVR12 was achieved in 99% (122/123) of participants aged younger than 65 years and 93% (70/75) of those aged 65 years and older (7/8 participants who failed to achieve SVR12 were aged $\geq$65 years). All 5 patients with HCV GT1a infection and 34/35 cirrhotic participants (97.1%) achieved SVR.

In the randomized phase 3 part of the study (Part 2), drug-related adverse events were reported by 58 participants in the immediate-treatment group (25.6%) and 14 (18.9%) in the deferred-treatment group during the placebo phase; serious adverse events were reported by 11 (4.8%) and 1 (1.4%) participants, respectively. In the immediate-treatment group, cataract was the only serious adverse event reported by more than one participant ($n = 2$), and two drug-related serious adverse events of cerebral infarction and increased ALT/AST occurred. Three participants (13%) in the immediate-treatment group discontinued treatment because of an adverse event (cardiac sarcoidosis, cerebral infarction, and increased ALT/AST level) compared with one participant (1.4%) in the deferred immediate-treatment group who discontinued owing to hepatocellular carcinoma. Four of 227 participants in the immediate-treatment group had late ALT/AST elevations above 5× ULN between treatment weeks 8 and 12. Late ALT/AST level elevations above 5× ULN also occurred in 2 of 34 participants with cirrhosis (5.9%), and in both cases transaminase elevations were accompanied by slight increases in levels of bilirubin and eosinophils but no change in international normalized ratio.

4.10 C-ISLE: Elbasvir/Grazoprevir and Sofosbuvir in Participants with HCV GT3 Infection and Cirrhosis

C-ISLE was an open-label study in participants with HCV GT3 infection and compensated cirrhosis (Protocol MK-5172-083; NCT02601573) [26]. The study population included treatment-naive and treatment-experienced participants and both monoinfected and HCV-/HIV-coinfected individuals. All participants received elbasvir (50 mg)/grazoprevir (100 mg) plus sofosbuvir (400 mg) once daily. Treatment-naive participants were randomized to receive treatment for 8 weeks with ribavirin (8,000–1,400 mg) or 12 weeks without ribavirin; and treatment-

experienced participants were randomized to receive elbasvir/grazoprevir plus sofosbuvir with or without ribavirin for 12 weeks or elbasvir/grazoprevir plus sofosbuvir (without ribavirin) for 16 weeks.

One hundred predominantly white (69%) and male (68%) participants were enrolled. Among the treatment-naive participants, SVR12 was achieved by 91% (21/23) treated for 8 weeks with ribavirin and 96% (23/24) of those treated for 12 weeks without ribavirin. Two participants in the 8-week arm relapsed, and one participant in the 12-week arm was lost to follow-up. Among treatment-experienced individuals treated with elbasvir/grazoprevir for 12 weeks, SVR12 was achieved by 94% (17/18) and 100% (17/17) of those treated with and without ribavirin, respectively. The participant who did not achieve SVR in the 12-week elbasvir/grazoprevir plus ribavirin arm withdrew consent after 7 days of therapy. In the 16-week arm, SVR12 was 94% (17/18), with one participant discontinuing treatment because of adverse events of vomiting and cellulitis. Thus, overall, the only two participants with virologic failure in this study were treatment-naive individuals randomized to the 8-week treatment arm.

Adverse events tended to be more common among participants receiving ribavirin compared with those receiving elbasvir/grazoprevir plus sofosbuvir alone, with fatigue (56% [10/18] vs 34% [14/41]), nausea (33% [6/18] vs 15% [6/41]), and headache (61% [11/18] vs 29% [12/41]) all increased in participants who received ribavirin, when considering only participants treated for 12 weeks (ribavirin vs no ribavirin). Drug-related adverse events were reported by 60.9% (14/23) and 83.3% (15/18) of participants receiving a ribavirin-containing regimen for 8 or 12 weeks compared with 43.9% (18/41), and 61.1% (11/18) of those receiving ribavirin-free treatment for 12 or 16 weeks. Five participants reported serious adverse events: three were receiving ribavirin (pneumonia, chest pain, opiate overdose) and two were receiving elbasvir/grazoprevir plus sofosbuvir alone (cellulitis and decreased creatinine, with both considered to be drug-related). Three participants had on-treatment hemoglobin levels of less than 10 g/dL (two were receiving ribavirin and required ribavirin dose reduction), and no ALT/AST elevations above 5× ULN were reported.

5 Integrated Analyses

5.1 Patients with Compensated Cirrhosis

An integrated safety and efficacy analysis was performed that included 402 participants with compensated cirrhosis who received elbasvir/grazoprevir with or without ribavirin for 12, 16, or 18 weeks [28]. Most participants in this retrospective analysis were originally treated within the C-WORTHY, C-SALVAGE, C-EDGE Treatment-Naive, C-EDGE Treatment-Experienced, and C-EDGE CO-INFECTION studies. To be included in this analysis, participants had Child–Pugh class A compensated cirrhosis defined as: liver biopsy consistent with a METAVIR fibrosis

score of F4 at any time prior to entry into the study; FibroScan greater than 12.5 kPa within 12 months of starting treatment; or an AST-to-platelet ratio greater than 2.0 and FibroTest greater than 0.75 within 12 months of starting treatment.

Overall, 42% (169/402) of participants in this analysis were treatment-naive and 58% (233/402) were treatment-experienced. The treatment-experienced participants included 34 participants from the C-SALVAGE study who had failed previous treatment with PEG-IFN/ribavirin plus a first-generation protease inhibitor. Overall, 54% had HCV GT1a infection and 39% had HCV GT1b infection. Sixty-four percent of participants had cirrhosis diagnosed through FibroScan, of whom 36% had values greater than 25.0 kPa. In total, 6% of participants had albumin levels of less than 3.5 g/dL and 25% had platelet counts lower than 100,000 cells/μL.

Among the treatment-naive population, SVR12 rates were 97.8% (135/138) in those treated with elbasvir (50 mg)/grazoprevir (100 mg) for 12 weeks and 90.3% (28/31) in those treated with elbasvir/grazoprevir with ribavirin for 16 or 18 weeks. In the treatment-experienced population receiving elbasvir/grazoprevir for 12 weeks, SVR12 rates were 88.9% (48/54), while among treatment-experienced participants treated for 16 or 18 weeks, SVR12 was achieved by 100% (49/49) and 93.9% (46/49) of those receiving elbasvir/grazoprevir with or without ribavirin, respectively. Subgroup analyses showed uniformly high rates of SVR12 across a broad spectrum of participants. SVR12 rates were high regardless of severity of cirrhosis, as indicated by the generally high response rates in patients with albumin levels less than 3.5 g/dL (96%, 24/25), platelets less than 100×10^3 cells/μL (90%, 91/101), and FibroScan values greater than 25.0 kPa (89%, 83/93). All 69 participants with HCV GT1b infection and 10 of 12 (83%) participants with GT4 infection who received elbasvir/grazoprevir for 12 weeks achieved SVR. In treatment-naive and treatment-experienced cirrhotic participants with HCV GT1a infection, SVR rates were 96.1% (73/76) and 88.6% (31/35), respectively.

5.2 HCV GT1a-Infected Patients

In the clinical trials of elbasvir/grazoprevir, rates of virologic failure tended to be higher among participants with HCV GT1a infection compared with those with GT1b infection when treatment with elbasvir (50 mg)/grazoprevir (100 mg) for 12 weeks was administered; however, comparable efficacy was observed across both genotypes in those receiving elbasvir (50 mg)/grazoprevir (100 mg) plus ribavirin for 16 weeks. In an analysis performed by the US Food and Drugs Administration, the presence of baseline NS5A RASs was identified as a predictor of lower efficacy in patients with HCV GT1a infection but not in those with GT1b or GT4 infection receiving elbasvir/grazoprevir for 12 weeks [29]. This analysis revealed that SVR12 rates were ~25% lower in treatment-naive participants with HCV GT1a infection and baseline NS5A RASs compared with those with wild-type virus at baseline. However, all participants with HCV GT1a infection who received elbasvir/grazoprevir with ribavirin for 16 weeks achieved SVR12, regardless of the

presence of baseline NS5A RASs. As a result, in the United States testing for variants associated with resistance to EBR is routinely performed prior to the initiation of treatment with elbasvir/grazoprevir in people with HCV GT1a infection. People with HCV GT1a wild-type virus at baseline receive elbasvir/grazoprevir for 12 weeks, and those with RASs at the NS5A positions 28, 30, 31, or 93 receive elbasvir/grazoprevir with ribavirin for 16 weeks [30]. Stratification according to the presence of baseline NS5A RASs assigns approximately 11% of patients with HCV GT1a infection to the extended 16-week elbasvir/grazoprevir plus ribavirin treatment regimen [31].

In the European Union, testing for RASs at baseline is not adopted as standard practice in the treatment of HCV infection, and therefore an alternative approach using baseline viral load is employed to identify people with HCV GT1a infection who would benefit from an extended treatment regimen. European guidelines recommend that patients with HCV GT1a infection and a baseline viral load of 800,000 IU/mL or less receive treatment with elbasvir/grazoprevir for 12 weeks and those with a baseline viral load of more than 800,000 IU/mL receive elbasvir/grazoprevir plus ribavirin for 16 weeks [32]. This recommendation is based on an analysis of 506 participants with HCV GT1a infection who received elbasvir/grazoprevir for 12 weeks in five elbasvir/grazoprevir clinical trials. This analysis showed numerically lower SVR12 rates with increasing viral load strata, and no virologic failures among those who received elbasvir/grazoprevir with ribavirin for 16 weeks [33]. Overall, this approach has a high positive predictive value (98.9% of those with low baseline viral load achieve SVR12) but a very low negative predictive value (only 7.3% of those with high baseline viral load failed to achieve SVR12), resulting in a relatively weak overall accuracy for this approach of 38.9%. In this analysis, 331 of 506 participants were categorized as having high baseline viral load, of whom 307 (93%) achieved SVR12 when treated with elbasvir/grazoprevir for 12 weeks. If those with high baseline viral load had been stratified to receive elbasvir/grazoprevir with ribavirin for 16 weeks based solely on their viral load, 61% (307 of 506) of the population would have been over-treated.

5.3 HCV GT1b-Infected Patients

A retrospective analysis of data from participants with chronic HCV GT1b infection enrolled in 11 phase 2/3 clinical trials was performed [34]. One thousand and seventy participants who received elbasvir (50 mg)/grazoprevir (100 mg) once daily for 12 weeks without ribavirin in 11 phase 2/3 clinical trials were included in this analysis. A high proportion (43%) of those enrolled were from Asian countries, including Japan, Taiwan, and South Korea, 16% were from the United States, and 8% were from Russia. Most (80%) participants were treatment-naive. Comorbidities among the enrolled population included compensated cirrhosis (18%), HIV co-infection (5%), CKD stage 4–5 (10%), and inherited blood disorders (4%). Overall, the SVR12 rate was 97.2% (1,040/1,070). Of the 30 participants who failed to attain SVR12, 15 experienced relapse and 15 had nonvirologic failure. Among participant

subgroups, SVR12 rates were high in those with compensated cirrhosis (188/189, 99.5%), HIV coinfection (51/54, 94.4%), and baseline viral load of more than 800,000 IU/mL (705/728, 96.8%). Resistance-associated substitutions at NS5A positions 28, 30, 31, or 93 were present in 21.6% of participants at baseline. SVR12 rates were 99.6% (820/823) in participants without baseline NS5A RASs and 94.7% (215/227) in those with baseline NS5A RASs. A total of 104 participants in this analysis had variants at the Y93 position (primarily Y93H), of whom 99 (95.2%) achieved SVR12. This integrated analysis demonstrates that elbasvir/grazoprevir for 12 weeks represents an effective treatment option for people with HCV GT1b infection, regardless of baseline viral load or the presence of baseline NS5A RASs. Pretreatment resistance testing in individuals with HCV GT1b infection is not required prior to initiation of treatment with elbasvir/grazoprevir for 12 weeks.

5.4 HCV GT4-Infected Patients

One hundred and fifty-five participants with HCV GT4 infection were enrolled in eight international clinical trials across the elbasvir/grazoprevir phase 2/3 clinical program [35]. Most participants in this analysis had HCV GT4a (47%) or 4d (41%) infection, and this was a primarily white (85%) and male (68%) population. Approximately 21% of the population had cirrhosis and 22% had HCV/HIV coinfection. In total, 111/117 (95.0%) of treatment-naive and treatment-experienced participants with GT4 infection achieved SVR12. Of the six participants who failed to achieve SVR12, three experienced relapse, two were lost to follow-up and one participant died. SVR12 rates were comparable in cirrhotic and noncirrhotic participants (91% vs 96%), those with baseline viral load of 800,000 or less and greater than 800,000 IU/mL (94% vs 95%), those with HCV monoinfection and HCV/HIV coinfection (94% vs 97%), and those with HCV GT4a, GT4d, or GT4-other infection (96% vs 94% vs 93%). NS5A RASs at positions 24, 28, 30, 31, 32, 38, 58, 92, or 93 were present in 42 of 114 (37%) participants who received elbasvir/grazoprevir for 12 weeks. SVR12 rate was 97.2% (41/42) in those with baseline NS5A RASs and 97.6% (70/72) in those with no baseline NS5A RASs. In the United States, elbasvir/grazoprevir for 12 weeks is a recommended treatment regimen for people with HCV GT4 infection, regardless of the presence of NS5A RASs or other baseline demographic characteristics.

5.5 Integrated Safety Analysis

A comprehensive integrated analysis of 1,690 participants who received elbasvir/grazoprevir with or without ribavirin in five phase 2 and three phase 3 clinical trials has also been reported (Table 3) [36]. This analysis included 1,033 participants who

Table 3 Integrated safety summary [36]

	EBR/GZR ($n = 1,033$)	EBR/GZR + RBV ($n = 657$)	Placebo ($n = 105$)
General safety overview			
≥1 AE	738 (71.4)	549 (83.6)	72 (68.6)
Fatigue	167 (16.2)	187 (28.5)	18 (17.1)
Headache	186 (18.0)	137 (20.9)	19 (18.1)
Nausea	82 (7.9)	100 (15.2)	8 (7.6)
Insomnia	42 (4.1)	71 (10.8)	6 (5.7)
Drug-related[a] AE	414 (40.1)	44 (67.6)	41 (39.0)
SAE	25 (2.4)	17 (2.6)	3 (2.9)
Drug-related SAE	1 (0.1)	3 (0.5)	0 (0.0)
Death	2 (0.2)	1 (0.2)	0 (0.0)
Discontinued[b] due to an AE	5 (0.5)	11 (1.7)	1 (1.0)
Discontinued due to a drug-related AE	3 (0.3)	5 (0.8)	1 (1.0)
Discontinued due to an SAE	1 (0.1)	2 (0.3)	0 (0.0)
Discontinued due to a drug-related SAE	0 (0.0)	0 (0.0)	0 (0.0)
Hepatic laboratory abnormalities			
ALT (IU/L)			
Grade 3: 5.1–10.0× ULN	11/1,033 (1.1)	3/656 (0.5)	9/105 (8.6)
Grade 4: >10.0× ULN	6/1,033 (0.6)	1/656 (0.2)	0/105 (0.0)
AST (IU/L)			
Grade 3: 5.1–10.0× ULN	6/1,033 (0.6)	1/656 (0.2)	2/105 (1.9)
Grade 4: >10.0× ULN	3/1,033 (0.3)	0/656 (0.0)	1/105 (1.0)
Total bilirubin[c]			
Grade 3: 2.5–5.0× ULN	3/1,033 (0.3)	37/656 (5.6)	0/105 (0.0)
Grade 4: >5.0× ULN	0/1,033 (0.0)	2/656 (0.3)	0/105 (0.0)

Every patient is counted a single time for each applicable row and column. A specific AE appears on this report only if its incidence in one or more columns meets the incidence criterion in the report title, after rounding

AE adverse event, *ALT* alanine transaminase, *AST* aspartate transaminase, *EBR* elbasvir, *GZR* grazoprevir, *IU* international unit, *RBV* ribavirin, *SAE* serious adverse event, *ULN* upper limit of normal

[a]Determined by the investigator

[b]Study medication withdrawn

[c]No patient had drug-induced liver injury; total bilirubin occurred early in the course of treatment

received elbasvir/grazoprevir alone and 657 who received elbasvir/grazoprevir plus ribavirin. A further 105 participants who received placebo for 12 weeks prior to deferred therapy with elbasvir/grazoprevir in the C-EDGE Treatment-Naive study were also included to provide a direct comparison of safety events. The analysis population included participants with compensated cirrhosis, HIV coinfection, prior treatment failure, and infection with HCV GT1–6.

In participants receiving elbasvir/grazoprevir, the most frequent adverse events were fatigue (71.4%), headache (16.2%), nausea (18.0%), and insomnia (4.1%).

Drug-related adverse events were reported in 40.1% of participants, and five participants discontinued treatment because of an adverse event (in three of these cases, the adverse event was considered related to study medication [ALT level increase, $n = 2$; anxiety, $n = 1$]). In this integrated population, three deaths occurred among participants receiving elbasvir/grazoprevir (post-appendectomy complication, $n = 1$; coronary artery disease, $n = 1$) or elbasvir/grazoprevir plus ribavirin (motor vehicle accident, $n = 1$). The overall safety profile of elbasvir/grazoprevir for 12 weeks was generally similar to that of placebo, with similar frequencies of drug-related adverse events (40.1% vs 39.0%), serious adverse events (2.4% vs 2.9%), and discontinuations due to adverse events (0.5% vs 1.0%).

During the phase 2/3 trials of elbasvir/grazoprevir, 13 of 1,690 (0.8%) participants experienced elevation of ALT levels above 5× ULN. These events occurred generally at or after treatment week 8 (mean onset, 10 weeks; range, 6–12 weeks) and were typically asymptomatic. Most late ALT elevations resolved with ongoing therapy or after completion of therapy; however, in three participants (0.2%), treatment was discontinued early. The incidence of late ALT elevations was not affected by treatment duration, and the presence of compensated cirrhosis was not a risk factor for late ALT elevations. Clinically significant elevations of bilirubin or changes in liver function were also not observed.

6 Summary

The objective of the elbasvir/grazoprevir clinical development program was to develop a well-tolerated, convenient, and simple regimen highly effective in clearing HCV infection. Following an extensive program of clinical trials encompassing a broad spectrum of participants with HCV infection, elbasvir/grazoprevir was approved for the treatment of people with HCV GT1 and GT4 infection. These studies showed consistently high rates of SVR of more than 90% in participants with HCV GT1 and GT4 infection, together with an acceptable safety profile. In addition, this clinical development program also provided unique insights into the management of several important HCV populations, including those with stage 4–5 CKD in the C-SURFER study and those receiving opioid agonist therapy in the CO-STAR study. Overall, all-oral DAA regimens have revolutionized the treatment of HCV infection, offering the hope of virologic cure to the vast majority of affected individuals. Elbasvir/grazoprevir represents an important all-oral DAA treatment option for many people with HCV infection, combining high rates of sustained virologic response with a well-established safety profile across a broad patient population.

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Compliance with Ethical Standards

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Conflict of Interest Drs Robertson and Barr are employees of, and hold stock in, Merck & Co., Inc., Kenilworth, NJ, USA.

Ethical Approval All studies were carried out in accordance with the Declaration of Helsinki, current guidelines on Good Clinical Practices and local ethical and legal requirements. For each study, independent institutional review boards or ethics committees reviewed and approved the protocol and applicable amendments.

Informed Consent In all studies, all participants gave written informed consent.

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Part IV
Impact of Curative Therapies

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Real-World Evidence and Hepatitis C

Michael W. Fried and David R. Nelson

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Abstract Direct-acting antiviral agents (DAAs) are the treatment of choice for patients with chronic hepatitis C. Their efficacy across diverse patient populations and safety among those with all stages of liver disease, including cirrhosis, have been repeatedly demonstrated in studies encompassing all classes of DAAs. Real-world evidence has confirmed that DAA therapies used in usual clinical practice achieved similar rates of sustained virological response when compared to those reported in rigorously controlled clinical trials. These data, developed from large cohort studies performed around the world, have instilled greater confidence in the management of patients with chronic hepatitis C using DAAs. Furthermore, real-world evidence contributed to better understanding the strengths and limitations of DAA treatment

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among unique populations of patients with chronic hepatitis C who were underrepresented in the original registration trials of these agents.

Keywords Hepatitis C, Observational studies, Real-world data, Real-world evidence

1 Introduction

Phase 3 clinical trials demonstrated the remarkable efficacy and safety of various classes of direct-acting antiviral agents (DAAs) for the treatment of chronic hepatitis C (CHC) [1–4]. Regardless of hepatitis C virus (HCV) genotype or level of disease severity (cirrhotic vs non-cirrhotic), all-oral DAA regimens achieve sustained virological responses in more than 90% of treated patients. These therapies were rapidly adopted, and many thousands of patients have been successfully treated since the first protease inhibitors, in combination with peginterferon and ribavirin, were approved in 2011 [5, 6]. As all-oral regimens debuted and utilization increased, additional questions arose regarding the safety and effectiveness in populations of patients that were less well studied in traditional phase 3 clinical trials. This chapter will discuss the important role played by real-world evidence in informing gaps in knowledge of safety and effectiveness across broad populations and in optimizing treatment for patients with hepatitis C in the era of direct-acting antiviral agents.

2 Identifying and Filling Knowledge Gaps for Approved HCV Therapies

Gaps in knowledge often exist between the evidence generated during clinical trials and the information needed for clinical practice, especially in the immediate period after medications are approved for general use [7–9]. Rigorous, controlled phase 3 clinical trials do provide the highest level of evidence regarding the safety and efficacy of new medications. However, these studies, specifically designed to achieve market authorization in the shortest time frame, generate clear answers to narrowly focused questions in selected populations [7–9]. Practicing clinicians, in contrast, are usually called upon to make treatment decisions in patients whose demographics or clinical status does not completely align with the patients who were enrolled in phase 3 trials. Thus, patients at the extremes of age, non-Caucasian race, those with more severe liver disease, patients in whom other medical comorbidities exist, and for which numerous concomitant medications are being administered were underrepresented or entirely excluded in the phase 3 registration trials of DAAs for HCV.

The eligibility criteria for the initial phase 3 trials of ledipasvir/sofosbuvir, for example, required participants to meet at least seven inclusion criteria, in addition to being within specified ranges for nine laboratory tests, and not meeting any of at least six exclusion criteria [2]. In usual clinical practice, the eligibility criteria of HCV treatment are many fewer: patient desire to be treated, a reasonable expectation that medications will be effective, the absence of absolute medical contraindications to the planned regimen, and access to medications. Thus, a much wider spectrum of patients are being treated for hepatitis C that is very different from the phase 3 trial populations upon which initial approval was granted.

Optimizing clinical use of new medications often requires additional information to be developed in the post-marketing period. Specific post-approval phase 4 studies could be designed to meet post-marketing requirements and expand the knowledge base around previously underrepresented populations, such as patients with cirrhosis. However, these studies are often plagued by delays in enrolling, high costs, and insufficient power to confidently answer the prespecified question and may be irrelevant by the time the studies are completed [7]. Numerous alternatives to traditional clinical trials exist and are becoming increasingly important as a source of "real-world" evidence to augment information derived from phased drug development programs.

3 FDA Commitment to Real-World Evidence

In December 2016, the 21st Century Cures Act was signed into US law with the goal of accelerating drug development by, among other things, innovating clinical trial design and clinical outcome measures. One key facet of 21st Century Cures Act required the "FDA to evaluate the use of real world evidence to help support the approval of a new indication for a previously approved drug and to help support or satisfy post-approval study requirements" [10]. Furthermore, "By no later than the end of FY 2021, FDA will publish draft guidance on how RWE can contribute to the assessment of safety and effectiveness in regulatory submissions, for example in the approval of new supplemental indications and for the fulfillment of post-marketing commitments and requirements" [10].

4 Sources of Real-World Evidence

Real-world data (RWD) can be derived from a wide range of sources, including information gathered from medical and pharmacy claims, electronic health records, pharmacy data, electronic health devices, social media, and prospective observational registry data [11]. Real-world evidence (RWE) is the clinical evidence derived from the analysis of RWD [11]. RWE can contribute to all phases of drug development by defining the natural history of disease, identifying medical comorbid

conditions that could impact a product profile, characterizing current practice patterns, and quantifying risks and benefits in certain subpopulations [7, 11]. In the post-marketing period, real-world evidence has provided important insights into the safety of new drugs in diverse populations and has supported new indications for previously approved medications [11].

5 HCV-TARGET and Other Real-World Cohorts

Multiple prospective, longitudinal observational registries were initiated shortly after the approvals of the first oral protease inhibitors, circa 2011. Nearly every continent has contributed important real-world evidence demonstrating the safety and effectiveness of DAAs for the treatment of hepatitis C across diverse populations (Fig. 1). To date, these registries have cumulatively enrolled tens of thousands of patients whose insights have had a substantial impact on optimal management for patients with hepatitis C.

HCV-TARGET (Hepatitis C Therapeutic Registry and Research Network) was established as an academic collaboration between the University of Florida (David R. Nelson, PI) and University of North Carolina (Michael W. Fried, PI) to better understand the impact of new therapies on the management and long-term outcomes of patients with hepatitis C. It was evident that there were many unanswered questions as these new classes of drugs were increasingly utilized in populations that were different from those studied in phase 3 registration trials. Thus, patients with cirrhosis (compensated and decompensated), African American race, elderly populations, and those with many comorbid medical conditions were being treated with DAA regimens despite a paucity of clinical data regarding safety and effectiveness in these populations.

Fig. 1 Real-world cohorts from around the globe have provided RWE regarding the safety and effectiveness of DAAs

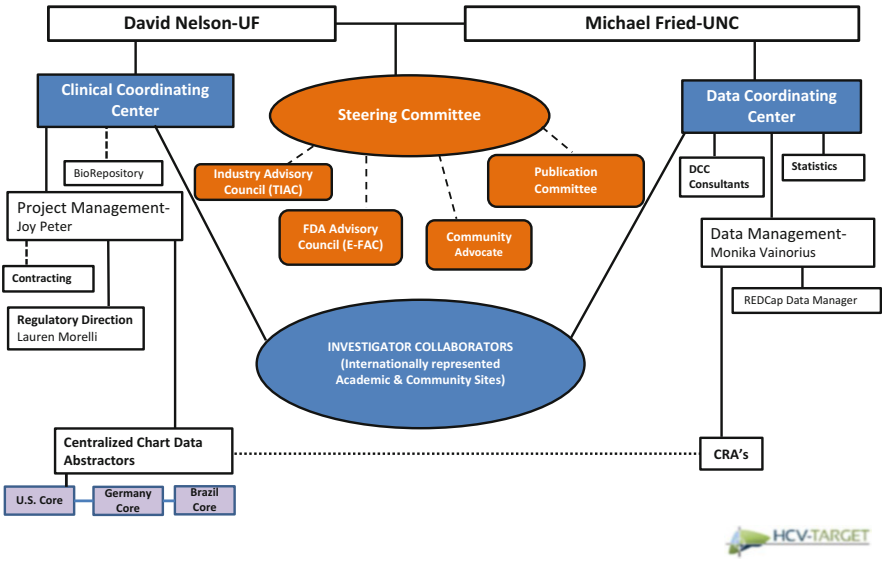

Fig. 2 Organizational structure of HCV-TARGET

HCV-TARGET is a unique collaboration between academia, industry, and community working together to fill in knowledge gaps about the rapidly evolving HCV treatment landscape (Fig. 2). A memo of understanding with the FDA (MOU 225-13-0012) was executed in 2013 which allowed members of the Division of Antiviral Products to participate in HCV-TARGET steering committee meetings, query the database, and exchange scientific insights with the network [12]. HCV-TARGET is led by an academic steering committee composed of global experts in viral hepatitis (Fig. 2). The steering committee works closely with the industry advisory board to establish the research agenda, implement policies, and plan for abstracts, presentations, and manuscripts that served to disseminate important clinical findings to the scientific community.

HCV-TARGET has focused on data quality with a REDCAP-based data platform that was compliant with 21CFR part 11 standards for electronic data capture, met CDISC standards compatible for data exchange, and incorporated industry recognized WhoDrug coding for concomitant medications and MEDDRA for classifying adverse events. Furthermore, HCV-TARGET utilizes a novel data capture process whereby sites upload the entire redacted health record (structured and unstructured data, lab and x-ray reports, telephone messages, biopsy results) from consented patients which is then abstracted and entered into the database by a team of trained abstractors. This centralized method minimized the burden to sites and allowed for greater consistency of data entry than traditional distributive models that relies on individual sites and variably experienced study staff completing case report forms. HCV-TARGET also employed an independent monitoring core that compared source documents with database entries using a risk-based strategy for key outcome variables.

The HCV-TARGET consortium includes over 60 sites throughout the United States, Germany, and Israel and enrolled over 12,000 patients treated with every generation of DAA medications. The unique rolling design allowed for rapid acquisition of data as new medications were approved and began to be utilized in usual clinical practice. Evidence generated from HCV-TARGET informed treatment guidelines for AASLD, EASL, and the World Health Organization [13, 14].

Other important RWD cohorts include

1. the French cohort ANRS C022 HEPATHER, a national 32-center prospective observational cohort that included up to 15,000 HCV-infected patients and was established to identify prognosis factors, including response to treatment and long-term impact of viral clearance. Demographic and history of liver disease were collected at entry into the cohort. Clinical, adverse events, and virological data were collected throughout treatment and posttreatment follow-up [15].
2. The Veterans Affairs Healthcare System, which includes 167 medical centers and 875 ambulatory care and community outpatient clinics throughout the United States [16]. It is the largest integrated healthcare provider for HCV-infected patients in the United States, with over 175,000 pts diagnosed with HCV infection in VA care in 2014. The VA utilizes electronic medical records and electronic clinical data, and HCV treatment regimens are collected into the VA Corporate Data Warehouse, a national repository of data from VA's computerized patient records. Data extracted includes all pt pharmacy prescriptions, demographic characteristics, inpatient and outpatient visits, problem lists, procedures vital signs, diagnostic tests, and laboratory tests.
3. German Hepatitis C Cohort (GECCO), which is a multicenter prospective database from 9 German HCV treatment centers [17];
4. TRIO Health Cohort, which comprises patients treated in approximately 500 community and academic practices affiliated with the TRIO Health Innovation Platform [18]. Baseline information as well as outcomes data are collected through both specialty pharmacies and clinicians, allowing the evaluation of concomitant medications and the evaluation of compliance using pharmacy dispense data; however no safety data are collected; and
5. United Kingdom cohort, comprising 10,184 patents with a history of HCV infection enrolled through attendance at one of 56 UK HCV clinics between 2012 and 2013 [19].

6 Real-World Evidence Finds an Important Safety Signal in Patients with Cirrhosis

The approval of the first-generation HCV protease inhibitors, telaprevir and boceprevir in combination with peginterferon and ribavirin, began the transformation of HCV therapies and served as an important interim step toward the development of all-oral regimens. The registration trial treated 363 patients with 12 weeks of peginterferon, ribavirin, and telaprevir and yielded a remarkable 75% sustained

virological response, compared to only 44% in those treated with peginterferon and ribavirin alone [20]. However, there was evidence of an altered safety profile in patients treated with triple therapies. Most evident was the increased frequency of anemia in patients treated in the triple therapy arm compared to peginterferon/ribavirin, 37% vs 19%, respectively, with 5% of patients requiring blood transfusion. Of note, only 6% of patients enrolled in the registration trial had evidence of cirrhosis [20].

Perhaps the earliest demonstration of the importance of real-world evidence to inform HCV therapy was the French CUPIC study, an open-label, real-world early access protocol that enrolled over 600 cirrhotic patients treated with triple therapy (telaprevir or boceprevir) [21]. Among the patients treated with telaprevir-based therapy, 23% discontinued treatment due to adverse events. Thirty-one percent of patients developed treatment-emergent anemia (hemoglobin <9.0 mg/dL), including 54% who received RBC growth factors and 16% blood transfusions. Thus, the safety signal of anemia was greatly amplified in patients with cirrhosis, leading to immediate changes in clinical practice with more vigilant monitoring as well as early and rapid dose reductions in ribavirin to mitigate development of anemia [21].

In the US cohort, HCV-TARGET similarly demonstrated that patients treated with these first-generation protease inhibitors had high rates of advanced disease (38%), had lower SVR rates, and were more likely to experience significant adverse events compared to patients in registrational trials [22]. The lower SVR rates in HCV-TARGET were likely explained by the high proportion of patients with cirrhosis and African American race, factors that have been associated with a lower SVR with interferon-based therapies [12, 22].

7 RWE Contributes to the Approval of the First All-Oral DAA Regimen Commonly Prescribed

The near simultaneous approval in 2013 of two triple therapy regimens (sofosbuvir + peginterferon/ribavirin and simeprevir + peginterferon/ribavirin) set the stage for the first commercially available, but unapproved, all-oral regimen for the treatment of hepatitis C [23, 24]. Simeprevir, a first-generation protease inhibitor with once daily administration and a better safety profile than earlier HCV protease inhibitors, was immediately an attractive candidate to be combined with the nucleoside analogue, sofosbuvir. Indeed, a small phase 2 study treated 167 patients with the combination of simeprevir and sofosbuvir yielding SVR in over 90% of patients with negligible side effects [25]. These encouraging results provided reassurance that combining two classes of DAAs and shedding peginterferon and ribavirin was a viable alternative to triple therapy regimens, and simeprevir plus sofosbuvir became the first commonly used all-oral regimen.

This seismic shift in HCV treatment paradigms was immediately captured across multiple ongoing real-world cohorts, and evidence was rapidly developed regarding the safety and effectiveness of this treatment regimen that was routinely being utilized in an “off-label” manner. Between 2014 and 2015, HCV-TARGET enrolled

~1,400 patients treated with simeprevir/sofosbuvir. Sulkowski and colleagues reported the final results of more than 800 genotype 1 patients treated in the HCV-TARGET prospective observational study [26]. The study included treatment-naïve or treatment-experienced, cirrhotic and non-cirrhotic patients. Overall SVR was 88% and was higher in non-cirrhotic vs cirrhotic patients (94% vs 84%, respectively). The regimen was also demonstrated to be safe with only 2% discontinuing treatment prematurely due to adverse events [26]. Several other real-world cohorts provided additional evidence regarding the effectiveness of simeprevir and sofosbuvir [27].

When the manufacturer of simeprevir submitted an efficacy supplement to the FDA to support the use of this combination based on the prior phase 2 results, the sponsor also included a robust dossier of safety and effectiveness data from HCV-TARGET in support of this application, which was ultimately approved [12]. Interestingly, the HCV-TARGET results that had been generated in real-world settings were comparable to subsequent phase 3 confirmatory trials generated by the sponsor to fulfill specific post-marketing commitments [12, 28, 29].

8 RWE Confirms Safety and Effectiveness of DAA Regimens

In late 2014, a single tablet regimen, ledipasvir/sofosbuvir, was approved for treatment of HCV based on the remarkable results of several phase 3 trials. In treatment-naïve patients, SVR rates ranged from 97–99% to 94–99% in treatment-experienced patients [1, 2]. Moreover, efficacy was demonstrated across a wide spectrum of patients who previously had lower response rates with interferon-based medications, such as those with cirrhosis and African American patients. With nearly 1,300 patients enrolled, confidence intervals for most subpopulations were quite small with rare exceptions. However, only 181 patients with cirrhosis were included which comprised approximately 14% of the study population (in contrast to a 40–50% prevalence of cirrhosis in patients being treated in real-world cohorts) [1, 2].

Numerous real-world cohorts quickly augmented, and largely confirmed, the results of these phase 3 clinical trials (Table 1). Sustained virological response rates in a per protocol analysis for patients with cirrhosis treated with ledipasvir/sofosbuvir were 94% in the HCV-TARGET study ($n = 677$) and 92% in the TRIO cohort [18, 39]. Similar results were obtained from the Veterans Administration cohort and a large number of international cohorts [30, 40]. In an ongoing study from the German Hepatitis C Registry, 93/96 patients (97%) treated with glecaprevir/pibrentasvir, one of the newest DAA regimens, achieved SVR without any virological failures reinforcing the real-world effectiveness of this regimen [41].

In addition to safety and efficacy, RWE has subsequently shown improved clinical outcomes from DAA therapy in cirrhotic HCV-infected patients. In a comparison between 6,460 patients who received DAA vs 2,835 who did not receive a DAA, DAA use was associated with a decrease in deaths (HR 0.65; more pronounced for liver-related deaths) and no increased risk of HCC and hepatic

Table 1 Efficacy of DAA regimens in real-world cohorts (from [30])

Cohort (ref)	Regimen	No. of patients (treatment-naive/ treatment-experienced/ cirrhosis)	SVR treatment naïve (%)	SVR treatment experienced (%)	SVR cirrhosis (%)
Genotype 1 patients					
Target [31]					
	LDV/SOF 8 weeks	154/–/–	97	NR	NR
	LDV/SOF 12 weeks	627/–/239	97	NA	96
Trio [32, 33]					
	LDV/SOF 8 weeks	263/–/–	95	NR	NR
	LDV/SOF 12 weeks	632/–/121	95	NA	84
	LDV/SOF 24 weeks	–/–/329	NA	NA	92
VA [7]					
	LDV/SOF 8 weeks	2,027/–/–	94	NR	NR
	LDV/SOF 12 weeks	2,899/933/925	95	96	92
	LDV/SOF 24 weeks	141/479/473	92	95	93
Hepather [34]					
	SOF/ DCV ± RBV 12 weeks	66/82/118	89	91	90
	SOF/ DCV ± RBV 24 weeks	59/349/442	88	97	96
HCV Research UK Registry/Expended Access Program [12]					
	SOF/DCV/ RBV 12 weeks	–/–/30	NA	NA	88
	LOV/ SOF + RBV 12 weeks	–/–/136	NA	NA	91
Israel Cohort [35]					
	PrOD ± RBV	–/–/253	NA	NA	99
German Cohort Register [36]					
	PrOD ± RBV	208/322/252	96	97	95
Genotype 2 or 3 patients					
Target G2 [37]					
	SOF/RBV 12–16 weeks	198/97/80	89	84	80

(continued)

Table 1 (continued)

Cohort (ref)	Regimen	No. of patients (treatment-naive/ treatment-experienced/ cirrhosis)	SVR treatment naïve (%)	SVR treatment experienced (%)	SVR cirrhosis (%)
VA G2 [7]					
	SOF/RBV 12 weeks	1,910	88	80	77
VA G3 [7]					
	SOF/RBV 24 weeks	630	75	61	62
	LDV/ SOF + RBV 12 weeks	344	78	77	65
French multicenter compassionate use program G3 [38]					
	SOF/ DCV ± RBV 12 weeks	–/–/37	NA	NA	73
	SOF/ DCV ± RBV 24 weeks	–/–/183	NA	NA	85
HCV Research UK Registry/Expended Access Program genotype 3 [12]					
	SOF/ DCV + RBV 12 weeks	–/–/75	NA	NA	71
	LDV/ SOF + RBV 12 weeks	–/–/37	NA	NA	65

decompensation [42]. The US Veterans Affairs Healthcare System analysis of 62,354 patients who initiated antiviral therapy found that SVR was associated with significantly decreased HCC risk in multivariable models, irrespective as to whether the antiviral treatment was IFN-based (HR 0.32) or IFN-free (HR 0.29) [43].

9 RWE Reassures and Refines Criteria for Shortened Treatment Duration

The ION-3 trial of ledipasvir/sofosbuvir randomized treatment-naïve, non-cirrhotic patients to either standard 12 weeks of treatment or an abbreviated 8-week regimen [44]. SVR rates were similar for both groups 93% with 8 weeks of treatment and 95% with 12 weeks of treatment [44]. Post hoc analysis completed by FDA and study sponsors demonstrated that the relapse rate varied by the pretreatment level of HCV RNA. Among those with HCV RNA <6 million IU, the relapse rate was 2% in

both the 12-week and 8-week treatment arms. However, for patients with HCV RNA >6 million IU at the start of treatment, the relapse rate was 1% for those treated with 12 weeks duration but increased substantially to 10% for those treated with the shortened 8-week regimen [12]. Thus, the initial label for ledipasvir/sofosbuvir included the following language: "LDV/SOF for 8 weeks can be ***considered*** in treatment-naïve genotype 1 patients without cirrhosis who have pre-treatment HCV RNA less than 6 million IU/mL."

Despite the remarkable effectiveness in both arms of the study and the relapse rate that could be mitigated by stratifying by viral load, clinicians remained concerned that the body of evidence was insufficient and that some patients would be disadvantaged by a shorter treatment duration. Payers, already balking at the high cost of this medication at the time of its introduction, usually *mandated* that patients who met the above criteria be treated for 8 weeks rather than 12 weeks as a cost-saving measure [12]. Clinicians countered that in the absence of any compelling safety signal shortening treatment duration was unnecessary. These competing interests, cost vs perceived optimized patient care, created great tension and clinicians regularly appealed denials for 12 weeks duration of therapy (M. Fried, personal communication).

Real-world evidence helped to reassure clinicians that patients could be considered for 8 weeks of treatment without sacrificing outcomes. Terrault and colleagues in HCV-TARGET analyzed patients who met the criteria for 8 weeks of treatment but received either 8 or 12 weeks based on patient choice, physician choice, or payer factors. Among 586 patients who *qualified* for 8 weeks of therapy (treatment-naïve, non-cirrhotic, with baseline HCV RNA <6 million IU) but *actually received either* 8 weeks or 12 weeks of treatment, SVR was 96% and 98%, respectively, further instilling confidence in the abbreviated regimen [39]. A similar analysis in the TRIO cohort demonstrated that 98% (95% CI 96.8–99.1) of patients treated with 8 weeks of ledipasvir/sofosbuvir achieved SVR, which was further supported by an accompanying meta-analysis.

The VA cohort further refined the criteria for shortening therapy with the ledipasvir/sofosbuvir regimen. Overall sustained virological response rates for patients treated with a variety of all-oral DDA regimens generally paralleled those in registration trials [40]. Furthermore, no significant difference was found between white and black patients treated with these regimens [45]. However, African American patients treated for 8 weeks with LDV/SOF had lower SVR (93%) compared to whites with the same baseline characteristics (96%). This suggestion that African American patients may be disadvantaged by a shorter duration of therapy was adopted by the HCVguidelines.org who recommended against the abbreviated regimen for African American patients.

10 RWE Characterizes Impact of Proton Pump Inhibitors on Outcomes

Phase 1 studies of ledipasvir indicated that bioavailability was reduced during coadministration with H2 blockers and proton pump inhibitors (ledipasvir has a pH-dependent solubility whereby it is essentially insoluble when pH $\geq$ 4) and original US labeling for ledipasvir/sofosbuvir suggested limiting exposure to concurrent acid-reducing agents during the course of therapy [46]. It was unknown whether this interaction was a clinically significant effect in light of the stellar cure rates in most patients treated with these agents in phase 3 trials where exposure to acid-reducing medications had been limited. Terrault and colleagues performed the most detailed investigation of this issue in over 1,700 patients treated with LDV/SOF regimen. The unadjusted SVR was modestly lower in patients who took PPIs (94%) compared to those who did not take PPIs (97%) [39]. It was recognized that numerous factors besides PPI use could impact SVR in this nonrandomized real-world cohort and, therefore, a rigorous secondary analysis incorporating inverse probability weighting was performed. Despite very few overall failures, any PPI use, PPI use at beginning of therapy, PPI use >20 mg daily, as well as twice daily PPI use remained significantly associated with treatment failure [39]. A similar analysis in the TRIO cohort confirmed that high-dose PPI was associated with lower SVR, although lower doses of PPI did not impact treatment response [47].

11 RWE Contributes to Safety and Efficacy Profile of DAAs in Unique Populations

As reported in multiple phase 3 clinical trials, DAAs have outstanding safety profiles, highlighted by treatment discontinuation rate for adverse events below 1% for non-cirrhotic populations [1, 2, 4, 48]. Given this favorable safety profile of DAAs, there has been dramatic expansion of HCV treatment into populations of patients that have been historically underserved by previous interferon-based regimens: chronic kidney disease, liver/kidney transplant, decompensated cirrhosis, and HCV/HIV co-infected patients.

Chronic Kidney Disease The large registration trials of DAAs for HCV infection have generally excluded patients with significant renal impairment, with the exception of glecaprevir-pibrentasvir [49, 50]. Nevertheless, the available evidence suggests that patients with renal impairment can expect a virological response rate to a given regimen similar to that observed in the general population, as long as the regimen is tolerated. In an international cohort study of patients treated with DAA-based regimens in real-world settings, SVR rates were similar, among patients across all eGFR spectrums (<30, 31–45, 46–60, and >60 mL/min per 1.73 m^2) [51]. In an observational VA cohort study of almost 14,000 persons treated with a

ledipasvir-sofosbuvir regimen, SVR for those with stage 3 CKD who completed treatment was 97%, while those with stage 4–5 was 94% [52].

Transplantation HCV is a common comorbidity in patients who have undergone kidney and/or liver transplantation and is associated with increased morbidity and mortality compared with recipients who do not have chronic HCV infection. Recent reports in the literature from clinical trials and real-world cohorts demonstrate that direct-acting antiviral therapies effectively cured HCV liver and kidney transplant recipients (>95%); the majority were treated with sofosbuvir-based regimens [53]. Smaller numbers of transplant recipients have been treated with paritaprevir-ritonavir, ombitasvir and dasabuvir, elbasvir-grazoprevir, or glecaprevir-pibrentasvir with excellent success [54, 55]. DAA therapies were well tolerated and did not increase the rate of acute rejection. For example, the HCV-TARGET cohort study evaluated 347 liver, 60 kidney, and 36 dual liver kidney transplant recipients [56]. Among the 279 participants treated with ledipasvir/sofosbuvir for 12 weeks or 24 weeks, the SVR rates were 97% for those also taking ribavirin and 95% for patients not taking ribavirin. The rate of therapy discontinuation due to an adverse event was 1.3%, highlighting the safety of the drug combination. Acute graft rejection occurred in only 1.4% of patients and serve to remind clinicians of the need to monitor immunosuppressive agent levels during DAA therapy [56].

Decompensated Cirrhosis Clinical trial data indicate that persons with decompensated cirrhosis who receive DAAs have high rates of SVR and that SVR can lead to improvement in clinical and biochemical indicators of liver disease, including patients with CTP class C cirrhosis [57–59]. Both the UK and HCV-TARGET observational cohorts evaluated decompensated cirrhotic patients treated with sofosbuvir/ledipasvir +/− ribavirin and showed high SVR (86–90%) and relatively low rates of treatment-related adverse events [39, 60, 61]. Furthermore, the predictors of improvement or decline in liver disease are now being evaluated in observational cohorts, though patients with Model for End-Stage Liver Disease (MELD) score of >20 or severe portal HTN complications (ascites, encephalopathy) may be less likely to improve and are potentially better served by transplantation than HCV treatment [54, 61].

HIV Coinfection The introduction of DAAs has changed the landscape of therapy for persons with HCV and HIV coinfection. Several studies using DAA-based therapy have demonstrated SVR rates among individuals with HCV-HIV coinfection that are comparable to those with HCV monoinfection, providing convincing evidence that persons with HCV-HIV coinfection no longer require the designation of a "special" population. It should be noted that these trial participants in registration trials included primarily individuals without cirrhosis and those with CD4 counts usually well above 200 cells/mm^3 [62, 63]. Several observational cohort studies have shown comparable clinical efficacy in more heterogeneous cohorts of persons with HCV and HIV coinfection, including those with lower CD4 cell counts [64, 65].

12 Summary

Direct-acting antiviral agents have demonstrated remarkable rates of cure of hepatitis C infection across all patient populations studied in phase 3 clinical trials. Real-world evidence derived from global cohorts evaluating the safety and effectiveness of DAAs in usual clinical practice generally paralleled those impressive results. RWE contributed to optimizing treatment paradigms when gaps in knowledge existed and in expanding utilization to populations underrepresented in registrational trials.

Compliance with Ethical Standards

Conflict of Interest: MWF received research grants to his institution from AbbVie, BMS, Gilead, Merck. He serves as unpaid consultant to AbbVie, BMS, Merck, and TARGET PharmaSolutions. Stock in TARGET PharmaSolutions is held in an independent blind trust.

DRN received research support from AbbVie, BMS, Gilead, and Merck paid to his institution and is a stockholder of TARGET PharmaSolutions.

Ethical Approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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The Benefit of Direct-Acting Antiviral HCV Cure Therapies

Paul J. Pockros

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Abstract Although more than 600,000 patients in the USA have now been cured of HCV, the clinical benefits of cure have been seen primarily in patients with more advanced liver disease. This has resulted in a reduced incidence of liver cancers and reduced liver-related mortality. Benefits of cure in patients without cirrhosis have not been seen yet in systemic review, but are likely to be seen in the future years. There remain unresolved issues regarding patients with advanced fibrosis or cirrhosis who are cured of their HCV. It is not clear if fibrosis reverses after cure in everyone, if so by how much, and for how long patients with cirrhosis need to be monitored after cure. We have shown that 62% had improved liver stiffness measured by transient elastography (TE) that was consistent with regression of at least one stage of fibrosis over 1 year. Fifteen patients with matched liver biopsies prior to SVR underwent a biopsy after SVR. However, the post-SVR liver biopsies of only 4 patients showed

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F1–F2, while 11 patients still showed F3–F4, indicating that TE improvements are overstated when compared to histologic staging and that patients with cirrhosis before DAA therapy need to be monitored for hepatocellular carcinoma after cure.

Keywords Chronic hepatitis C, Cirrhosis regression, Hepatocellular carcinoma, Morphometry, Transient elastography

Abbreviations

DAA	Direct-acting antiviral therapy
F3	Advanced fibrosis
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
kPa	Kilopascals
SVR	Sustained virologic response
TE	Vibration-controlled transient elastography

1 Introduction

More than one million patients with HCV worldwide and over 600,000 patients in the USA have now been cured of HCV infection using DAA therapies. In our own hepatology unit at Scripps Clinic in La Jolla, California, we have successfully cured over 150 patients in clinical trials using DAAs and over 1,000 patients since the approval of the first DAA regimen in 2011. Between 2011 and 2013, we needed to treat 156 patients in order to achieve an SVR in 107 of them, with an SVR rate of less than 70%. In retrospect, these were low rates of cure but represented an incremental improvement over Pegylated interferon (PegIFN) and ribavirin (RBV) regimens which had been in use since 2000. Besides these relatively low SVR rates, the toxicities of the first-line protease inhibitors telaprevir and boceprevir were an extreme challenge to patients and their physicians.

Once combination regimens using sofosbuvir became available in 2014, we witnessed cure rates at or above 95% with minimal side effects and we were successful in treating and curing over 250 patients per year in our practice. The number of patients we treated began to decline in 2016 and has now been reduced to $<$100 cases annually, as most of our population with HCV have now been cured, transplanted, or died in earlier years. The benefit of cure for these patients has been a remarkable achievement to witness for any provider who has been entrusted with their care. The gains in quality of life, reduced healthcare expenses, reduced hospitalizations, and reduced mortality that cure has given to these patients are incalculable. I would never try to estimate such benefits that have now been shared by more than one million patients.

An evidence-based analysis of literature done by the Cochrane Systematic Review group in 2017 concluded there were no clinical benefits of DAA therapies for chronic HCV [1]. This review was subsequently refuted by a number of societies and physicians as being incorrect [2]. I believe the reason for this discrepancy is relatively simple to explain. The clinical benefits of cure seen thus far have been primarily in patients with more advanced liver disease. A significant number of decompensated HCV patients have now been delisted for transplantation, and there is a reduced incidence of HCV-associated liver cancers as well as reduced liver-related mortality. However, the true benefit of DAAs and cure will likely be seen in the future years and has not been fully realized this soon after the availability of DAAs. The most likely reason why the Cochrane analysis in 2017 concluded there was no evidence of clinical benefit of DAAs is that they were premature in their review. Further, they could only utilize evidence-based literature for their analysis. It may take another 5 years before there are significant clinical benefits seen in the evidence-based literature.

Nonetheless, we are now seeing publications that demonstrate curative therapy has markedly reduced the number of patients listed for transplantation in the USA for HCV [3, 4] (Fig. 1). Recent modeling studies have shown a reduction in the expected death rates in decompensated HCV cirrhosis attributed to DAA therapies [5] (Fig. 2). At our own liver center in Southern California, we have rarely transplanted a patient for decompensated HCV cirrhosis in the last 2 years. This is a remarkable change from the last two decades when decompensated HCV cirrhosis was the major indication for transplant in our center. Nonalcoholic steatohepatitis (NASH) and alcoholic liver disease are now quickly becoming the most common indications for liver transplantation in the USA [3]. SVR is conferring a reduced mortality not just from liver-related deaths but also from cardiovascular-related deaths [6] (Fig. 3a, b). Prior studies have demonstrated a reduced all-cause mortality in HCV patients who

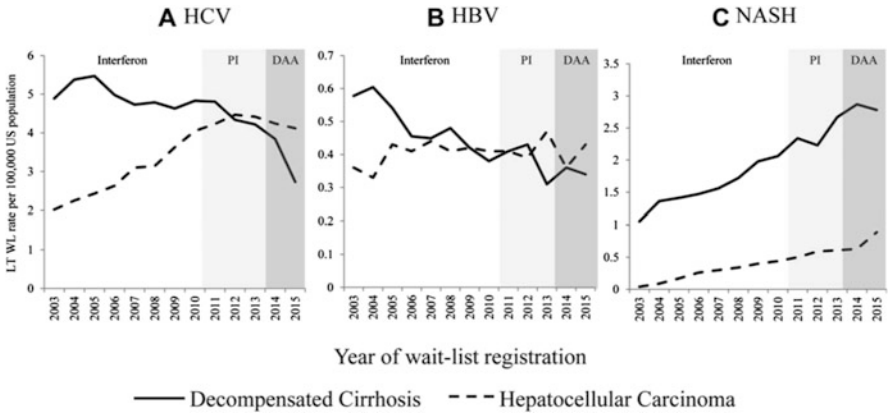

Fig. 1 Annual standardized incidence rates (ASIR) of LT wait-listing per 100,000 US population by etiology of liver disease and indication for wait-listing. *X*-axis is the year of LT wait-listing registration. *PI* protease inhibitor, *DAA* direct-acting antiviral [3]. (**a**) HCV. (**b**) HBV. (**c**) NASH

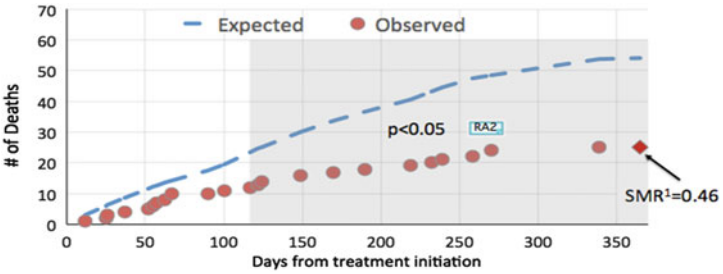

Fig. 2 Observed versus expected deaths in DAA-treated patients [5]. 54 deaths expected from survival model. [1]Standardized mortality ratio

were cured with IFN-based regimens, so this is not surprising data [7, 8]. We can expect to eventually see data that shows a reduction in mortality due to non-liver-related cancers as well [9].

There is a remaining issue that confronts a significant number of these patients, especially those who had advanced fibrosis or cirrhosis (METAVIR F3/F4), that is, how long do they require monitoring for complications of portal hypertension (esophageal varices) and HCC? Fibrosis stage and liver stiffness are predictors of adverse outcomes in chronic HCV, but it is not clear if fibrosis reverses after cure in everyone, and if so by how much, and whether their risk for liver cancer is reduced to a level that is safe to stop screening. A comprehensive study utilizing the Veterans Affairs Hospital database in 22,500 patients has now demonstrated a reduced risk for HCC following SVR achieved by DAAs in patients with F3/F4 at the beginning of treatment [10] (Fig. 4). However, this risk has been reduced to 0.5–1.0% per year range but not to zero. Further, patients who start therapy with decompensated cirrhosis do not seem to reduce their HCC risk below 2.5% annually. Neither of these populations can stop their screening according to the American Association for the Study of Liver Disease (AASLD) guidelines [11].

Healthcare providers and patients thus have no guidance as to when to discontinue monitoring for hepatocellular carcinoma and complications from portal hypertension. The most recent estimate of the number of patients that fall into this category is 25–37% of all HCV-infected individuals in the USA [12]. Therefore, this is not a trivial concern as it has significant clinical impact on 200,000 or more successfully cured patients.

Vibration-controlled transient elastography (TE) has been shown to accurately detect advanced fibrosis and cirrhosis in patients with chronic hepatitis C virus (HCV) infections [13]. However, the use of TE is limited by active inflammation and/or edema of the liver, which can cause overestimation of the degree of fibrosis [14]. Other factors known to confound TE include obesity, waist circumference, ascites, hepatic congestion, extrahepatic cholestasis, and eating within 4 h of the exam [15]. Despite these limitations, TE offers a simple and rapid bedside assessment of fibrosis for many patients.

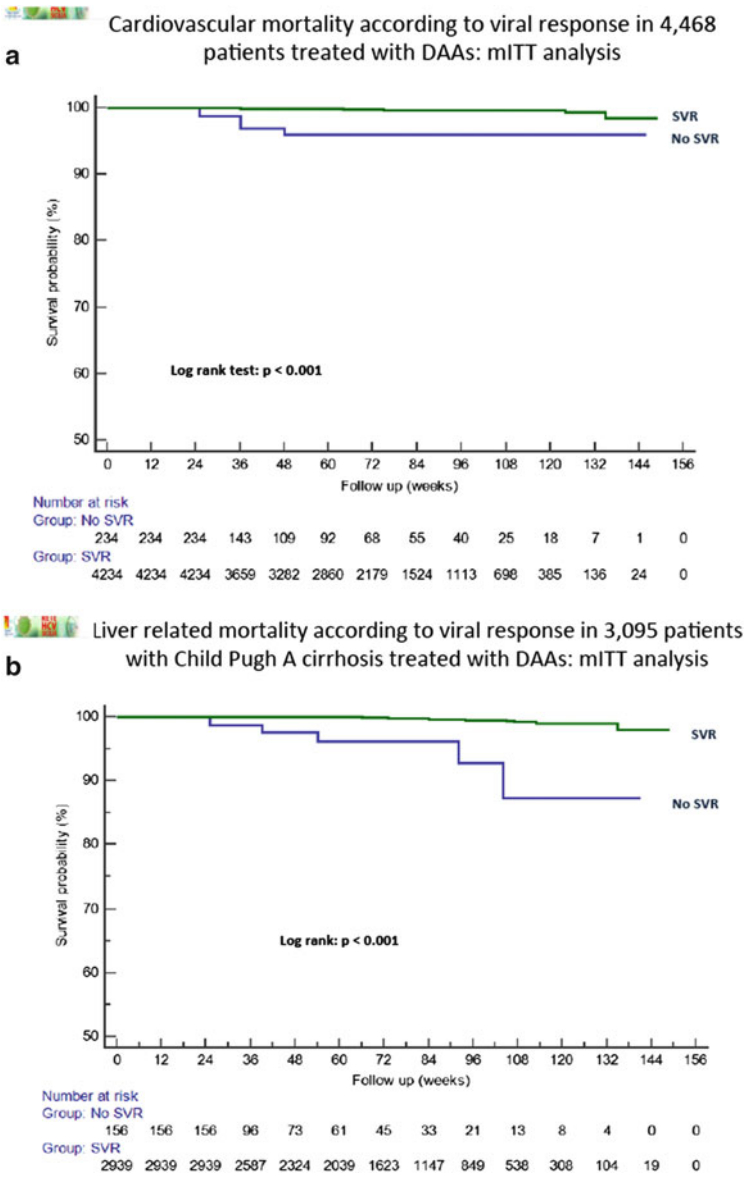

Fig. 3 (**a**, **b**) Disease outcomes after DAA-induced SVR: data from the resist-HCV cohort [6]

Multiple reports have demonstrated that novel direct-acting antiviral (DAA) therapies for HCV result in dramatic improvement in liver stiffness measured by TE in patients with sustained virologic response (SVR) [16–19]. Improvements in liver stiffness can be observed as early as the end of treatment and continue even 12 months after therapy completion, with liver stiffness improvements ranging from

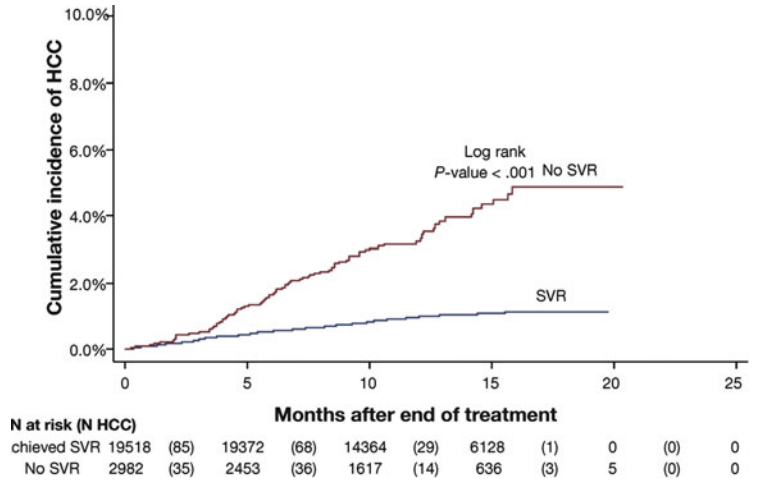

Fig. 4 Cumulative incidence of HCC among 22,500 patients treated with DAA agents [10]. *SVR* sustained virological response

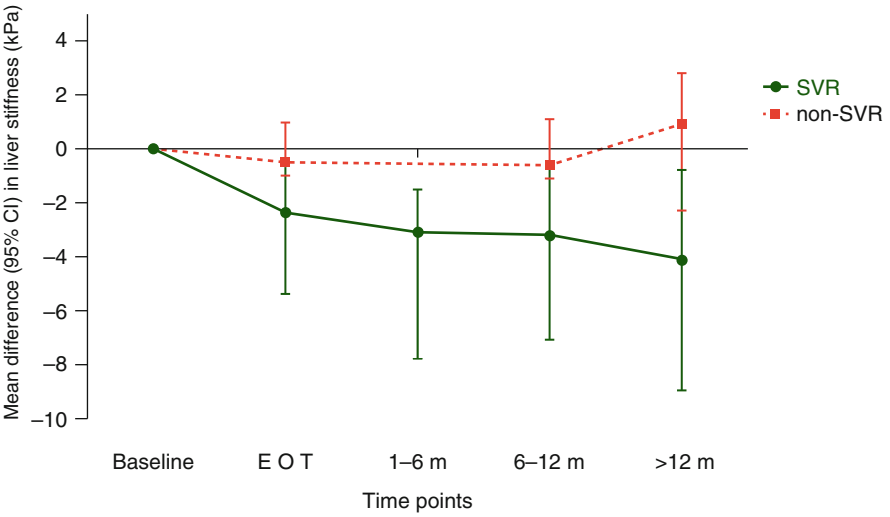

Fig. 5 Difference in liver stiffness at different time points in patients who achieve SVR vs. those who do not achieve SVR, demonstrating net decline in liver stiffness [16]

−2 to −10 kilopascals (kPa) [16] (Fig. 5). This decrease in liver stiffness is associated with lowering of liver enzymes, improvements in FIB-4 and APRI fibrosis scores, and an increase in platelets [18, 19]. A recent report from Georgia [20] demonstrated reversal of TE scores in 304 patients with advanced fibrosis or cirrhosis following SVR at a similar rate to that reported by our group in 2015 [21].

However, a correlation between fibrosis regression by TE and histology is lacking in the DAA era literature. These critical data are needed for physicians who are monitoring patients with advanced fibrosis or cirrhosis after SVR following DAA treatment. Previous evidence shows a lower risk of hepatocellular carcinoma (HCC) and other liver-related complications with fibrosis regression following interferon-based regimens [22], but the regression of fibrosis seems to be a very slow process [23]. Further, some patients did not regress after SVR, and some even worsened, with an increased risk for HCC [24]. This potential complication has created an abundance of caution from clinicians and updates to guidance documents recommending indefinite screening for HCC in patients who achieved SVR who had advanced fibrosis or cirrhosis [25]. The guidelines issued by the European Association for the Study of the Liver (EASL) and the Asociación Latinoamericana para el Estudio del Hígado (ALEH) have indicated that the routine use of noninvasive tests during treatment or after SVR in non-cirrhotic patients does not add to clinical disease management and that the routine use of noninvasive tests after SVR in patients with HCV cirrhosis has a high false-negative rate and cannot be used to determine which patients no longer need HCC screening or for the diagnosis of cirrhosis reversal. They further indicate that the routine use of noninvasive tests after SVR has not yet established thresholds that predict low risk of liver-related events [26]. Despite these recommendations, TE is being done routinely in the community post-SVR, so demonstrating the correlation between biopsy and TE has important implications for clinical practice.

In May 2017, the American Gastroenterological Association (AGA) published guidelines recommending a TE cutoff of <9.5 kPa to rule out advanced liver fibrosis in non-cirrhotic chronic HCV patients who achieved SVR after antiviral therapy [27]. The cutoff of <9.5 kPa would be expected to misclassify only 1% of patients as not having advanced fibrosis in a low-risk population and 7% in a high-risk population, which would include patients with liver stiffness >12.5 kPa before therapy or other risk factors for chronic liver disease. This conditional recommendation was rated to have low-quality evidence per the GRADE framework, as actual comparative post-SVR data have not been available.

2 Methods

Patients with advanced fibrosis or cirrhosis prior to treatment in the Hepatology division at Scripps Clinic were identified and screened for the study between 2010 and 2015. Patients were included in the study if they were at least 18 years old with chronic HCV with fibrosis of at least F3 staging who achieved SVR at 12 and 24 weeks' posttreatment. Exclusion criteria included liver transplant prior to therapy, SVR without DAA therapy, failure to achieve SVR, age less than 18 years old, liver fibrosis less than F3, and additional causes of liver fibrosis other than HCV. Patient's baseline fibrosis prior to DAA therapy was assessed by liver biopsy, TE, and/or clinical signs. Patients with clinical evidence of portal hypertension (varices, portal

hypertensive gastropathy, ascites, and/or hepatic encephalopathy) or baseline TE greater than 12 kPa were categorized as having cirrhosis; otherwise, patients were placed in the advanced fibrosis (F3) group.

In patients for whom SVR was achieved at 12 and 24 weeks, fasting TE measurements consisting of ten measurements with an interquartile range of <25% were collected at 6–12-month intervals for up to 5.5 years. Fibrosis staging cutoffs for TE were as follows: F0–F1 < 7 kPa, F2 7–9.4 kPa, F3 9.5–11.9 kPa, and F4 > 12 kPa, derived from values used in meta-analyses and systematic reviews [28, 29]. Hepatocellular carcinoma was screened regularly with ultrasound, CT, and/or MRI as needed.

Fourteen patients who had improvement in their fibrosis by at least one stage by TE had additional informed consent discussions regarding obtaining liver biopsies to confirm improvement. One patient enrolled in the study who showed unchanging liver stiffness also wished to pursue liver biopsy. Liver biopsies were processed and evaluated using standard techniques; sinusoidal fibrosis was additionally evaluated. The biopsies were all read independently by two expert pathologists who were blinded to any clinical information and to the timing with respect to antiviral treatment. Discrepancies were solved by consensus reading.

Liver biopsy fibrosis stage was assessed according to the Batts-Ludwig scoring system as follows [30]: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with septal fibrosis; F3, bridging fibrosis with architectural distortion; and F4, cirrhosis. Similarly, hepatic inflammation was evaluated according to both the METAVIR and Batts-Ludwig grading systems [30, 31]. The 11 biopsies used for morphometric measurements were read by a third pathologist using the Batts-Ludwig and Ishak scoring systems.

For quantification of fibrosis, sections were stained with picrosirius red, which binds stoichiometrically to collagen. A digitized image of each entire stained section was acquired using an Aperio/Leica Scanscope XT scanner at 20X magnification. The image analysis process also included a manual editing step to determine the total stained area of the section and to eliminate image artifacts. An area quantification algorithm (Indica Labs, Inc.) was used to quantify number of red-stained pixels of the collagen fibers. Accuracy of classification was confirmed by visual inspection and results expressed as a fraction of the total pixels positive for picrosirius red (communication from Zachery D Goodman).

Descriptive statistics were calculated for the primary outcome of changes in liver stiffness and improvements in fibrosis by biopsy. Changes in liver stiffness and liver chemistries following SVR were compared to pre-SVR levels by paired *t*-tests. Time to improvement, defined as the time from SVR to one stage of fibrosis regression on TE, was assessed in both the cirrhosis and advanced fibrosis groups using Kaplan-Meier curves and compared using the log-rank test. Statistical significance was defined as a p-value <0.05. The primary outcomes were liver stiffness in kPa, and subsequently fibrosis staging predicted by TE after SVR was achieved compared to liver biopsy fibrosis staging and morphometry in a subset of patients. Secondary outcomes included the analysis of pre- and post-therapy FIB-4 and APRI and the prevalence of HCC in this post-SVR population.

3 Results

Two hundred twenty-four patients from a single center were originally eligible for the study. Three patients declined to participate. Twenty-eight patients were excluded; 1 had a liver transplant, 10 had multiple etiologies for their liver disease, and 17 were treated without DAA therapy. One hundred and nine patients who were treated in 2015 and later are still in follow-up, pending final analysis. Eighty-four patients were included in this analysis.

The mean age was 60 years old, and mean BMI was 28 kg/m^2 (standard deviation 4.6 kg/m^2). The study population was non-Hispanic white (87%), Hispanic (10%), or Asian (3%). The majority of patients were genotype 1A or 1B. Fifty-seven percent (57%) of the patients were treatment-experienced, and most had previously received interferon and ribavirin. Fifty-five percent (55%) of patients were treated with sofosbuvir-based regimens. Thirty-five percent (35%) of patients received telaprevir-based therapy, and 10% were in DAA clinical trials, which included NS3/4A protease inhibitors and/or NS5A/B inhibitors, sometimes in combination with ribavirin and/or interferon.

Fifty-six patients were in the cirrhotic group; 28 patients were in the F3 group. The cirrhosis group had a statistically significantly higher mean FIB-4 scores ($p < 0.01$) and lower platelets ($p < 0.001$) than the F3 group. The cirrhotic group also had a lower mean albumin, but this was not statistically significant. None of the patients had decompensated cirrhosis at the time of enrollment. Three patients developed ascites and hepatic encephalopathy during their antiviral therapy with telaprevir, Pegylated interferon, and ribavirin; however, all three had compensated cirrhosis at the time this study began, and resolution of ascites was documented by ultrasound or CT scan prior to enrollment.

Among the 56 patients who had cirrhosis prior to SVR, 23 patients (41%) continued to have cirrhosis ($\geq$12 kPa) predicted by TE. Thirty-three patients (59%) had improved by at least one stage ($<$12 kPa), and 27 patients (48%) had improved by at least two predicted stages of fibrosis ($<$9.5 kPa). The median time to improvement was 1 year (95% confidence interval, 1.2–1.9 years; Figs. 6 and 7). Among the 28 patients who had F3 fibrosis prior to SVR, 9 patients (32%) had either worsened or unchanged stiffness ($\geq$9.5 kPa) by TE. Seven patients progressed to cirrhosis ($\geq$12 kPa), and two patients remained in F3 (9.5–11.9 kPa). Nineteen patients (68%) had improved liver stiffness by at least one stage of fibrosis ($<$9.5 kPa), and 11 patients (39%) had improved liver stiffness by at least two stages of fibrosis ($<$7 kPa). Median time to improvement was 1.5 years (95% confidence interval, 1.1–1.8 years; Figs. 6 and 7). Overall, among the 84 patients with advanced fibrosis or cirrhosis, 52 patients (62%) had decreased liver stiffness. There were statistically significant changes in liver chemistries and FIB-4 scores between pre-SVR and the most recent post-SVR values. The mean FIB-4 score among patients with cirrhosis decreased from 4.7 to 2.6 ($p < 0.001$). In the F3 group, the FIB-4 score in the advanced fibrosis group decreased from 2.8 to 1.4 ($p < 0.01$). Of the 84 patients, 4 (4.8%) were found to have hepatocellular carcinoma (HCC). Prior to DAA, two patients had cirrhosis, and two had F3 fibrosis.

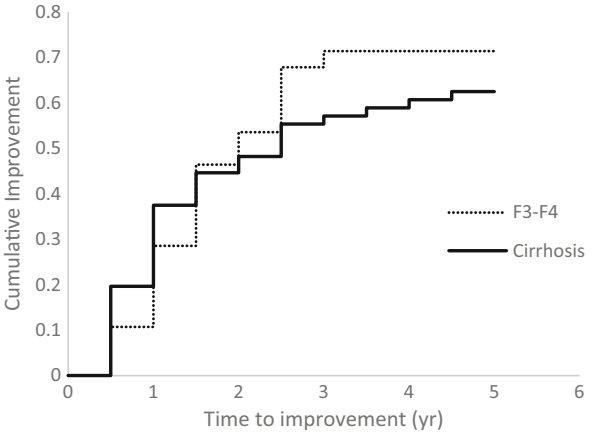

Fig. 6 Time to improvement from SVR by one stage of fibrosis estimated by transient elastography. Median time to improvement was 1.5 years for F3–F4 (F3–F4, 1.1–1.8 years, 95% CI) and 1 year for the cirrhosis group (1.2–1.9 years, 95% CI). Log-rank statistic p-value = 0.08 [32]

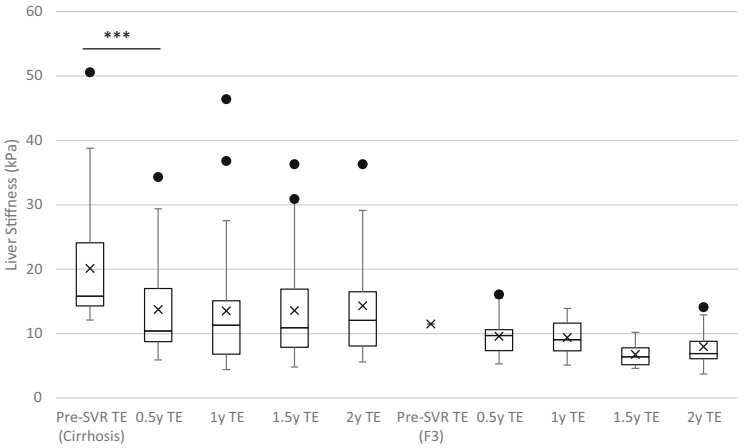

Fig. 7 Pre- and post-SVR transient elastography values (kPa) for cirrhotic group and F3 group. Box plots of pre-SVR and post-SVR TE values from 0.5 to 2 years after SVR. Box consists of first to third quartile values, with the median line marked. Whiskers extend to the farthest data point within 1.5 of the interquartile range. Outliers outside of 1.5 of the interquartile range are marked with a circle, and the mean of each group is marked by an "X." The three asterisks denote statistical significance with p-value <0.001 for a paired t-test comparison between the pre-SVR and 0.5 years after post-SVR samples. Unfortunately, there was only one baseline TE reading in the F3 group, so statistical testing was unable to be done [32]

Fifteen patients underwent a repeat liver biopsy after achieving SVR. Prior to DAA therapy, eight patients had cirrhosis, and seven patients had F3 fibrosis. In the cirrhosis group, the highest level of fibrosis observed on repeat biopsy after SVR was

F3, and the lowest was F0–F1. These biopsied cirrhotic patients were predicted per AGA guidelines by TE to have at least one stage improvement (<12.5 kPa). Six cirrhotic patients were predicted by TE to have F0–F1 fibrosis (<7 kPa) and two cirrhotic patients to have F2–F3 fibrosis (7–11.9 kPa) after SVR. Their liver biopsies revealed that two patients had F1–F2 fibrosis and six patients had features of F3–F4 fibrosis. In this group with dramatic improvements on TE, the pathologist also reported that three patients had reduced sinusoidal fibrosis compared to their prior biopsy, and two patients did not have any sinusoidal fibrosis in their current biopsy (but their prior biopsy was not available to determine sinusoidal fibrosis regression). Among the seven F3 patients, six patients were predicted by TE to have F0–F1 fibrosis (<7 kPa), but only two post-SVR liver biopsies were noted to be F1–F2. The other five post-SVR biopsies contained features of F3 fibrosis. A reduction in sinusoidal fibrosis was appreciated in three patients in the F3 group. Of the 15 patients with cirrhosis and advanced fibrosis, 13 had significant improvements in their liver stiffness by TE (<9.5 kPa) after SVR was achieved. The liver biopsies of four patients had a staging of F1–F2; the other nine patients had liver biopsies with features of F3 or F4 fibrosis (Fig. 8).

Morphometric analysis was done on the first 11 of the 15 patients who underwent post-SVR biopsy. Of these 11 patients, 10 had a decline in collagen by an average of 46% over varying time intervals (Figs. 9 and 10). The one subject with the increase in collagen had the lowest amount of baseline collagen and had demonstrated improvements in liver biopsy (F3 to F1–F2) and TE (post-SVR TE <9.5 kPa). The mean percent collagen decreased from 7.1 to 3.8% ($p < 0.01$).

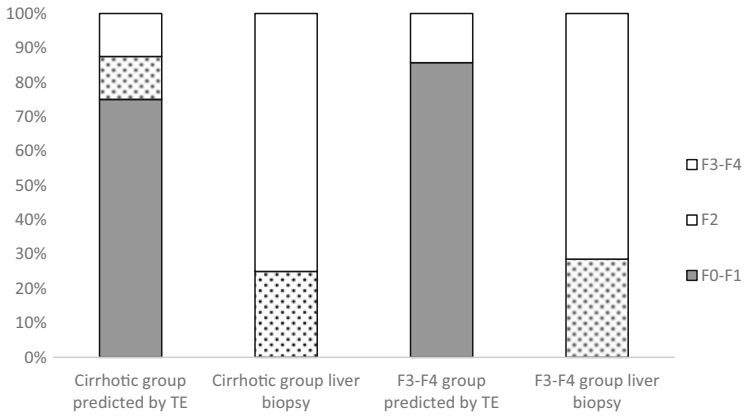

Fig. 8 Post-SVR fibrosis stage predicted by TE compared to liver biopsy [32]

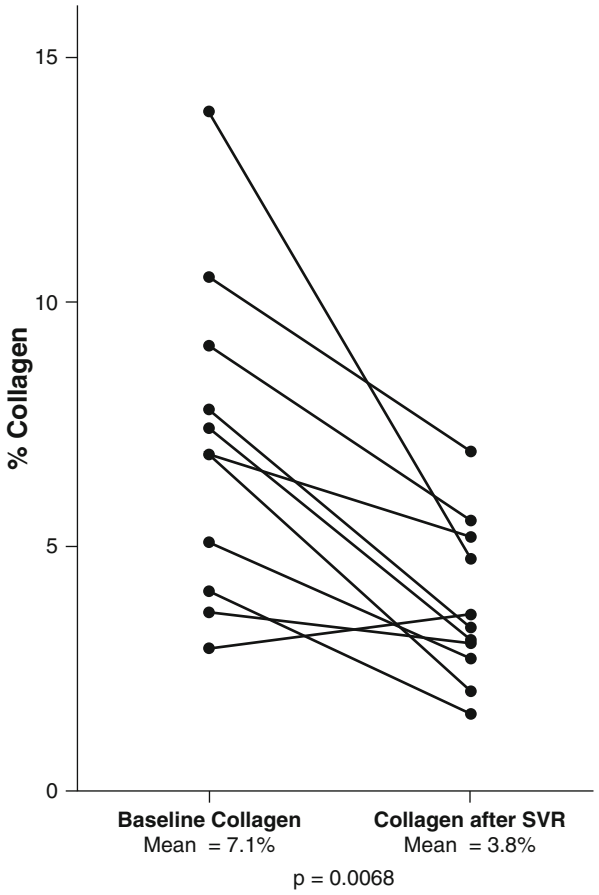

Fig. 9 Collagen quantitation morphometric analysis before and after SVR [32]

4 Discussion

TE with a cutoff of 9.5 kPa and 12.5 kPa has been recommended to stratify patients into advanced fibrosis and cirrhosis, respectively, including patients who have been treated with DAAs [27]. In our study, 13 of the 15 patients with advanced fibrosis or cirrhosis who underwent a repeat liver biopsy had liver stiffness <9.5 kPa at the time of their repeat biopsy. However, the biopsies of only four patients (31%) had fibrosis without F3–F4 features. In the two patients with liver stiffness >9.5 kPa, both continued to have F3 fibrosis, suggesting that TE remains an effective tool for confirming advanced fibrosis but may not be sensitive enough to confirm resolution of advanced fibrosis.

A previous histologic study utilizing morphometric analysis in paired liver biopsies from 37 patients with cirrhosis due to HCV demonstrated reversal of fibrosis and morphometry scores following SVR after interferon-based therapy [33]. The regression of area of fibrosis measurement in this previous study was

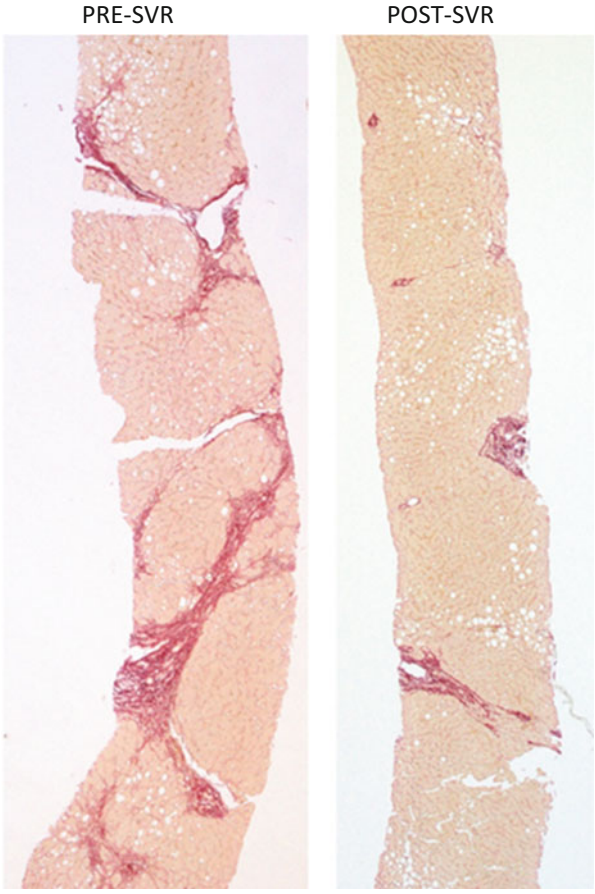

Fig. 10 Pre- and post-SVR liver biopsies show regression of fibrosis [32]

similar to that reported in our study. These findings suggest that, similar to patients treated with interferon-based regimens, the degree of liver fibrosis regression from DAA therapy is overestimated by TE compared to liver biopsy, which remains the only reliable and practical approach to stage liver fibrosis after SVR is achieved.

Hepatocellular carcinoma (HCC) screening should still be considered in non-cirrhotic patients with HCV status post-SVR after DAA therapy, despite their liver stiffness being <9.5 kPa, because they may continue to have F3 fibrosis and thus be at increased risk of HCC development. Four patients were found to have HCC. The two patients who had cirrhosis at baseline did not demonstrate liver stiffness improvement, but the two F3 patients with HCC diagnosed at 6 months and 2 years after therapy demonstrated liver stiffness improvement consistent with F0–F1 staging.

The sensitivity and specificity of TE to determine the correct fibrosis stage typically ranges from 78 to 89%, depending on the actual stage seen on liver biopsy in viremic HCV patients [28]. Although there is a range of cutoffs that may be used

for fibrosis stages, the discordance between TE and liver biopsy after SVR from DAA therapy in this study exceeds expectations (Fig. 8). These findings suggest that a distinction must be made between total amount of hepatic fibrosis reflected in liver stiffness measurement and the histopathologic features seen on liver biopsies used in staging.

In our study, the repeat biopsies of seven patients revealed major reductions in sinusoidal fibrosis. Sinusoidal fibrosis regression was recently correlated with patients who had significant TE decreases in a study where liver transplant patients underwent DAA therapy for recurrent HCV [34]. Current histologic staging systems do not utilize sinusoidal fibrosis, which has been traditionally associated with non-alcoholic steatohepatitis and perivenular fibrosis. However, hepatitis C is associated with sinusoidal fibrosis, which has been suggested to be an early histopathologic sign of hepatitis C recurrence in liver transplant recipients [35]. The reduction in sinusoidal fibrosis may reflect an overall reduction in total collagen and hepatic fibrosis; sinusoidal fibrosis has previously been shown to be an independent variable that correlates with hepatic fibrosis [36]. This hypothesis is further supported by the morphometric analysis that was completed on the first 11 biopsied patients from the present study, where all but 1 had significant reductions in total collagen. Although there may be sampling variability, morphometric analysis has been shown to be a more sensitive tool for tracking changes in fibrosis than numerical scoring systems [37]. Although morphometry is a more specific and quantifiable measurement, the lack of its widespread availability makes it an impractical test for clinical use.

The limitations of our study included limited sample size of patients undergoing a repeat biopsy and consequently possible sampling error. It is feasible that a larger study would show a closer correlation between TE scores <9.5 kPa after SVR and resolution of advanced fibrosis or cirrhosis. However, it may prove impractical or even impossible to convince a significant number of patients who have accomplished a virologic cure and have no evidence of portal hypertension to undergo a liver biopsy years after SVR. Necro-inflammation and transaminase flares before treatment could lead to an increase of stiffness not related to liver fibrosis (confounders). However, acute inflammation is unlikely to have a role in the TE and biopsy changes because the findings were consistent over a prolonged period during normal AST/ALT levels (Fig. 2) and because the average time to follow-up biopsy was 3 years. Unfortunately, no TE studies were performed immediately after DAA therapy, but previous studies have shown that there is an immediate improvement of 2 or more kPa due to resolution of inflammation during antiviral treatment [34, 38]. TE studies were not conducted prior to antiviral therapy in patients who were treated before 2013 when FibroScan® was approved for use in the USA. This limited the ability to assess TE measurement and histology pre-therapy, but the aim of this study was to assess fibrosis reversal after therapy. Multiple studies have previously shown that this correlation in chronic HCV pre-therapy is valid [13–15, 25–29].

Ours is the first study that has also paired liver biopsies showing a regression of liver fibrosis with histology as well. By obtaining follow-up liver biopsies on a select group of patients with dramatic TE improvements, this study also demonstrated that

these TE-predicted improvements, especially with cutoffs of <9.5 kPa, were not seen in histologic staging but in morphometric analysis. Of the 13 patients who had a repeat biopsy and displayed significant improvement in liver stiffness (<9.5 kPa), only 31% showed improvement to less than F3 staging on pathology. There is a discordance between the level of liver stiffness improvement measured by TE and fibrosis regression seen on liver biopsies, using these simple histologic scoring systems. Morphometry demonstrates 46% reduction in fibrosis with SVR over a relatively short time period and is a more accurate measure of improvement in fibrosis regression. However, morphometry is not a practical test for the clinic as it is done by only a few specialty centers and pathologists in the world.

It is therefore currently impossible for one to determine the ultimate benefit of cure in patients who had advanced fibrosis or cirrhosis before their cure. I anticipate that this issue will not be resolved until enough patients have been followed for enough time that we can conclusive say they are free of liver-related mortality. Hopefully this will be clearer within the next decade.

Until that time patients with TE scores <20 kPa and platelet counts ≥150,000 can be monitored without repeat endoscopy, however all patients will continue to require HCC screening at 6–12-month intervals.

Compliance with Ethical Standards

Conflict of Interest Paul J. Pockros has received research grants from Gilead, AbbVie, BMS, Merck, Conatus and has received a honorarium for speaking and consulting to PJP from Gilead, AbbVie, Merck, Conatus. He is Intercept Board of Directors member at Conatus.

Ethical Approval All procedures were performed in accordance with the ethical standards of the Scripps institutional research committee and with the 1964 Helsinki declaration and its comparable standards.

Informed Consent Informed consent was obtained from all individual participants included in the study which was approved by the Scripps Human Subject committee IRB.

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Cure and Control: What Will It Take to Eliminate HCV?

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Abstract In May 2016, the World Health Organization adopted the first global hepatitis strategy, setting the ambitious goal of "elimination of viral hepatitis as a public health threat" by 2030. HCV-specific targets included a 65% reduction in HCV-related mortality and an 80% reduction in HCV incidence. Globally, an estimated 71 million people were living with chronic HCV infection in 2015. Approximately two million new HCV infections occur annually, with injecting

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drug use and unsafe health-care practices (including unsterile health-care injection), the predominant modes of HCV transmission.

The development and availability of highly effective direct-acting antiviral agents (DAAs) have revolutionised HCV management and provide the therapeutic tools required to strive for elimination. For HCV treatment as prevention to have greatest impact, HCV testing and treatment coverage must be high, with new diagnoses linked expediently to care and treatment. In 2015, of the 71 million people living with HCV infection, only 20% (14 million) were diagnosed, and of those diagnosed, 13% (1.1 million in 2015, 1.76 million in 2016) had initiated DAA treatment.

This chapter outlines the United Nations and World Health Organization elimination targets; defines the epidemiological concepts of control, elimination and eradication; and discusses the important lessons learnt from control and elimination efforts in other infectious diseases epidemics. The biological and technical feasibility of HCV control and elimination is discussed, followed by related financial, political and social considerations. Examples of national HCV strategies are presented, highlighting the facilitators and barriers to successful implementation of HCV elimination strategies. Control and elimination of HCV infection will require an enormous public health, political and economic commitment. The costs and risks may be high, but so too are the potential benefits.

Keywords Diagnosis, Direct-acting antiviral, Elimination, Hepatitis C, Prevention, Screening, Treatment

1 Introduction

In recognition of the significant global disease burden attributed to communicable diseases, the United Nations Sustainable Development Goals proposed to "end the epidemics" of HIV, tuberculosis and malaria and "combat" viral hepatitis [1]. In May 2016, the World Health Organization (WHO) responded by adopting the first global hepatitis strategy, setting the ambitious goal of "elimination of viral hepatitis as a public health threat" by 2030 [2]. HCV-specific targets included a 65% reduction in HCV-related mortality and an 80% reduction in HCV incidence (Table 1) [1, 2].

Globally, an estimated 71 million people were living with chronic HCV infection in 2015 [2–4]. Approximately two million new HCV infections occur annually, with injecting drug use and unsafe health-care practices (including unsterile health-care injection) the predominant modes of HCV transmission [2, 3]. People living with chronic HCV infection are at risk of cirrhosis and its complications, including hepatocellular carcinoma, which are associated with an estimated 250,000–400,000 deaths per year [3, 5]. Ageing HCV-infected populations and ongoing HCV transmission among at-risk populations are contributing to the growing burden of chronic HCV infection and advanced liver disease in many settings [6–8].

Table 1 World Health Organization HCV-specific elimination targets

	2015, Baseline	2020, Target	2030, Target
Impact targets			
Incidence New cases of HCV infection, *n*	–	30% reduction	80% reduction
	1,750,000	1,230,000	<350,000
Mortality HCV-related deaths[a], *n*	–	10% reduction	65% reduction
	399,000	359,100	139,650
Service delivery targets			
Diagnosis Proportion diagnosed with HCV infection	20%	30%	90%
Treatment uptake Proportion diagnosed with HCV initiated on treatment	7%	Not specified (three million[b])	80% (cumulative)
Blood safety Donations screened with quality assurance	97%	95%	100%
Injection safety Proportion of unsafe injections	5%	0%	0%
Harm reduction Number of sterile needles and syringes distributed per PWID per year	27	200	300

Compiled from [1–3]
[a]Death predominantly due to hepatocellular carcinoma and cirrhosis
[b]Total cumulative HCV treatment uptake target by 2020

The development and availability of highly effective direct-acting antiviral agents (DAAs) has revolutionised HCV management and provides the therapeutic tools required to strive for elimination [9]. In the absence of a vaccine, the cornerstone of HCV elimination will be DAA therapy (treatment as prevention) [10], in combination with infection (and reinfection) prevention. To achieve elimination, populations with high HCV prevalence and incidence will require targeted interventions [11]. For HCV treatment as prevention to have greatest impact, HCV testing and treatment coverage must be high, with new diagnoses linked expediently to care and treatment [11, 12]. In 2015, of the 71 million people living with HCV infection, only 20% (14 million) were diagnosed, and of those diagnosed, 13% (1.1 million in 2015, 1.76 million in 2016) had initiated DAA treatment [13, 14] (Fig. 1). As such, in 2016, new HCV infections continued to exceed DAA-based cure [15].

This chapter outlines the United Nations and World Health Organization elimination targets; defines the epidemiological concepts of *control*, *elimination* and *eradication*; and discusses the important lessons learnt from control and elimination efforts in other infectious diseases epidemics [16, 17]. The biological and technical feasibility of HCV control and elimination is discussed, followed by related financial, political and social considerations. Examples of national HCV strategies are presented, highlighting the facilitators and barriers to successful implementation of HCV elimination strategies. Control and elimination of HCV infection will require

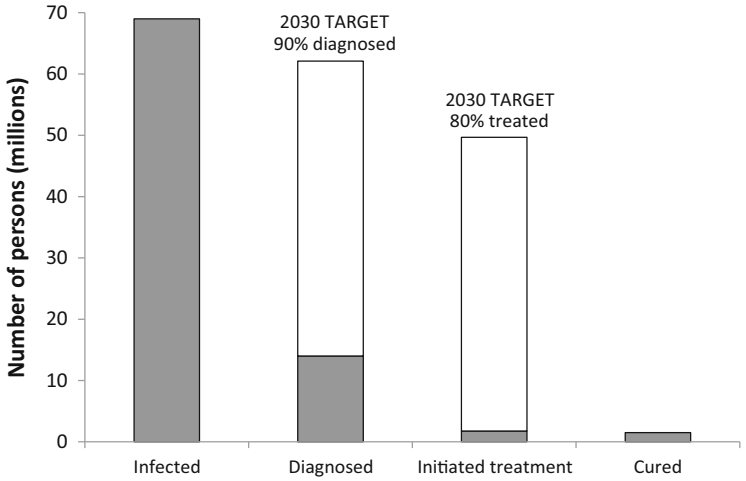

Fig. 1 Cascade of care for people living with HCV infection, 2016. In 2016, an estimated 69 million people were living with HCV infection, of whom 14 million were diagnosed and 1.76 million had commenced HCV treatment. Based on the number of people infected, diagnosed and treated in 2016, approximately 48 million additional people will need to be diagnosed with HCV infection and commenced on DAA therapy, in order to meet the WHO 2030 elimination targets. Adapted from [13, 14]

an enormous public health, political and economic commitment. The costs and risks may be high, but so too are the potential benefits.

2 Global HCV Epidemiology

2.1 HCV Prevalence

Globally, an estimated 71 million people were living with chronic HCV infection in 2015 (HCV RNA [viraemic] prevalence, 1%), with an estimated 115 million people having ever been infected with HCV (anti-HCV antibody prevalence, 1.6%) [2–4]. HCV prevalence varies considerably by country and region (Fig. 2), with the majority of people living with HCV infection (72%) residing in low-income and middle-income countries (LMIC) [3]. Thirty countries account for 80% of viraemic HCV infections, with China (9.8 million), Pakistan (7.2 million), India (6.2 million) and Egypt (5.6 million) accounting for more than 40% of viraemic HCV infections [4]. The greatest burden of HCV infection is in countries in which transmission was primarily related to past or current unsafe health-care procedures (including blood transfusion and unsafe medical injection) [4]. The initial driver of high HCV prevalence in Egypt (anti-HCV antibody prevalence, >10%) was a mass campaign

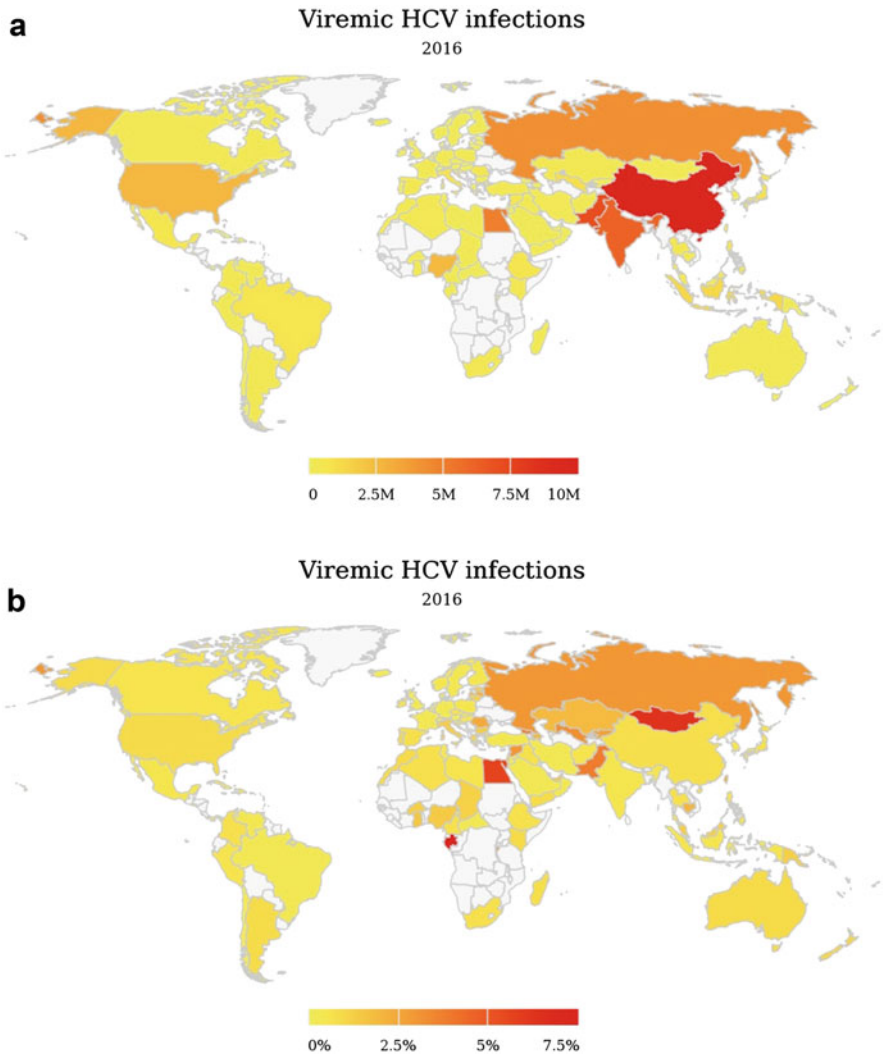

Fig. 2 Burden of HCV infection in 2016, by country. Panel (**a**) Number of people estimated to be living with HCV infection (millions). Panel (**b**) Prevalence of HCV infection. From [14]

of parenteral anti-schistosomiasis treatment, administered in rural areas in the 1960s and 1970s.

Seven HCV genotypes have been identified. HCV genotype 1 predominates in North and South America, Europe, Australia, New Zealand, Central Asia and East Asia, while HCV genotype 3 is most common in India and Pakistan [4]. HCV genotype 4 dominates in Egypt and Central sub-Saharan Africa, HCV genotype 5 accounts for more than one-third of infections in South Africa, and HCV genotype 6 is found in South East Asia [4].

2.2 HCV Incidence and Risk Factors for Acquisition

In 2015, an estimated 1.75 million new HCV infections occurred worldwide [3, 4]. Substantial regional variation existed, with the highest HCV infection incidence in Europe (62 per 100,000) and the Eastern Mediterranean region (63 per 100,000) [3]. In high-income countries, the key at-risk populations for HCV acquisition are people who inject drugs (PWID) and HIV-positive gay and bisexual men (GBM) [18–20], although the burden of infection among GBM remains markedly lower than among PWID. In LMIC, unsafe health-care practices (including unsafe health-care injection, blood transfusion and other invasive medical procedures) account for a large proportion of new HCV infections [21–24], with an increasing burden of HCV infection related to injecting drug use [3, 20, 25].

Country-specific data demonstrated that annual HCV incidence appeared to have peaked in the vast majority of countries between 1970 and 2005, with the notable exception of Russia [6–8]. However, the opioid epidemic and increases in injecting drug use appear to be associated with a recent rise in HCV incidence in the United States, particularly among young white adults (20–39 years) in non-urban communities [26]. After the initial dramatic decline (more than 90%) and then plateau in HCV incidence in the 1990s and early-mid 2000s, the estimated number of new HCV infections in the United States rose from approximately 10,000 in 2009 to 40,000 in 2016 [27]. Reliable current epidemiological data is required to design appropriate public health interventions and tailor national HCV elimination strategies to respond appropriately to established and emerging epidemics.

Of the estimated 1.75 million new HCV infections in 2015, 23% were attributable to current injecting drug use [3] (estimated number of PWID aged 15–64 years in 2015, 15.6 million; anti-HCV antibody prevalence among PWID, 53% [20]). The majority of new (60%) and existing (50%) HCV infections in developed countries occur among PWID [20], with higher incidence in specific populations, including young adults (aged <30 years) [26, 28, 29] and those who are incarcerated [30, 31]. Although stable or declining HCV incidence among PWID has been reported in some jurisdictions (including Western Europe and Australia) [32, 33], sustained high or increasing incidence has been reported in other regions, including some LMIC and the United States [26, 34].

3 Infectious Diseases Control, Elimination and Eradication

In theory, if the right tool were available, all infectious diseases would be eradicable [16].

3.1 Definitions

In 1998, Dowdle proposed a definition of *control* as a reduction in the incidence, prevalence, morbidity or mortality of an infectious disease to a locally acceptable level, *elimination* as reduction to zero of the incidence of disease or infection in a defined geographical area and *eradication* as permanent reduction to zero of the worldwide incidence of infection [16] (Box 1).

Box 1 Dahlem Workshop on the Eradication of Infectious Diseases: Definitions

- *Control:* The reduction of disease incidence, prevalence, morbidity or mortality to a locally acceptable level as a result of deliberate efforts; continued intervention measures are required to maintain the reduction.
  Example: diarrhoeal diseases and schistosomiasis.
- *Elimination of disease:* Reduction to zero of the incidence of a specified disease in a defined geographical area as a result of deliberate efforts; continued intervention measures are required.
  Example: neonatal tetanus.
- *Elimination of infection:* Reduction to zero of the incidence of infection caused by a specific agent in a defined geographical area as a result of deliberate efforts; continued measures to prevent re-establishment of transmission are required.
  Example: measles and poliomyelitis.
- *Eradication:* Permanent reduction to zero of the worldwide incidence of infection caused by a specific agent as a result of deliberate efforts; intervention measures are no longer needed.
  Example: smallpox.
- *Extinction:* The specific infectious agent no longer exists in nature or in the laboratory.
  Example: none.

Adapted from [16].

While the definition of eradication emphasises that routine intervention measures are no longer required once interruption of transmission has been certified worldwide, inherent in the definitions of control and elimination is the need for continued intervention measures to prevent re-emergence and re-establishment of transmission.

3.2 Treatment as Prevention

The concept of “treatment as prevention”, used initially in the context of HIV, incorporates treatment as a tool for limiting spread of an infection in generalised

epidemics, by reducing the pool of infection in the community and subsequent risk of transmission [35, 36]. The demonstration that antiretroviral therapy reduced HIV transmission between sexual partners provided evidence and enormous impetus for HIV treatment as prevention [37]. Similarly, randomised controlled trials provided evidence for HIV pre-exposure prophylaxis (PrEP) [38, 39], further strengthening the overall feasibility of HIV treatment as prevention. Finally, the reduction in HIV-related morbidity and mortality associated with early antiretroviral initiation provided additional support for universal treatment access as clinical and public health policy [40].

3.3 Micro-elimination

Attempts at infectious disease control and elimination often commence with targeted interventions in defined populations or geographic areas. "Micro-elimination" refers to targeted tailored treatment and prevention interventions, which are implemented and evaluated in specific populations [41]. A successful (micro)elimination effort requires a thorough understanding of the target population, including the barriers faced in accessing care and treatment.

As much of the burden of HCV infection and ongoing transmission occurs among specific populations with defined risk behaviours, targeted treatment and prevention strategies could be effective. In order to achieve HCV elimination, populations with high prevalence and incidence, including PWID, people who are incarcerated and people living with HIV, will require focussed interventions.

3.4 Lessons Learnt from Other Infectious Diseases

Over the last century, morbidity and mortality from infectious diseases, particularly acute infections, has progressively declined. Chronic infections have emerged as major global health threats, with annual deaths from HIV, tuberculosis and chronic viral hepatitis (HBV and HCV) of 1.0 million, 1.2 million and 1.3 million, respectively, in 2016 [5]. With smallpox the only human pathogen on the list of eradicated infections, the prospect of any of these chronic infections being added this century appears remote. Even with rapidly increasing infant coverage of a highly effective vaccine for HBV infection, it will be difficult to achieve HBV eradication by 2099, due to the largely asymptomatic nature of chronic infection, lack of highly curative therapy and improving overall life expectancy.

Control and elimination strategies for other infectious diseases can assist in designing the optimal approach to HCV infection and highlight potential implementation challenges (Boxes 2 and 3). In the response to HIV, the success of antiretroviral therapy scale-up (with 17 million people in LMIC on combination antiretroviral therapy in 2015), with declining HIV-related mortality (1.9 million to 1.0 million

deaths 2005–2016) and reduced HIV transmission [42], stands as testament to the impact of concerted advocacy, global leadership and large-scale health investment. This success in HIV has been achieved in spite of potential negative predictors, including lack of a vaccine, requirement for lifelong therapy, high burden of infection in low-income countries (particularly sub-Saharan Africa) and imperfect behavioural risk reduction strategies. In contrast, global tuberculosis control has been impeded by ongoing transmission (particularly in LMIC), lack of effective and affordable diagnostics, prolonged complicated antituberculous therapy (at least 6 months), lack of a highly effective vaccine and inadequate coordination and funding. Tackling tuberculosis must occur in concert with sustainable financial investment, access to quality essential health-care services, political will and multi-sectoral engagement to ensure progress at global, national and local levels. The contrasting fortunes of HIV and tuberculosis serve as important reminders in the global response to HCV.

Box 2 Schistosomiasis in China: An Example of Disease Control

Three phases of schistosomiasis control have been carried out for over 50 years:

- First phase (1950s–1970s): snail control
- Second phase (1980s–2004): chemotherapy for humans and animals
- Third phase (2005–current): transmission source control
  - Snail surveys (vector control; freshwater snails are essential to the parasite's life cycle)
  - Chemotherapy for humans (praziquantel) and domestic animals
  - Health education:

    Including educating fishermen and boatmen about the dangers of infested water
  - Engagement of specialists from agriculture, forest, water conservancy and land
  - Mechanisation of agriculture (replacing buffalos with tractors, fencing pasture areas and banning grazing in snail-infested areas)
  - Improving household sanitation and access to clean water

Compiled from [43, 44].

The need for continued intervention after reaching control or elimination targets can be problematic, with misunderstanding leading to neglect or cessation of intervention activities and resultant re-emergence of the target disease (Boxes 2 and 3). In the case of schistosomiasis control in the Sichuan province in China, control and interruption of transmission were achieved through a mixture of interventions including mollusc (freshwater snail) control, chemotherapy (praziquantel), health education and provision of clean water. However, while surveillance continued in

most counties after attainment of control targets, most other interventions to control infection in the snail vector and human host were discontinued, permitting re-emergence of schistosomiasis years later [43, 44]. The spread of poliomyelitis from Nigeria between August 2003 and July 2005 provides another example of the reintroduction of an infectious disease to areas where control interventions were neglected [45–48]. Wild poliovirus genetically linked to endemic poliovirus in northern Nigeria was reintroduced into polio-free countries in Africa, the Middle East and Asia. In many of these regions, routine polio vaccination programmes had been neglected after being declared polio-free, resulting in a large population of susceptible human hosts and polio re-emergence in 18 polio-free countries. Surveillance and continuation of control interventions are necessary to maintain achievements in infectious disease control, highlighting need to for ongoing commitment, political will and adequate financial resources.

Box 3 Poliomyelitis: An Example of Infectious Disease Elimination

- Global Polio Eradication Initiative (commenced 1988):
  - International partnership led by five organisations: the World Health Organization, Centers for Disease Control and Prevention, United Nations Children's Fund, Rotary International Bill and Melinda Gates Foundation
  - Multi-sectoral collaboration – government and non-government donors and ministries of health of all affected nations
  - Cost: >$1 billion per year
- Global eradication programme:
  - Routine infant immunisation
  - Supplementary immunisation activities (at-risk LMIC)
  - Surveillance (acute flaccid paralysis in children and adolescents; environmental surveillance by periodic sampling of sewage effluent in high-risk areas)
  - Mop-up campaigns
- Decline in annual global incidence of poliomyelitis (>99.9%):
  - 1988: >350,000 cases.
  - Wild polio, 2017, 22 cases (Afghanistan, Pakistan); vaccine-derived polio, 2017, 96 cases (Syria, Democratic Republic of Congo).
  - Three countries have not eliminated polio: Nigeria, Pakistan and Afghanistan.
  - More than 20 countries have experienced reintroduction of polio from endemic areas because of low population immunity.

(continued)

Box 3 (continued)

- Operational challenges:
  - Variable commitment from national authorities
  - Religious and cultural opposition
  - Inadequate funding
  - Poor campaign quality
  - Surveillance gaps
  - Slow response to outbreaks
  - Armed conflict, including extremist groups targeting vaccine campaign workers
- Scientific challenges:
  - Reduced effectiveness of oral polio vaccine (areas with high burden of enteric pathogens)
  - Circulating vaccine-derived poliovirus outbreaks

Compiled from [45–47].

Global progress in combating communicable diseases has been uneven, with millions (predominantly in LMIC) unable to access appropriate treatment and prevention. From the outset, the fight against infectious diseases has been dogged by social, legal and economic barriers, with significant funding gaps. These issues are paramount in designing national and global HCV elimination strategies, to ensure equitable access to HCV care and treatment.

4 Feasibility of HCV Elimination: What Is Required to Achieve the WHO 2030 Targets?

In striving for infectious diseases control, elimination and eradication, three factors are of primary importance, when considering biological and technical feasibility [16, 17]:

1. The availability of an effective intervention (to disrupt transmission)
2. The availability of practical, sensitive and specific diagnostic tools
3. The role of humans in the life cycle of the pathogen (with no other vertebrate reservoir and no amplification in the environment)

In relation to HCV, effective therapeutic and prevention interventions are available to curb HCV transmission, HCV diagnostics are evolving to allow broader access and implementation, and infected humans appear to be the exclusive reservoir of HCV in nature.

However, biological and technical feasibility alone is not sufficient; a successful elimination strategy must also consider contemporary economic, social and political factors [16, 17]. Globally, financial, political, structural and social barriers exist which complicate efforts to achieve HCV elimination in many countries and regions. Challenges to HCV elimination include limited reliable epidemiological data; inadequate HCV testing and resultant poor levels of diagnosis; limited access to care, treatment and harm reduction (particularly for PWID); a lack of HCV education and training for health-care providers; continued health-care-associated transmission (in LMIC); prevailing stigma and discrimination against people living with HCV and people at high risk of HCV (including PWID and GBM); variable leadership and commitment from policy-makers and governments; and financial barriers to testing, treatment and care.

While HCV control and elimination is conceptually simple, with a clear unequivocal outcome, global operational challenges make implementation difficult.

4.1 Direct-Acting Antiviral Therapy: Facilitating "Access for All"

The development and availability of highly effective, well-tolerated DAA therapy have revolutionised the clinical management of HCV and fostered optimism regarding HCV elimination [49]. With high efficacy (cure of HCV infection, >90%) after only 8–12 weeks of oral treatment, DAAs provide the therapeutic tools required to reverse the growing burden of infection and HCV-related liver disease [10]. Importantly, DAA therapy is safe and effective among priority populations, including PWID and people living with HIV (reviewed in [50, 51]).

The goal of treatment is cure of HCV infection (with achievement of a sustained virological response [SVR], defined as undetectable HCV RNA in blood 12 or 24 weeks after treatment) in order to prevent HCV-related hepatic and extrahepatic complications, prevent onward transmission and improve quality of life [52]. SVR is associated with favourable clinical outcomes, including improvements in liver fibrosis stage, quality of life and survival and reduction in HCV-related morbidity [53, 54].

4.1.1 Reducing HCV Incidence and HCV-Related Mortality: Insights from Mathematical Modelling Studies

Mathematical modelling suggests that substantial reductions in HCV incidence and prevalence could be achieved by targeted HCV treatment scale-up among those at highest risk of ongoing transmission, including PWID and HIV-positive GBM, across a wide range of settings [11, 12, 55–66]. In addition, burden of disease models have shown that targeted DAA treatment among people with more advanced

liver disease at existing or only modestly increased levels can achieve reductions in mortality, in line with the WHO target [66], but these models do not account for transmission and incidence reduction.

Regardless of prevalence, modelling studies show that treating less than 100 per 1,000 PWID per year results in considerable HCV prevalence and incidence reductions [57, 64–67]. Using HCV treatment uptake data from seven sites in the United Kingdom, Martin et al. demonstrated that treating 26 per 1,000 PWID per year with DAA therapy could achieve a 15–50% decrease in chronic HCV prevalence within 10 years [57]. Similarly, a modelling study by Scott et al. evaluating DAA treatment uptake among PWID in Australia indicated that treating 4,700 PWID per year (59 per 1,000 PWID per year) for the next 15 years would reduce HCV incidence by 80% in 2030, in line with WHO elimination targets [66]. Additionally, modelling has highlighted the need for tailored interventions in different populations depending on the burden of infection in a given setting. Among PWID in three cities with different chronic HCV prevalence – Edinburgh, Scotland (25%); Melbourne, Australia (50%); and Vancouver, Canada (65%) – it was estimated that a 50% reduction in HCV prevalence would be achieved within 15 years if annual DAA treatment uptake increased to 15 per 1,000, 38 per 1,000 and 75 per 1,000 PWID in Edinburgh, Melbourne and Vancouver, respectively [68].

Unsurprisingly, strategies that combine DAA treatment scale-up and HCV prevention interventions appear to deliver greater benefit. Among PWID, modelling has shown that higher harm reduction coverage (NSP and OST) allows for lower DAA treatment uptake to achieve specific reductions in HCV prevalence [64, 65, 67]. Among HIV-positive GBM, the importance of behavioural risk reduction in HCV elimination strategies has been highlighted [58–60]. Data from the UK Collaborative HIV Cohort predicted that the greatest effect on HCV incidence and prevalence would be achieved if DAA scale-up was prioritised to those with recently diagnosed (<1 year) HCV infection and occurred in combination with behavioural risk reduction [12]; while DAA scale-up alone was predicted to reduce incidence (by over 60%), without behavioural risk reduction, the WHO elimination targets would not be met by 2030.

4.1.2 Broad Unrestricted Access to DAA Treatment

DAA treatment uptake must improve if the WHO 2030 elimination targets are to be met. In 2015 and 2016, almost three million people initiated DAA treatment [13]. While this is a marked improvement on historical interferon-based HCV treatment uptake, treated HCV infections are only keeping pace with new HCV infections [15], and those treated represent the minority of those infected who are diagnosed and linked to care. The vast majority of people living with HCV infection remain undiagnosed and untreated.

DAA treatment uptake varies considerably by country and region, with a small number of countries accounting for the majority of people treated [13] (Table 2). With high infection burden and a very active national DAA treatment initiative,

Table 2 HCV treatment uptake in selected countries in 2016

Country	Number of people commencing HCV treatment, *n*	Proportion treated of total HCV population, %
HCV treatment uptake >7% per year		
Iceland	420	55
Australia	32,000	16
Egypt	577,000	12
Georgia	21,000	12
Japan	90,000	12
The Netherlands	2,000	12
France	17,000	8
Spain	26,000	8
United Kingdom	10,000	8
United States	231,000	8
HCV treatment uptake 3–7% per year		
Austria	1,500	7
Brazil	41,000	6
Germany	15,000	6
Mongolia	10,000	5
Portugal	4,800	5
Canada	9,500	4
Italy	28,400	4
Pakistan	161,000	3
HCV treatment uptake <3% per year		
India	110,000	2
China	100,000	1
Russia	12,000	<1

Compiled from [13, 14]

Egypt accounted for almost half of all people starting DAA treatment in 2016 [13]. Countries with higher DAA treatment uptake have demonstrated a strong coordinated government response, with national HCV treatment strategies, allocation of funding and resources, integration into broader health systems and active pursuit of measures to improve DAA access and lower costs [15, 49, 69, 70].

Unrestricted access to DAA therapy provides the opportunity for broad treatment scale-up among people living with HCV [70]. In jurisdictions with unrestricted access to DAAs, encouraging initial reports highlight high DAA uptake among HIV-positive GBM with corresponding reductions in HCV viraemic prevalence [71–73] and incidence [71], providing preliminary empirical evidence in support of HCV treatment as prevention. As linkage to care and engagement with health services are generally high among HIV-positive GBM, the short- and long-term success of HCV elimination service implementation in this population should

provide important information for use in other settings. However, providing unrestricted access to DAA therapy in isolation will not be sufficient, as exemplified by the recently reported experience in Germany, where DAA treatment initiation numbers have markedly declined [74].

Several HCV treatment as prevention projects are in progress, predominately among priority populations, including PWID, people who are incarcerated and people living with HIV [10]. In Iceland, a national HCV treatment as prevention project is underway (TraPHepC; clinicaltrials.gov registry identifier: NCT02647879) [75]. Most of estimated 800–1,300 people living with HCV in Iceland are current or former PWID, of whom 80% are diagnosed [75]. By increasing DAA uptake to 188 per 1,000 PWID per year (equivalent to 75 of the estimated 400 PWID in Iceland per year), an 80% reduction in incidence will be achieved by 2020 [67]. However, testing, diagnosis and harm reduction will need to increase in concert with DAA scale-up. The small population size and high proportion diagnosed are strong foundations on which to build a national HCV elimination programme. Effective field-proven treatment as prevention strategies will help further efforts to implement HCV elimination strategies globally.

4.1.3 Reduce Prices for DAA Treatment

Price remains a significant impediment to HCV treatment scale-up in most countries, regardless of the countries income status. Even with low price generic DAAs for low-income and some middle-income countries, price remains a barrier to broad treatment scale-up [13].

To reduce the financial impact of DAA therapy, governments in many countries have restricted access to DAA therapy, based on liver disease stage, drug and alcohol use and prescriber type [76, 77]. However, this is not consistent with international recommendations from peak bodies, which support treatment for all people living with HCV [52, 78], nor is it a sensible public health approach, based on epidemic modelling [11, 66] and empirical DAA treatment uptake data [70]. Modelling estimates show that restricting DAA therapy by liver disease stage or drug use status will have limited impact on HCV transmission. Even in settings in which substantial transmission occurs among the general population (as opposed to specific risk populations), restricting DAA treatment to those with advanced liver disease is not cost-effective and will require greater numbers of people treated to reach the WHO elimination targets. Several states in the United States have removed fibrosis stage restrictions following potential lawsuits from patients [79], highlighting the need for continued advocacy and societal will in the response to HCV.

Substantial reductions in DAA pricing have occurred over the previous year (discounting of >50%) [13]. Depending on the setting, DAA treatment prices have been reduced through price negotiation with pharmaceutical companies, availability of generic DAAs, increased competition among pharmaceutical companies and generic suppliers, acquisition of voluntary licences and occasional compulsory licences. In order to scale-up DAA treatment, most countries will require further

price discounting to ensure unrestricted access (through either risk-sharing arrangements as in Australia or volume taxation as in France). However, in some settings, there is no facility for government-based health-care plans (e.g. Medicaid in the United States) to negotiate with pharmaceutical companies. Legislative changes may be required in order to improve access to DAA therapy, as has occurred in Canada and the United States [80, 81]. Increased transparency could assist in global and regional reductions in DAA prices.

4.2 Screening, Diagnosis and Linkage to Care

High levels of HCV diagnosis and linkage to care are a prerequisite to a successful HCV elimination strategy, with modelling studies demonstrating the limited impact of DAA therapy on HCV epidemics in settings of low HCV diagnosis and treatment uptake [82–84]. In 2015, of the 71 million people living with HCV infection, only 20% (14 million) were diagnosed, with marked disparities between high-income countries (43% diagnosed) and LMIC (8% diagnosed) [13]. In China, Pakistan, Egypt, India and Russia, the five countries with the largest number of individuals living with HCV infection [85], less than 25% of those infected are diagnosed [14]. Additionally, the number diagnosed with HCV among priority populations for elimination, including PWID, appears to be even lower than among the general population, particularly in low-income settings [86].

Practical sensitive tests are available for the diagnosis of HCV infection, a prerequisite for an elimination strategy, with commercial assays available to detect both anti-HCV antibodies [87, 88] and HCV RNA [87, 89–93]. While anti-HCV antibody testing is relatively inexpensive (laboratory-based and rapid diagnostic tests on capillary whole blood: US$0.50–US$2.00; rapid diagnostic tests on oral fluids: US$10), HCV RNA testing is still expensive (US$30–US$200). In addition, HCV RNA testing requires specific laboratory equipment and qualified personnel. The complexity and price of HCV diagnostics are barriers to large-scale testing, impacting considerably on budget considerations in planning elimination interventions [94].

4.2.1 Increase HCV Testing, Diagnosis and Linkage to Care

The current gold-standard diagnosis of current (viraemic) HCV infection is based on the detection of HCV RNA in serum or plasma by a sensitive molecular method, with a recommended lower limit of detection of 15 IU/mL. The current HCV testing algorithm is a two-step process, with anti-HCV antibody testing (to confirm exposure) followed by HCV RNA (to confirm current infection). Standard of care testing for anti-HCV antibodies and HCV RNA requires the collection and processing of serum and plasma via venepuncture, with use of a centralised laboratory. A significant number of people who are anti-HCV antibody positive never

receive confirmatory HCV RNA testing [95–100]. While education [101–105] and innovative laboratory testing algorithms (involving reflex HCV RNA testing [106]) may improve HCV diagnosis, further diagnostic simplification would be ideal for broad implementation and elimination efforts.

Several strategies have demonstrated effectiveness in increasing HCV diagnosis and linkage to care (reviewed in [107, 108]). In primary care and hospital-based settings, automated HCV testing reminders and screening based on risk assessment or birth cohort have increased HCV testing and diagnosis [108–110]. In drug and alcohol services, utilising comprehensive systematic multidisciplinary programmes, including counselling, education and peer support, can achieve high HCV testing and assessment [111, 112]. Other strategies that have facilitated linkage to HCV care and treatment include non-invasive liver disease screening using transient elastography (FibroScan®) [104, 113, 114] and patient navigation programmes [115, 116]. Simplified HCV diagnostic procedures, including point-of-care and dried blood spot testing, have also been effective in increasing HCV testing, diagnosis and linkage to care [107, 117–123].

4.2.2 Develop Low Price, Simple HCV Diagnostics

Point-of-care tests for HCV infection, particularly for HCV RNA, have the potential to simplify testing algorithms, increase diagnoses and facilitate linkage to care and treatment [124, 125]. Simplified point-of-care diagnostic testing across a number of platforms has provided significant advances in other infectious diseases, including HIV [126, 127], tuberculosis [126, 128], chlamydia [129, 130], syphilis [127, 131] and gonorrhoea [129, 130]. Countries could potentially reduce costs by using existing infrastructure and equipment available for other infectious diseases, including HIV and tuberculosis.

Point-of-care HCV testing can include oral fluid rapid diagnostic testing [132–134], finger-stick whole-blood rapid diagnostic testing [133–136], on-site venepuncture-based testing [92, 137] and finger-stick capillary whole-blood testing [138, 139]. Although most of these tests detect anti-HCV antibody, point-of-care HCV RNA assays are available or are in late-stage development, including the Xpert HCV Viral Load (Cepheid), HCV ID Kit (Genedrive) and Truenat HCV (Molbio Diagnostics). Simplified sample collection with limited processing could improve HCV testing uptake, with the potential for HCV point-of-care testing to be made available in a variety of settings, including community health centres, drug treatment clinics, prisons, remote and rural regions, homelessness settings, supervised drug consumption rooms and residential rehabilitation/detoxification facilities.

HCV diagnostics and testing algorithms are evolving to allow broader access and implementation. Assays need appropriate sensitivity and specificity to detect infection that can result in transmission, with sufficient simplicity to be applied globally, accounting for a wide range of capabilities, resources and health-care infrastructure. In recognition of the need for large-scale testing uptake, cheaper (less than US $5–10) and less sensitive (lower limit of detection 1,000 IU/mL) diagnostic HCV RNA tests have recently been recommended by peak bodies [52]. There has also

been renewed interest in HCV core antigen, as a stable, affordable (US$25–US$50) alternative to HCV RNA testing, particularly in LMIC [140–142]. In addition, the availability of well-tolerated highly effective pan-genotypic DAA therapy should markedly simplify diagnostic and monitoring requirements and reduce cost, by removing the need for HCV genotyping, quantitative HCV RNA assessment and on-treatment HCV RNA monitoring. For example, a two-test strategy using qualitative HCV RNA testing prior to treatment (to diagnose current infection) and at post-treatment week 12 (to assess for SVR) could become the standard of care. Further, there is discussion regarding the absolute requirement for HCV RNA testing at post-treatment week 12 (to asses for SVR), given very high effectiveness of current DAA regimens and global DAA scale-up needs.

4.2.3 Optimise HCV Screening Strategies

Different screening strategies for HCV infection have been recommended based on regional epidemiology and include screening of at-risk populations, birth cohorts and general populations in areas with intermediate (2–5%) to high prevalence (>5%) [52, 78, 94, 143–145]. In the United States, the Centers for Disease Control and Prevention and the Preventive Services Task Force currently recommend one-time anti-HCV antibody testing for all people born between 1945 and 1965 (birth cohort screening) and targeted testing for people at high risk of HCV acquisition (risk-based screening) [78]. However, if HCV prevalence is high or elimination is the ultimate goal, cost-effectiveness analysis supports one-time screening of all adults (≥18 years) in addition to risk-based screening [146]. In a given setting, the optimal regional or national screening strategy should be determined according to local epidemiology and incorporated into the regional or national HCV elimination strategy.

Populations at high risk of HCV infection required targeted screening interventions. PWID should be screened for HCV with anti-HCV antibody, and in the context of ongoing injecting drug use, 6–12 monthly screening with anti-HCV antibody should be performed to assess for incident infection [52, 78, 147]. In some high-income settings with high chronic HCV prevalence among PWID, qualitative HCV RNA testing could be justified for screening. All newly diagnosed HIV-positive individuals should be screened for HCV antibody [52, 78]. HIV-positive GBM at risk of HCV acquisition should be reviewed 6–12 monthly with assessments for ALT levels and anti-HCV antibody [52, 148]. After potential exposure (via injecting drug use and/or high-risk sexual behaviour) or diagnosis of a sexually transmitted infection in GBM, additional HCV screening with anti-HCV antibody should be considered and repeated 3 months later if negative. In at-risk individuals, HCV RNA or HCV core antigen assays should be performed if transaminases (particularly ALT) are elevated or if HCV reinfection is suspected. Screening protocols for HCV infection in specific high-risk populations, including young PWID, PWID in incarceration and HIV-positive GBM, should be considered, potentially utilising point-of-care diagnostics [139, 149], to enhance HCV diagnosis,

prevention and surveillance as part of a "micro-elimination" strategy, which could have national benefit.

4.3 Prevention of HCV Infection and Reinfection

Elimination of an infectious disease through deliberate intervention is much more feasible if humans are essential to the pathogens life cycle, as is the case with HCV. However, there is no or limited protective immunity gained following HCV infection, and at present, no vaccine is available to prevent acquisition [150]. Interventions to prevent HCV infection are available and cost-effective and must be included in a national elimination strategy, tailored to the epidemiology.

4.3.1 Facilitate Access to Harm Reduction for PWID

Access to health services and implementation of evidence-based harm reduction programmes is necessary to reduce the burden of HCV among PWID. The combination of OST and high-coverage needle and syringe programmes (NSP; adequate needles or syringes to cover all injecting episodes) can reduce HCV incidence by up to 80% [151–153]. However, despite favourable modelling studies, cost-effectiveness analysis [154] and empirical evidence [32, 33], global coverage of harm reduction services remains poor, with less than 1% of PWID residing in countries with high coverage of both NSP and OST (high-coverage NSP: >200 needles/syringes distributed per PWID per year; high-coverage OST: >40 OST recipients per 100 PWID) [155]. In many countries, political resistance to harm reduction services, stigma, discrimination and criminalisation has reduced accessibility and limited coverage.

Engagement with harm reduction programmes provides a means to prevent both HCV primary infection and reinfection. As an example, a marked reduction in HCV incidence was demonstrated among PWID in Scotland between 2008 and 2012 following changes to government policy and provision of harm reduction interventions [33]. While many countries have not achieved an adequate level of coverage to curb HCV transmission, the Scottish example highlights the potential positive impact of broad harm reduction strategies in only a short period in the context of political will, widespread availability and high end-user uptake.

High-coverage harm reduction among PWID could provide some of the essential components for a successful HCV elimination strategy. Harm reduction services provide an access point for PWID to engage in HCV education and counselling and could facilitate HCV testing and (ideally) HCV treatment provision. Education and counselling can reduce high-risk injecting behaviours among people with HCV infection [156, 157] and should be offered to PWID commencing DAA therapy by health-care providers, peer support workers and community drug user organisations. While robust evidence exists for HCV infection prevention among people who use

opioids, little evidence exists for those who predominantly inject (meth)amphetamine or cocaine, with no licenced pharmacotherapies. Evaluation of novel prevention strategies should be a priority.

Drug policy reform and implementation of evidence-based harm reduction programmes will be required to support efforts to eliminate HCV infection as a global public health threat [158]. Restrictive drug policies and criminalisation of drug use have unintended implications for harm reduction and blood-borne virus prevention. Fear of arrest and prosecution may reduce uptake of prevention services resulting in decreased needle-syringe distribution and increased needle-syringe sharing [159]. Incarceration of PWID places them in an environment with high HCV prevalence and incidence and no to limited HCV treatment or harm reduction services [30]. Consulting and involving community drug user organisations in the design and implementation of HCV prevention strategies will be essential, ensuring public health efforts meet the needs of the target population.

4.3.2 Behavioural Risk Reduction Among Gay and Bisexual Men

Increasing HCV infection incidence and prevalence has been reported among HIV-positive GBM over the past decade [18, 160], although the overall burden of disease remains markedly lower than among PWID (estimated number of people living with HIV/HCV coinfection, 2.3 million [including 1.4 million PWID]; anti-HCV antibody prevalence among people living with HIV, 6.2% [GBM 6.4%, PWID 82.4%] [161]).

The reported increase in HCV infection incidence in HIV-positive GBM has been associated with an increase in sexual risk behaviour and drug use [160]. Permucosal (sexual) HCV exposure (with blood as the medium) facilitates HCV transmission among HIV-positive GBM, with risk factors for HCV acquisition including condom-less traumatic anal intercourse, higher number of sexual partners, group sex, ulcerative sexually transmitted diseases and sexual acts that involve trauma and bleeding [160, 162, 163]. Additionally, the increase in HCV infection incidence has occurred in parallel with certain behavioural trends in GBM communities, including use of social media sexual networking applications, “serosorting” sexual behaviours (use of HIV serostatus in decision-making regarding sexual behaviour) and the phenomena of “chemsex” (illicit [stimulant] drug use before or during sex, by both injecting and non-injecting routes of administration) [163–168].

Although similar sexual risk behaviours have been reported in HIV-positive and HIV-negative GBM, HCV infection incidence seems to be markedly lower in HIV-negative GBM [169–171]. However, with increasing use of HIV PrEP, there is the potential for a reduction in serosorting of sexual partners and increased sexual risk behaviour and transmission of HCV among HIV-positive and HIV-negative GBM populations, with incident HCV infections observed in GBM receiving HIV PrEP [168, 171, 172]. Phylogenetic analysis of NS5B sequences obtained from HCV-positive HIV-negative GBM receiving PrEP in Amsterdam, the Netherlands, suggested that HCV transmission was occurring within discrete populations, with

GBM-specific HCV clusters containing both HIV-positive and HIV-negative individuals [168]. While current guidelines do not advise routine HCV screening of HIV-negative GBM, increasing use of HIV PrEP and overlapping behavioural networks support monitoring of HCV incidence among high-risk GBM to guide policy.

In the context of HCV treatment as prevention among HIV-positive GBM, mathematical modelling indicated that while DAA treatment scale-up (80% among new diagnoses) could decrease HCV prevalence from 9 to 3% over 10 years, a further reduction to less than 2.5% would require additional behaviour change interventions [12]. Evidence supporting sexual behavioural interventions for HCV prevention among GBM is lacking. In addition, HIV-positive GBM who report injecting drug use may exhibit different drug use behaviours, as compared with non-GBM PWID populations traditionally reported in the HCV literature. Evaluation of novel prevention strategies should be a priority.

4.3.3 Pre-exposure or Post-exposure Prophylaxis?

There is no role for pre-exposure or post-exposure prophylaxis of HCV infection [52, 78]. The natural history of HCV infection, extremely high curative potential of DAA therapy and cost-effectiveness modelling do not support pre-emptive therapy [173]. Instead, appropriate testing and expedient treatment, if required, is recommended.

In the future, prevention of HCV infection may include a prophylactic vaccine strategy, although this is likely to prevent progression to chronic HCV rather than provide sterilising immunity. A double-blind, randomised, placebo-controlled clinical trial of a prophylactic HCV vaccine (AdCh3NSmut-MVANSMut HCV vaccine) among recent PWID at high risk for HCV infection is ongoing in the United States (NCT01436357).

4.3.4 Address HCV Reinfection

One challenge to achieving HCV elimination through therapeutic intervention is reinfection [174]. The risk of HCV reinfection after treatment is higher in those who report ongoing high-risk behaviour (such as injecting drug use or high-risk sexual behaviour) [51, 175–180]. Additionally, specific drug use behaviours, including frequency of injection drug use and predominant type of drug injected, impact HCV reinfection risk [175, 176]. Higher reinfection incidence following treatment for HCV infection in individuals with ongoing risk behaviour emphasises the need for education, harm reduction, post-treatment surveillance, rapid diagnosis of reinfection and access to retreatment.

People at risk for HCV reinfection should have at least annual monitoring with HCV RNA and ALT [52, 78]. The optimal testing interval for detection of (clinically significant) reinfection is under investigation; more frequent testing may identify a

greater number of reinfections [181] and provide the potential for earlier retreatment. Routine post-treatment surveillance and adherence to international guidelines should ensure that reinfection is diagnosed within the first year of reacquisition. However, recent data would suggest that monitoring for HCV reinfection following treatment occurs infrequently, with only 61% of PWID in a Scottish cohort study screened at least once in 4.5 years of follow-up post-SVR [180]. If testing rates remain low, reinfection incidence will be under-reported, and the impact of DAA treatment scale-up will be unclear.

Efforts directed at addressing, preventing and managing HCV reinfection should be incorporated into individual- and population-level HCV strategies, with multicomponent interventions likely to be most effective [152]. Potential constructive management options include education and counselling [59], optimal harm reduction [153], treatment of the individual and their injecting (or sexual) partner or people in their network [182], management of medical and psychiatric co-morbidity [183] and post-treatment surveillance [181]. Most importantly, retreatment for reinfection should be offered, without stigma or discrimination. Shortened duration DAA treatment (6 weeks) for those with recently acquired HCV reinfection is under evaluation [184].

4.3.5 Prevent Health-Care-Associated HCV Transmission

Unsafe health-care procedures (including unsafe health-care injection, blood transfusion and other invasive medical procedures) account for a substantial proportion of incident HCV infections in LMIC [22–24]. In 2010, an estimated 5% of all health-care injections were given with unsterilised or reused equipment, resulting in an estimated 315,000 new HCV infections, most of which were in the Eastern Mediterranean and Southeast Asia [24]. Coupled with poor injection practices, excessive medication administration by injection contributes to transmission in these regions [21, 23].

Broad implementation of infection control procedures has reduced HCV incidence among haemodialysis recipients in high-income countries [185, 186]. However, high HCV infection incidence and prevalence among haemodialysis recipients in LMIC highlight the need for universal implementation of these procedures [185].

Training of health-care providers, structural changes to health-care models, effective screening and investment in HCV diagnostics, disposable materials (ideally with reuse-prevention devices) and effective sterilisation procedures will be required to reduce health-care-associated HCV transmission [21] and meet the WHO HCV elimination targets regarding blood product and infection safety.

4.4 Monitoring and Evaluation

Surveillance data is needed to direct policy change and health service implementation. Evaluation of HCV elimination will require monitoring of DAA uptake and effectiveness, particularly among populations at risk of transmission, monitoring of HCV viraemic prevalence (HCV RNA) and incidence (primary infection and reinfection) and monitoring of the population-level impact of DAA therapy on HCV-related morbidity and mortality. Many countries will need to implement new or improve upon existing surveillance networks to gain reliable epidemiological data, identify and respond to new epidemics and accurately analyse the change in burden of HCV infection in response to public health interventions [187].

4.5 Economic Considerations

The cost-effectiveness of control methods is often a deciding factor in implementation, particularly with limited health resources. Economic considerations include the estimated direct economic burden, estimated annual productivity loss savings, cases averted per year and cost-effectiveness of diagnostic tests and treatment [17]. However, it can be difficult to accurately estimate costs of control efforts and their benefits, particularly when attempting to calculate future benefits, future costs and long-term impact. In addition, there are intangible benefits, like quality of life, which are very difficult to quantify. High short-term costs, the potential risks and consequences of failure and competing national and regional health-care priorities impact on decision-making.

Economic analyses related to the cost-effectiveness of HCV screening and treatment in different settings can help inform politicians and administrators of the value gained by considered investment.

4.5.1 Cost-Effectiveness of Direct-Acting Antiviral Treatment for HCV

In general, economic analyses have supported scale-up of DAA therapy among the general population with HCV infection, including in LMIC with generic DAA treatment [66, 188–193]. In high-income countries, despite the cost of DAA therapy, treating HCV-infected PWID and HIV-positive GBM with early liver disease appears to be cost-effective compared to delaying until cirrhosis, given the reduction in liver-related complications and additional benefit of averting secondary infections [11, 66, 188, 194]. Despite restrictions on DAA access in some settings related to liver disease stage, modelling suggests that deferral of treatment until advanced liver disease increases liver-related morbidity and mortality, fails to halt transmission and, ultimately, is not cost-saving [194]. Modelling and cost-effectiveness estimates support broad access to DAA therapy, without limitations based on liver disease

stage, duration of infection or drug use, to gain the greatest individual- and population-level benefits [11]. A "test-and-treat" strategy may be one of the most cost-effective public health strategies in attempts to eliminate HCV [188].

4.5.2 Cost-Effectiveness of HCV Diagnostics

For low-income and some middle-income countries to achieve the WHO elimination target of 90% diagnosed by 2030, the prices of HCV diagnostics must fall. With the availability of low-cost generic DAA therapy, the cost for diagnostic testing may exceed the cost of therapy, depending on the setting. While many diagnostic companies offer discounts to high disease burden countries, strategies for lowering the costs for LMIC, while maintaining incentives for companies to invest in diagnostics for sale in high-income countries, are required.

HCV screening and diagnostic algorithms need to be reviewed relative to the setting to improve cost-effectiveness. For example, in countries with high HCV prevalence, cost-effectiveness analysis supports one-time screening of all adults ($\geq$18 years) in addition to risk-based screening [94, 146]. In addition, a modelling study in Australia indicated that replacing anti-HCV antibody testing with point-of-care HCV RNA testing for screening PWID would save AUD$62 million and would gain 11,000 quality-adjusted life years [195].

4.6 Social and Political Will

4.6.1 Social and Political Support for HCV Elimination

The success of an elimination or eradication initiative, as with any public health programme, is dependent on high levels of sustained societal and political engagement. As such, the disease must be considered to be of significant public health importance, with broad (international) relevance. Globally, there is support for viral hepatitis elimination. However, much needs to be done; as of March 2017, of 194 WHO member states, only 43 (22%) had formulated national viral hepatitis elimination plans, and an additional 36 (19%) reported that they were in development [3]. National viral hepatitis strategies are critical to define national priorities, outline public health interventions, enable the effective and efficient use of resources, allocate roles and responsibilities to stakeholders and enable measurement of progress.

Elimination efforts require a broader focus on equity, health systems strengthening, universal health coverage and multi-sectoral action. Political commitment to HCV elimination must be gained with allocation of adequate funding, infrastructure and personnel. Resolutions from peak bodies, including the WHO, in support of HCV elimination provide a vital boost to countries seeking to implement appropriate public health interventions. In many settings, lack of promotion and public

awareness regarding HCV infection has contributed to inadequate funding. Regional support for HCV elimination should be established, given the potential for cross border transmission of HCV in countries with different levels of DAA availability and harm reduction. Several countries have shown that rapid scale-up of testing and treatment can be achieved through committed political leadership and a reduction in the prices of essential medicines and diagnostics to expand testing and treatment services [49, 69].

4.6.2 Adequate Health Service Provision

HCV elimination implementation should not occur in isolation. While control programmes are often integrated horizontally with a focus on strengthening primary care and universal health care, elimination and eradication efforts may require a targeted "vertical approach", possibly at the expense of other public health issues. Given limited resources in many countries, HCV elimination will only be possible through an integrated public health approach that strengthens the existing health system, as opposed to establishing a new disease-specific programme. Elimination efforts can support primary health care by providing basic services and improving surveillance, training personnel and expanding immunisation programmes or establishing a global laboratory network, as seen in the response to polio (Box 3).

To increase access and reduce health inequities, delivery of hepatitis and harm reduction services can be tailored to different populations and settings through integration, decentralisation and task-shifting. HCV care and treatment among PWID are feasible and successful across a broad range of multidisciplinary health-care settings, including hospital-based specialist clinics, community health centres, drug and alcohol clinics, prisons, needle and syringe programmes and primary care [196]. Substance use, mental health and medical co-morbidity should be addressed concurrently, with increased HCV treatment uptake and adherence [108] and lower risk of HCV reinfection among PWID receiving OST and mental health counselling services [183]. Holistic models of care external to traditional tertiary hospital clinics may more effectively facilitate the ongoing health care needs of people living with HCV infection, particularly PWID. Models of care which include nurse-led education improve HCV treatment completion [108]. Acknowledgement of the individual circumstances of people living with HCV, particularly PWID, as opposed to rigid criteria will aid in the success of long-term HCV management strategies and drug user health overall.

4.6.3 Deliver HCV Education and Training to Health-Care Providers

Education needs to be delivered to health-care providers, regarding contemporary best practice in the diagnosis and management of HCV infection. Poor knowledge

and lack of competence among health-care providers regarding the natural history and testing algorithm for HCV infection may impede diagnosis [101–103]. Providers may fail to order confirmatory HCV RNA or HCV core antigen testing following a positive anti-HCV antibody test to accurately determine HCV viraemic status (and subsequent requirement for treatment) [103]. Lack of confirmatory testing risks inappropriately labelling people with prior exposure to HCV (anti-HCV antibody positive, HCV RNA negative; indicating spontaneous or treatment-induced clearance) as "hepatitis C positive" or risks failing to diagnose, educate and treat people with current (viraemic) HCV infection (who may be at risk of transmission). There may also be limited knowledge among people living with and at risk of HCV regarding screening, diagnosis and the difference between anti-HCV antibody and HCV RNA or HCV core antigen [104, 105].

Given the pace of change in HCV therapeutic development, targeted education programmes focussing on DAA therapy should be provided to a broad range of health-care providers. Many medical practitioners have previously reported being unwilling to treat PWID, with reinfection, adherence and medication price listed as important concerns when determining an individual's suitability for HCV treatment [197]. Education should be specific to the epidemiological setting, involve both health-care providers and the effected community and address issues of stigma and discrimination.

4.6.4 Reduce Stigma and Discrimination

Social stigma related to HCV infection and the associated risk populations has impeded meaningful policy change. Marginalised populations often fare less well in relation to access to health-care innovations. In particular, PWID are more likely to have socio-economic disadvantage, have considerable medical co-morbidities and experience disparities in health-care access [158]. Drug reform policies must be considered, including decriminalisation of drug use or alternatives to imprisonment, development of policies and laws that decriminalise use of needles and syringes (to permit NSP service provision) and legalising OST for those who are opioid-dependent. Continued community activism and advocacy will be required to ensure that that all people living with or at risk of HCV infection have access to HCV prevention, testing and treatment so that HCV elimination can be achieved.

5 National HCV Elimination Strategies

At the end of 2017, eight countries were on track to meet the 2030 HCV elimination targets, six high income (Australia, France, Iceland, the Netherlands, Spain, Switzerland) and two low to middle income (Georgia, Mongolia) (Table 3). Technically feasible, effective, field-tested HCV elimination strategies will provide a

Table 3 Countries on track to achieve the WHO 2030 HCV elimination targets

Countries on track to achieve WHO 2030 elimination targets	High-income countries						Low- to middle-income countries	
	Australia	France	Iceland	The Netherlands	Spain	Switzerland	Georgia	Mongolia
HCV-infected population, *n*								
2014	230,000	220,000	1,100	20,000	400,000	43,000	175,000	200,000
2016	200,000	200,000	750	17,000	350,000	40,000	150,000	190,000
Diagnosed in 2016, %	80	70	75	45	33	70	33	26
Proportion treated in 2016, %	16	8	40	12	8	5	12	5
New HCV infections in 2016, *n*	6,000	5,100	50	650	2,200	700	6,000	3,200
HCV-related deaths in 2016, *n*	800	800	2	80	1,450	230	210	1,300
National HCV strategy	✓	✓	✓	✓	✗	✗	✓	✓
National clinical guidelines for diagnosis and treatment of HCV	✓	✓	✓	✓	✓	✓	✓	✓
National expert advisory group	✓	✓	✓	✓	✓	✓	✓	✓
Main population group/s affected								
PWID	✓	✓	✓	✓	✓	✓	✓	
HIV-positive gay and bisexual men	✓	✓		✓		✓		
Prisoners	✓		✓					
Blood product recipients							✓	✓
Unrestricted DAA therapy (year)	✓ (2016)	✓ (2017)	✓ (2016)	✓ (2015)	✓ (2017)	✓ (2017)	✓ (2016)	✓ (2016)
DAA prescriber type								
Specialist	✓	✓	✓	✓	✓	✓	✓	✓
General practitioner/primary care	✓	✗	✗	✗	✗	✗	✗	✗
Harm reduction programmes for PWID								
Needle and syringe programme	✓	✓	✓ (limited)	✓	✓	✓	✓	✓
Opioid substitution therapy	✓	✓	✓	✓	✓	✓	✓	✗

Compiled from [4, 14, 49, 69, 75, 198–200]

template for other settings (Box 4). In general, those countries on track to achieve the 2030 HCV elimination targets have demonstrated a strong coordinated multi-sectoral response, with national HCV treatment strategies, allocation of funding and resources, integration into broader health systems, active pursuit of measures to improve DAA access and lower costs and political support (Table 3) [15, 49, 69, 70, 75, 198, 199]. Accumulated success in individual countries, regions or jurisdictions should generate the momentum required for ongoing international support.

Box 4 Key Components Required for National HCV Elimination Strategy

- Screening:
  - General population-based screening:

    Birth-cohort
    All adults (≥18 years), especially in intermediate (2–5%) and high prevalence settings (>5%)
  - Risk-based screening:

    Regular testing of high-risk populations:

    People who inject drugs
    People who are incarcerated
    HIV-positive gay and bisexual men
- Diagnosis:
  - Low-cost, simple, accurate HCV diagnostics
  - Efficient linkage to care
  - Education of health-care providers
- Treatment:
  - DAA therapy:

    Cost-effective; "access for all"
    Expansion of treatment services and capacity building:

    Specialist and non-specialist prescribers
    Education of health-care providers and affected community

    Targeted strategies among high prevalence and incidence populations
    Retreatment of reinfection
- Prevention:
  - Harm reduction:

(continued)

Box 4 (continued)

Needle and syringe programme and opioid substitution therapy

- Behavioural interventions
- Integrated care:

  Mental health assessment

- Education:

  Counselling
  Peer support

- Blood and injection safety

- Epidemiology and modelling:
  - Baseline epidemiology (including number of people living with HCV, HCV-related morbidity and mortality, transmission risks)
  - Modelling to plan beneficial and cost-effective strategies (including screening programmes)
- Surveillance:
  - Regular (at least annual) reporting
  - Monitoring of DAA uptake and effectiveness, HCV viraemic prevalence and incidence (primary and reinfection) and HCV-related morbidity and mortality
- Sustainable funding
- Social and political will:
  - Expert advisory panel
  - Decentralisation of programmes with establishment of local HCV networks
  - Education and public awareness campaign:

    Specific to epidemiological setting
    Involving community and health-care providers

  - Adequate infrastructure with dedicated personnel

Compiled from [13, 33, 52, 78, 94, 146, 152, 153, 156, 157, 182, 183, 196].

5.1 Australia

In 2015, an estimated 227,306 Australians were living with chronic HCV infection, with the vast majority (82%) having been diagnosed [201]. On March 1, 2016,

government-subsidised interferon-free DAA regimens were made available to all Australians (≥18 years of age) living with chronic HCV infection; with no liver disease stage, drug and alcohol or prescriber restrictions; and at no or minimal cost to the individual (AU$0–AU$39.50 per month). By the end of December 2017, an estimated 58,300 individuals had received DAA therapy (26% of people living with HCV in Australia), of which 53,970 received treatment between March 2016 and December 2017 during the first 22 months of the government-funded DAA programme (updated from [49, 69]). In regard to the WHO HCV mortality target, an estimated 70% of Australians with HCV-related cirrhosis initiated DAA therapy between 2014 and 2017 [49].

Rapid HCV treatment scale-up following unrestricted access to DAA treatment was achieved through a coordinated multi-sectoral public health approach, which had been evolving for two decades. Strong advocacy, bipartisan political support, robust existing epidemiology and surveillance, a high level of HCV screening and diagnosis [14], high-level harm reduction for PWID [155] and long-standing continued HCV education initiatives for health-care professionals [202] have all contributed to the successful response to HCV in Australia. One of the pillars on which this success has been built is the long-standing commitment to a comprehensive national strategy (first *National Hepatitis C Strategy* launched in 2000 [203]; fifth *National Hepatitis C Strategy* to be launched in 2018), with contributions from all major stakeholder, including government, drug user and hepatitis community organisations and medical and academic communities.

Unrestricted access to DAA therapy and a high rate of HCV diagnosis have established a foundation for achieving the 2030 WHO HCV elimination targets. However, after very high initial DAA treatment uptake in the early months of the DAA programme, a subsequent decline in the number treated per months was evident (Fig. 3) [69]. This "warehouse" effect will need to be countered by enhanced case finding outside of traditional tertiary clinics; encouragingly, the proportion of DAA initiations by general practitioners was seen to be increasing, and the age of

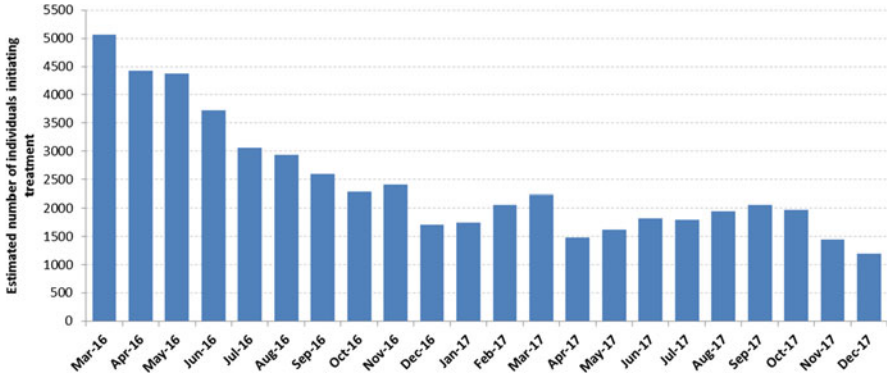

Fig. 3 Estimated number of people initiating direct-acting antiviral treatment per month in Australia between March 2016 and September 2017

people treated was declining, suggesting broader community access for potentially more "difficult-to-reach" populations. The key to HCV elimination in Australia will be sustained DAA treatment scale-up with equitable access and uptake.

5.2 *Georgia*

In 2014, an estimated 150,000–175,000 people were living with chronic HCV in Georgia (anti-HCV antibody prevalence 5%), most of who were current or former PWID [14]. In 2015, Georgia launched its national HCV elimination strategy in partnership with the US Centers for Disease Control and Prevention and Gilead Sciences, on the background of strong political and social support [198, 199]. The national programme incorporates universal access to DAA treatment, HCV screening, national surveillance and monitoring and public awareness campaigns [204]. DAA treatment was initially restricted to people with advanced liver disease, but in June 2016, eligibility criteria were expanded, such that all people living with HCV infection could access DAA treatment. In the first 20 months following launch of the national strategy, almost 28,000 people commenced HCV treatment (20% of the Georgian population living with HCV) [14, 198].

Unlike Australia, the proportion diagnosed with HCV prior to the launch of the national strategy was low (10–15%). A steady decline in the number of people commencing treatment was seen in the last 3 months of 2016, similar to the "warehouse" effect observed in Australia and other countries. In response, efforts to enhance HCV screening, linkage to care and treatment uptake have commenced, with provision of outreach services for at-risk populations, including PWID. Subsequently, the proportion of people living with HCV who are diagnosed has risen to over 30% [14]. As the Georgian experience to date highlights, unrestricted access to DAA therapy alone is insufficient to achieve elimination. A collaborative systematic approach is required, with high levels of HCV screening, linkage to care and treatment uptake, alongside transmission prevention.

5.3 *Egypt*

In 2014, 5.6 million people were estimated to be living with HCV infection Egypt [14]. While Egypt is not on track to achieve the WHO elimination targets by 2030, a strong government response to the HCV epidemic in the country has resulted in massive DAA treatment scale-up, with over one million people initiating treatment between 2015 and 2017 (2015: 200,000; 2016: 577,000; 2017: 400,000) [14, 70]. The comprehensive national HCV elimination programme supports initiatives to accelerate DAA access, including domestic generic production, fast-track registration and nationwide treatment facilities. The national HCV treatment programme has demonstrated the capacity to evolve, responding to local

requirements. In October 2014, DAA treatment was prioritised to those with advanced fibrosis (F3) or cirrhosis (F4). In May 2015, DAA treatment restrictions were lifted, and all people living with HCV were able to access therapy.

A significant issue facing Egypt in its attempt to achieve elimination is the need to rapidly scale-up screening and diagnosis. In 2016, approximately 4.5 million Egyptians were screened for HCV, with over 400,000 people newly diagnosed with current (viraemic) HCV infection [14]. Given the burden of infection, Egypt has entered into diagnostic price negotiations, reportedly obtaining significantly discounted oral rapid anti-HCV antibody tests (price: US$4 per test) [13]. Given the high prevalence, a national testing programme to screen all Egyptians is under consideration, with financial backing from the World Bank.

6 Conclusion

HCV elimination is possible. The absence of a non-human reservoir, the availability of sensitive diagnostic tools and the development of highly effective DAA therapy confirm the biological and technical feasibility. When coupled with prevention strategies including infection control, blood safety and harm reduction for PWID, the 2030 WHO elimination targets are achievable.

While considerable progress has been made in the global response to HCV, additional momentum will be required to foster ongoing political and societal will to allow all countries to act upon the lessons learnt so far, such that no one is left behind. Many countries have not yet seized the opportunity to initiate and scale-up HCV treatment services, while HCV testing coverage and diagnosis remain woefully low in most regions. Massively enhanced HCV screening and health system infrastructure development must be couple to provide expedient linkage to care and DAA treatment provision. Countries must push to achieve DAA “access for all” and, in the process, overcome barriers imposed by high drug pricing, liver disease stage and drug use restrictions and stigma. Countries which have made considerable headway are those with a strong government response, national elimination and treatment strategies and policies to improve HCV testing and DAA access. Ultimately, to maximise the population-level impact of DAA therapy and achieve the WHO elimination targets, HCV screening, diagnosis and linkage to care and treatment must dramatically improve.

HCV control and elimination is conceptually simple, with a clear unequivocal outcome. A concerted coordinated global public health response will be required from governments, researchers, health-care providers, policy-makers, community members, advocates and pharmaceutical and diagnostic industry partners. Optimism and aspiration fuel human effort. While the WHO has moved the epidemiological goalposts, striving for HCV elimination is a worthy endeavour.

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Perspectives on HCV Cure

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Abstract The development of interferon-free cures for hepatitis C has revolutionized the treatment of patients chronically infected with the hepatitis C virus. Since 2010, ten new curative regimens have been introduced into clinical practice. These new regimens have delivered cure rates in excess of 95% in as little as 8–10 weeks on therapy. Never before has there been an absolute cure for a chronic viral disease. This medical breakthrough has been made possible by the commitment of scientists and clinicians from both academia and industry working toward a common goal. Because of the availability of these curative regimens, it is now possible to contemplate eliminating HCV as a global public health problem as outlined by the World Health Organization. This perspective will give a brief commentary of the achievements and future possibilities provided by direct-acting antiviral interferon-free HCV cure therapies.

Keywords HCV cure therapies, HCV elimination, Hepatitis C, Hepatitis C virus, Interferon-free HCV therapy, WHO HCV elimination program

Until 2010 hepatitis C patients had only one choice in their quest to rid themselves of HCV and the specter of liver cirrhosis and liver cancer. That choice was to endure 48 weeks of grueling interferon therapy. Many patients could not endure the constant flu-like symptoms, the anemia, and the neurological side effects that accompanied this therapy with only a modest chance of achieving a cure. With the first introduction of the direct-acting antivirals, telaprevir and boceprevir, cure rates improved but interferon was still required, and additional side effects did not improve the patient experience and sometimes made it worse. Then in December 2013, sofosbuvir plus ribavirin was approved as the first interferon-free curative regimen for HCV and

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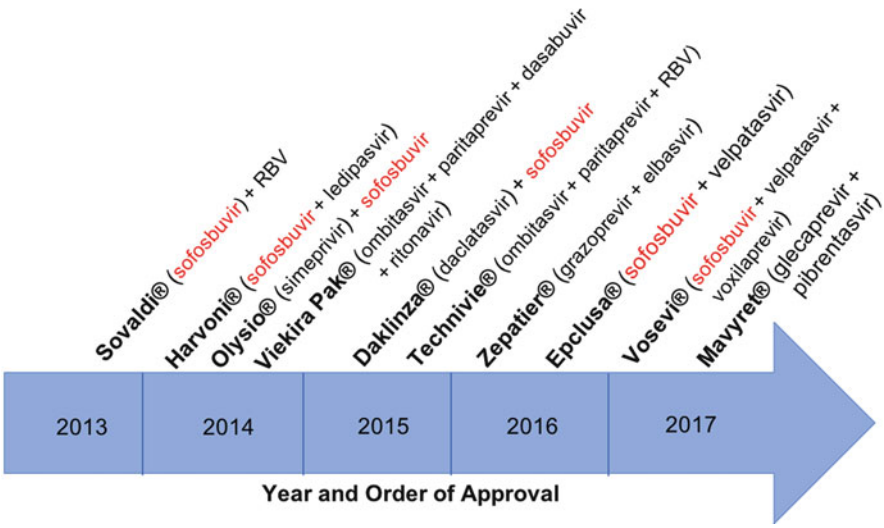

Fig. 1 HCV cure direct-acting antiviral drug combinations listed in chronological order of approval

subsequently transformed the treatment paradigm for HCV patients. Since 2013 ten new drug combinations have been approved as interferon-free HCV curative therapies (Fig. 1). Several of these drug regimens provide patients with high cure rates (95–100% SVR) and pan-genotypic coverage. This means that today patients irrespective of their genotype status can take a single pill once a day for only 8–12 weeks and be forever cured of their HCV infection. Never in the history of pharmacopeia has there been a therapy that can cure a chronic viral disease, and consequently, the delivery of an interferon-free HCV cure is undoubtedly a monumental achievement of modern medicine.

The impact of an HCV cure has been life changing for patients. Those patients cured of chronic HCV infection demonstrated improved liver function and improved liver fibrosis scores [1–3]. The specter of developing liver cirrhosis and liver cancer has been dramatically reduced [4, 5]. For patients, being cured of their HCV has resulted in a profound improvement in quality of life and afforded them the ability to live normal lives [6–8]. Patients awaiting liver transplants no longer have to be concerned with getting recurrent HCV because they can now be treated either prior to transplant or post-transplant to eliminate the chance of recurrent HCV [9]. In fact it is now possible to transplant an HCV-infected liver, thus expanding the available organs for transplant [10]. In addition, HCV patients coinfected with HIV are now curable without compromising their HIV treatment [11, 12]. Without a doubt DAA HCV cure therapies are having a major impact on public health.

With safe, highly effective, short treatment duration and convenient cures now available, the possibility exists that HCV can be eliminated as a major global public health problem. This has become an objective of the World Health Organization (WHO) which declared in 2017 the goal of eliminating viral hepatitis, including

HCV, by 2030 [13]. Many countries have established national initiatives to achieve this goal. According to the WHO, more than 80 countries have established HCV elimination programs; however, less than half have committed financial resources to achieve the objective. The national health authorities in Georgia, Egypt, Australia, the United Kingdom, Iceland, and several other countries have meaningfully signed onto this goal and have committed funding to make it happen. Yet, many countries including the United States have yet to fully embrace this initiative and thus continue to deny their citizens of the hope of eliminating this disease. In fact in many instances, the approach has been to only provide access to those who are the sickest and let the rest wait until they can't wait any longer. This wait-and-see approach along with other access restrictions only prolongs the overall burden to society, prolongs the HCV patient suffering, and increases the chances of disease spread.

There are many challenges to achieving the WHO goal of eliminating HCV by 2030. These challenges include implementing harm reduction approaches, identifying those who are infected, testing high-risk individuals and groups, and ultimately getting access to therapy for those who need it [13, 14]. To achieve the 2030 goal, a concerted effort by all countries must be realized across all facets of the elimination strategy. Having a cure is insufficient if the spread of HCV is not controlled and if those who are infected are not identified so that they can be treated. Today, of the 71 million individuals chronically infected with HCV, it is estimated that approximately 20% have been diagnosed (36% in the United States), and only a fraction of those have been treated [13]. To compound the problem, the opioid epidemic problem in the United States is leading to an alarming increase in the spread of HCV. Training and protecting healthcare workers with good practices and implementing needle exchange programs to protect those who inject drugs are ways to help curb the spread of HCV; however, there needs to be a political will to abandon parochial views that impede progress on these recommendations.

One key aspect to achieving the WHO viral hepatitis cure goal is access to curative therapies. This has been a topic of much debate as related to the cost of newly developed DAA therapies. The cost of these revolutionary therapies was strongly criticized by the medical community and patients, even though they delivered exceptionally high cure rates in all patient populations, were very well tolerated with virtually no significant side effects, and achieved cures after only 8–12 weeks of a one pill once-a-day oral regimen [15–17]. This criticism seemed to overlook the fact that patients were being forever cured and future costs to treat the symptoms of chronic HCV infection were no longer incurred. The cost coupled with a large demand led to rationing of access to contain costs. However, through increased competition and outcry from the medical community and patient groups, the cost of curative therapies began to fall substantially. The pharma industry gradually responded to the call for more affordable therapies with reduced pricing and with establishment of global programs that enables access to these drugs in underdeveloped regions of the world at much reduced prices. Yet the debate rages on as to what value to put on innovative therapies, especially those that cure a disease, and how should the innovators be compensated for the risk they take when the success rate of delivering an innovative drug to patients is generally less than 20% [18]. However,

this debate must be had so all groups become sensitized to the needs of the other and so that the development of innovative therapies to cure and treat debilitating diseases can continue to be delivered to the patients who need them. Extreme views on either side of the debate will not lead to a solution to this problem and will not result in the delivery of new innovative therapies.

It must be recognized that HCV curative therapies are a clear example of what can be achieved when academia and industry work synergistically to address a global health problem. Diseases like HCV are too complex and the challenges too great for any single group to "do it all." From the discovery of the HCV virus, to mapping its genome and identifying its gene products, to understanding its molecular virology and building a simple cell system to study the virus and screen drug candidates, to designing new drug candidates and perfecting them to be viable clinical candidates, and to the development of clinical strategies that assess candidate efficacy especially in drug combinations requires a symbiotic relationship between academia and industry. It is this continued collaborative relationship that will make other medical miracles like the cure for HCV a reality for patients.

The WHO goal of eliminating viral hepatitis by 2030 is a worthy objective. However, to truly achieve global HCV eradication, another piece of the puzzle will be needed, a preventive vaccine [19]. A vaccine will dramatically reduce the spread of HCV, especially because active carriers generally don't know they are infected since HCV is a disease that is asymptomatic for many years. Even patients who have been cured can become reinfected. To date such a vaccine has been elusive. This is attributed to the high sequence diversity seen with HCV resulting from its high mutational rate and a lack of complete understanding of what exactly drives protective immunity for this virus. This is an area of active research that once solved will provide the final tool in the toolbox needed to eradicate HCV.

It is not an exaggeration that as of today, at least several million HCV sufferers have been cured of their HCV infection and are living happier and more productive lives. What has been accomplished by those who committed their lives and talents to addressing a global health problem is nothing short of spectacular. The product of their efforts has spawned HCV elimination programs which are actively running in many parts of the world. However, HCV elimination needs to be fully embraced by all countries as were elimination programs for polio and small pox. Only then, millions more will be cured, and the fear of living with HCV will become a thing of the past.

Compliance with Ethical Standards

Conflict of Interest: Author is co-founder, Chief Scientific Officer and shareholder of Arbutus Biopharma, Inc. Author is a consultant for Gilead Sciences, Inc. Author was an employee of Pharmasset Inc. and Gilead Sciences Inc.

Ethical Approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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