
Keywords

HCV life cycle • Targets for direct antiviral therapy • Attachment inhibitors • Entry inhibitors • Translation inhibitors • Post-translational processing inhibitors • Replication and assembly inhibitors • NS3/4A inhibitors • NS5B polymerase inhibitors • NS5A inhibitors • Clinical development of direct antiviral therapy • Interferon-free combination therapy

Introduction

For almost one decade pegylated interferon alfa in combination with ribavirin has been the standard of care (SOC) in the treatment of patients with chronic hepatitis C [1, 2]. Despite numerous attempts to optimize peginterferon alfa/ribavirin therapy more than half of all patients with chronic HCV, genotype 1 cannot be cured with this treatment. The limitations of antiviral therapy with peginterferon alfa/ribavirin may be overcome by direct antiviral agents (DAA) which specifically target hepatitis C viral proteins.

Due to substantial progress in the development of DAA, treatment of chronic hepatitis C is about to enter a new era (Fig. 17.1). The most advanced DAA are directed against the NS3/4A protease or

the NS5B polymerase. Inhibition of these targets blocks post-translational processing of the viral polyprotein or inhibition of viral replication, respectively. Many more targets for DAA targeting viral entry, initiation of translation, virus assembly and release have been identified and may be the targets of future agents.

Development of DAA from a Historical Perspective

Discovery of the HCV Life Cycle

For many years, the understanding of the hepatitis C virus (HCV) life cycle has been hampered by the lack of reliable and efficient cell culture systems. Primary hepatocytes can be infected by serum-derived HCV, however, this system only supports low-level replication which is not sufficient to perform pharmacological studies [3]. In 1999, Lohmann et al. described a subgenomic HCV replicon that replicated at high levels upon transfection into a human hepatoma cell line [4]. The HCV replicon system provided the basis

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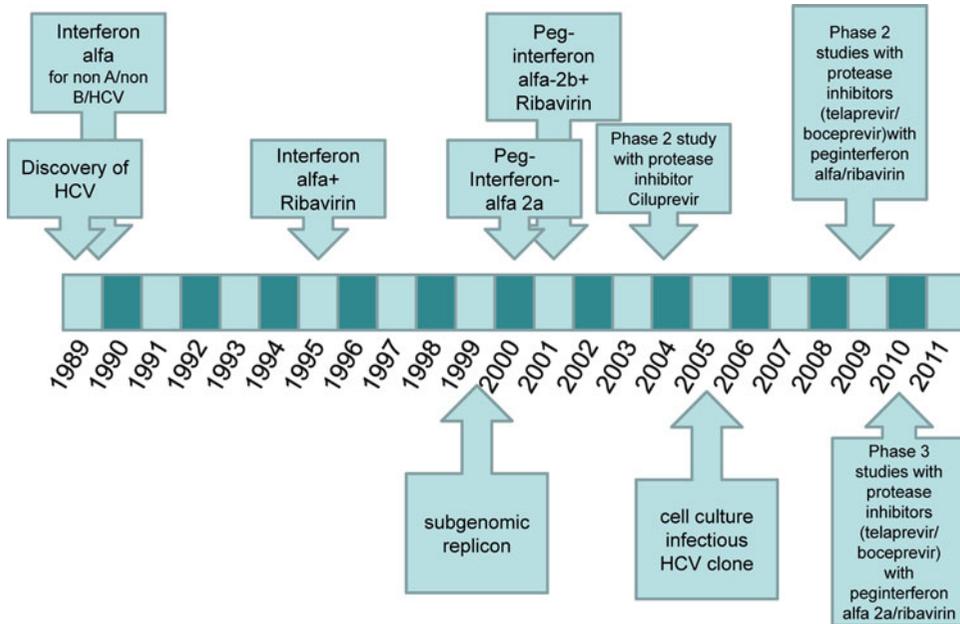


Fig. 17.1 Milestones in the development of HCV therapy

for detailed molecular studies of HCV and the development of antiviral drugs. The major limitation of the replicon system, however, is the lack of the structural proteins which are necessary for attachment, entry, and assembly.

To study attachment and cell entry, recombinant HCV-envelope glycoproteins, HCV-like particles produced by insect cells, and HCV pseudotypes particles consisting of HCV envelope glycoproteins assembled onto retro- or lentiviral core particles were developed [5]. In 2005, Wakita et al. described the isolation of a HCV genotype 2a JFH1 strain from a patient with fulminant hepatitis which replicated efficiently in Huh7 cells without cell culture adaptive mutations and resulted in secretion of viral particles that were infectious for cultured cells and a chimpanzee [6]. The cell culture infectious HCV clone enables the study of the whole HCV replication cycle. The subgenomic replicon and the cell culture infectious HCV are powerful tools for large-scale screening of HCV inhibitors against multiple viral targets.

Structure of the Hepatitis C Virus

Due to the development of *in vitro* models, tremendous progress has been made in the understanding of the HCV replication cycle since the description of the HCV in 1989. Nevertheless, the structure of HCV still is not completely elucidated. In analogy to other members of the flaviviridae family such as the dengue or tick-borne encephalitis virus, HCV is thought to adopt a classical icosahedral scaffold in which the two envelope proteins E1/E2 are anchored to the host cell-derived double layer lipid envelope. Underneath the membrane is the nucleocapsid composed of multiple copies of the core proteins in complex with the genomic RNA [7, 8].

Life Cycle of the Hepatitis C Virus

Infection starts with adsorption and entry of HCV to the target cell (Fig. 17.2) [8]. Adsorption and entry is the result of a complex interaction

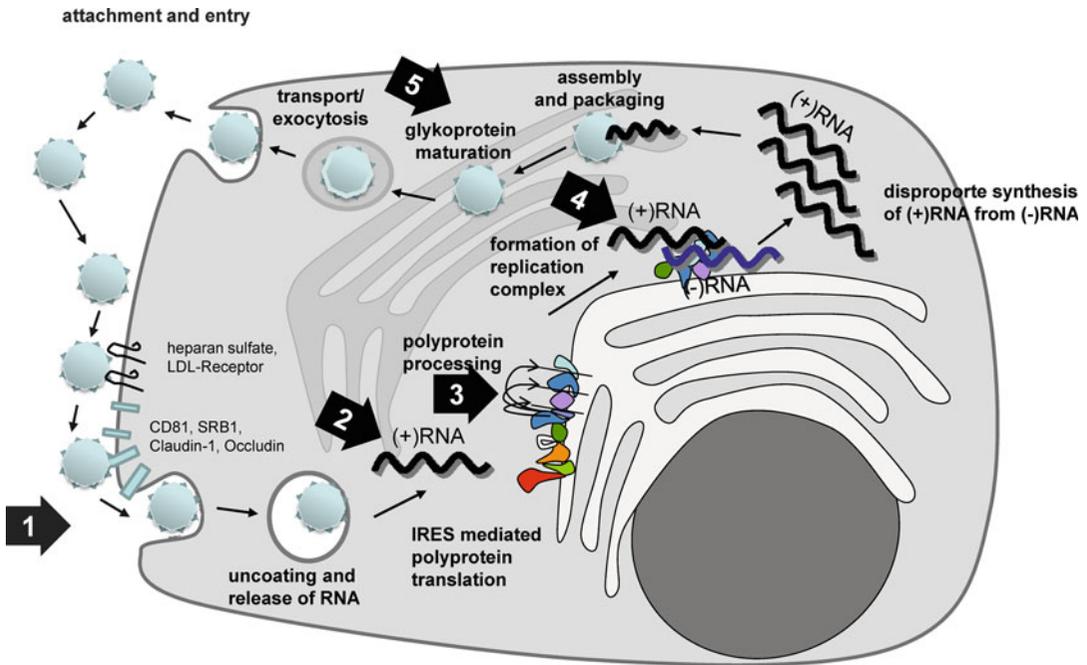


Fig. 17.2 HCV life cycle. The arrows indicate targets of antiviral therapy. (1) Entry inhibitor, (2) translation inhibitor, (3) post-translation processing inhibitors,

(4) replication inhibitors, (5) assembly and release inhibitors (based on data from Moradpour et al. [8] and Pawlotsky et al. [82])

between the envelope proteins E1/E2 and a couple of cellular receptors [9]. After cell entry the envelope proteins fuse with the membrane of the endoplasmic reticulum and release the HCV RNA into the cytoplasm. The HCV genome, a positive-sense 9.6 kb RNA molecule, encodes for a polyprotein of approximately 3,100 amino acids. Translation of the HCV polyprotein is initiated by an internal ribosome entry site (IRES) located in the 5' non-translated region (NTR) of the HCV genome. Host- and virally encoded proteases process the polyprotein into non-structural (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) proteins, which are required for HCV RNA translation and replication, and structural proteins (core, envelope E1, E2, and p7) (Fig. 17.3).

Attachment and Entry Viral Determinants

The envelope proteins E1 and E2 are type I transmembrane glycoproteins, with N-terminal ectodomains and a short C-terminal transmembrane domain and assemble as non-covalent heterodimers.

Experimental studies with pseudotypes lacking envelope proteins or bearing-mutated envelope proteins clearly demonstrated that envelope proteins are essential for infectivity [7].

HCV is the result of continuous de novo infection and elimination of infected cells. Due to the high de novo infection rate of this virus, blocking de novo infection could be a promising strategy to treat HCV. Antibodies directed against the hypervariable region (HVR1) within the envelope 2 protein or antibodies directed against the N-terminal E1 region have been shown to inhibit host cell recognition and attachment [10, 11]. Therefore, the envelope proteins are potential targets for antiviral therapy. However, the sequence of envelope proteins shows a high variability especially in the HVRs making it difficult to develop specific inhibitors.

Cellular Receptors

CD81 was the first cellular HCV receptor that was identified by expression cloning using a cDNA library derived from the human T cell

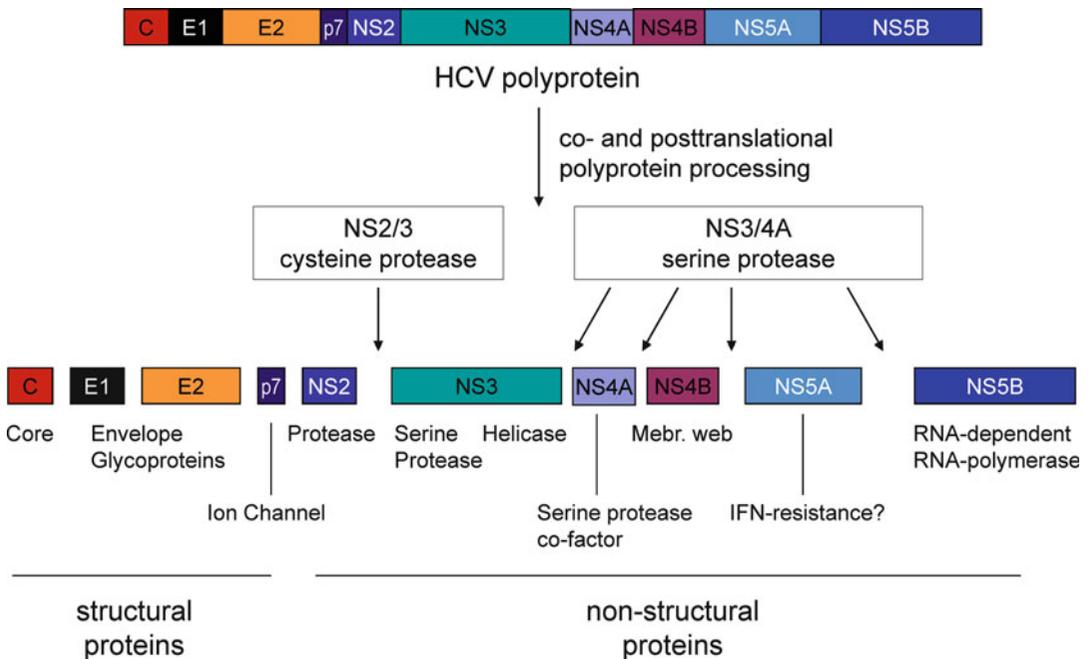


Fig. 17.3 Organization of the HCV polyprotein and co- and post-translational polyprotein processing

lymphoma cell line Molt-4 with the ability to bind recombinant E2 proteins [12]. The 25-kDa cell surface protein CD81 is widely expressed and is involved in pleiotropic activities. CD81 is essential for HCV but not sufficient for HCV infection. Due to the conserved structure of CD81, it appears as a promising target to block HCV entry. Anti-CD81 antibodies inhibit HCV pseudotype and cell culture infectious HCV particles to enter Huh-7 hepatoma cell lines [5, 6]. Furthermore, silencing of CD81 by small interfering RNAs (siRNAs) renders hepatoma cell lines resistant to HCV pseudotype and cell culture infectious HCV infection [13].

The scavenger receptor class B type I (SR-BI) was the second essential HCV receptor which was detected by recombinant E2 binding to the hepatoma cell line HepG2 lacking expression of CD81 [14]. SR-BI is a 82-kDa protein which is highly expressed in liver and steroidogenic tissues as well as in human monocyte-derived dendritic cells. SR-BI has the ability to bind HDL, LDL, and is involved in bidirectional cholesterol

transport at the cell membrane. Administration of antibodies against SR-BI as well as silencing of SR-BI is associated with loss of HCV pseudotype infectivity [15].

Other cell surface molecules such as heparan sulfate, the LDL-receptor, as well as the C-type lectins DC-SIGN and L-SIGN have been shown to bind the HCV E2 protein, however, all these factors were not sufficient to render HCV non-permissive cell line permissive to HCV infection [16]. Another important step in understanding the HCV entry mechanism was the identification of claudin-1, a tight junction component that is highly expressed in the liver [17]. Claudin-1 was shown to be required for HCV infection of human hepatoma cell lines and was the first factor to confer susceptibility to HCV when ectopically expressed in non-hepatic cells. Nevertheless, non-human cells were still not susceptible to HCV even when human CD81, SR-BI, and claudin-1 were expressed indicating that further essential receptors for HCV were required. Using a similar screen that has led to identification of claudin-1,

occludin was identified as a fourth host-cell protein essential for HCV entry [18]. Occludin renders mouse cells susceptible to HCV pseudoparticle infection. In addition to occludin, HCV pseudoparticle infection of murine cells required expression of CD81, SR-BI, and claudin-1.

Translation

The long open reading frame of the HCV genome is flanked at the 5'- and 3'-ends by short highly structured NTRs [7, 8]. The 5'-end contains an IRES which is required for the initiation of HCV polyprotein translation. The 5'-NTR consists of four highly ordered domains. Domains I and II are relevant for replication, domains II–IV together with the first 24–40 nucleotides of the core region constitute the IRES. HCV translation initiation occurs through the formation of a binary complex between the IRES and the 40S ribosomal subunit. Micro-RNA 122 was shown to bind to the 5'-NTR and enhance viral replication [19]. The 3'-NTR consists of a short variable poly U/UC region with a length of 80 nucleotides and an almost invariant RNA element of 98 nucleotides, the X-tail [7, 8]. The conserved elements are essential for HCV replication in cell culture. Detailed structural analyses have been performed of the 5'-NTR particularly with the IRES.

Post-translational Processing

Translation of the HCV open reading frame leads to the formation of a polyprotein precursor. The endoplasmic reticulum protease processes the structural proteins. The NS2/3 protease mediates a single cleavage at the NS2/NS3 junction, whereas the NS3/4A protease cleaves at four downstream sites in the polyprotein.

NS2

The crystal structure of the catalytic domain of the NS2 protease revealed a dimeric cysteine protease with two composite active sites [20]. NS2-deficient subgenomic replicons replicate efficiently in cell culture indicating that NS2 itself is not strictly required for genome replication.

New findings with the cell culture infectious HCV system indicate that NS2 is an essential cofactor for virus assembly [9, 21]. This function

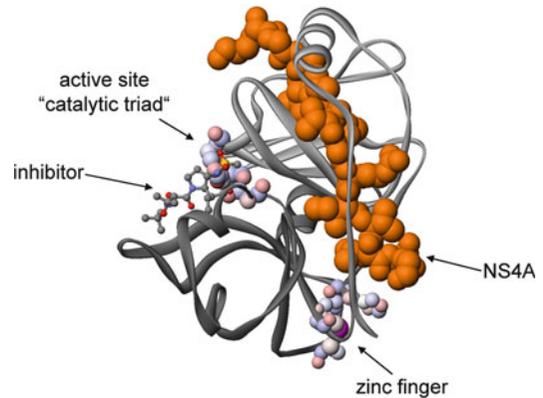


Fig. 17.4 Structure of the NS3/4A protease

involves interaction of NS2 with the core and the envelope proteins as well as with p7 [9]. In addition, NS2 interacts with cellular factors such as cyclophilin A. Additional findings report that NS2 is able to modulate apoptosis and gene expression [9].

NS3/4A

NS3 carries in the N-terminal part a serine-type protease [22]. The enzyme has a typical chymotrypsin-like fold and is composed of two beta barrel domains displaying on their interface the classical active site residues of serine-type proteases (Fig. 17.4). NS3 possesses intrinsic proteolytic activity. NS4A is a co-factor that increases NS3-associated polyprotein cleavage. NS4A anchors the protease to intracellular membranes, contributes to its complete folding, stabilizes the protease against degradation, and activates protease activity by changing the geometry of the catalytic triad. The NS3/4A cleaves at four downstream sites in the polyprotein to generate the N-termini of the NS4A, NS4B, NS5A, and NS5B proteins. The NS3/4A serine protease has also been shown to inactivate the host proteins Trif and Cardif which are involved in the interferon response mediated by toll-like receptor 3 (TLR3) and retinoic-acid inducible gene I (RIG-I), respectively [23]. Furthermore, it has been shown that NS3 is also an integral part of the viral RNA replication complex, functions as a RNA helicase and a nucleotide triphosphatase (NTPase) [24].

Replication

NS4B – Formation of the Replication Complex

NS4B is a highly hydrophobic molecule predicted to contain four transmembrane regions. NS4B induces the formation of an intracellular membrane structure, which represents the site of HCV replication, and is required to assemble the other NS proteins within these membrane-associated replication complexes [9]. An arginine-rich-like motif within NS4B that mediates binding to the 3'-terminus of the negative HCV strand is important for HCV RNA replication [9].

NS5A – Replication, Modulation of Cellular Processes

NS5A is a pleiotropic protein with key roles in both viral RNA replication and modulation of the physiology of the host cell. NS5A is composed of an N-terminal amphipathic alpha helix serving as a membrane anchor and three distinct domains that are separated by the low complexity sequences LC I and LC II [8, 9]. Domain I appears to be involved in RNA binding. Domain II may be involved in inhibition of interferon-induced dsRNA activated protein kinase (PKR). Domain III is poorly conserved and seems to be of minor importance for HCV replication. The X-ray crystal structure of domain I has been revealed [25].

NS5A is a phosphoprotein, basally phosphorylated and hyperphosphorylated forms have been identified [26]. The casein kinase CKII has been identified to be involved in NS5A hyperphosphorylation. The detailed role of NS5A phosphorylation is unclear. A model suggests that NS5A phosphorylation serves as switch between HCV replication and assembly [9]. NS5A is mostly known due to its potential effect on interferon-alfa signaling. Furthermore, NS5A has been shown to affect cell growth of target cells and apoptosis [9].

NS5B – RNA-Dependent RNA Polymerase

NS5B is the RNA-dependent RNA polymerase and the catalytic core of the replication complex. The polymerase activity appears to be modulated by interaction with the viral factors NS3 and NS5A and the host factor cyclophilin B. NS5B

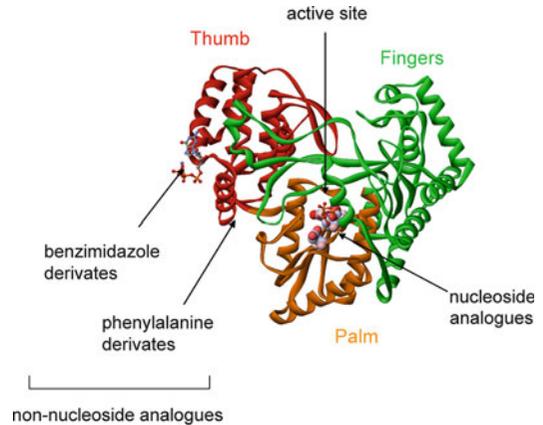


Fig. 17.5 Structure of the NS5B RNA-dependent RNA polymerase

reveals the typical polymerase structure, a classical “right hand” shape of thumb, palm, and finger (Fig. 17.5) [27]. Multiple interactions between the finger and thumb subdomains create a tunnel in which a single-stranded RNA molecule is directly guided to the active site.

Assembly and Release

HCV particles presumably form by budding into the endoplasmic reticulum or an endoplasmic reticulum-derived compartment and exit the cell through the secretory pathway [8]. Assembly and release of HCV particles involves a complex interplay between viral proteins that is not fully understood. Interactions between NS2, E1, and p7 as well as between NS2 and NS3 were shown to be essential for virus assembly [7]. The HCV core protein targets to early and late endosomes. Movement of core protein to the early and late endosomes and virus production were shown to require an endosome-based secretory pathway [28].

The small HCV protein p7 has the ability to form a membrane ion channel. Recently, Steinmann et al. showed that p7 has a central role in HCV assembly and release of cell culture infectious HCV particles [29]. p7 resembles viroporins, a class of proteins known from other viruses such as HIV-1 and influenza A virus. Members of this group of functionally related proteins form membrane pores that promote virus

Table 17.1 Entry inhibitors

Drug	Company	Target	Study phase (identifier)
Directed against viral structures			
MBL-HCV-1	University of Massachusetts	Anti-HCV antibody	Phase 1 (NCT01121185)
XTL-6865	XTL Biopharmaceuticals	Anti-HCV Antibody	Preclinical
HuMax-HepC Antibody	Genmab	Anti-HCV Antibody	Preclinical
Polyclonal Antibody	Civacir	Anti-HCV Antibody	Stopped (NCT00473824)
Directed against host structures			
ITX-5061	iTherX Pharmaceuticals	Downregulation of SR-B1 (Host target)	Phase 1 (NCT01165359)
ITX4520	iTherX	SR-B1	Preclinical
Anti-CD81	Non-commercial	CD81	Preclinical
Anti-Claudin	Non-commercial	Claudin-1	Preclinical
Unknown target			
PRO 206	Progenics	Entry inhibitor	Preclinical
SP-30	Samaritan Pharm.	Entry inhibitor	Preclinical
Therapeutic vaccination			
GI 5005	Globeimmune	Therapeutic vaccine	Phase 2 (NCT00606086)
IC41	Intercell	Therapeutic vaccine	Phase 2 (NCT00602784)
TG4040	Transgene	Therapeutic vaccine	Phase 2, planned (NCT01055821)
Hepavaxx C	ViRex Medical	Therapeutic vaccine	Preclinical

release and in some cases also virus entry. Conserved p7 residues crucial for functioning of this protein were identified that could be relevant for drug design.

Targets of DAA

Entry Inhibitors

Entry Inhibitors Against Viral Structures

As shown in Table 17.1, numerous compounds for entry inhibition have been developed. Approaches for entry inhibition include monoclonal or polyclonal antibodies against the envelope proteins or therapeutic vaccines. Proof of principle for in vivo inhibition of HCV infection by entry inhibitors was provided by the fully humanized monoclonal antibody MBL-HCV1 directed against a linear epitope of HCV E2 glycoprotein. Three chimpanzees received a single dose of the Anti-E2 antibody intravenously before challenge with HCV 1a strain H77. No HCV RNA was detected in the serum of the 250 mg/kg-dosed chimpanzees through week 20

while the 0 and 50 mg/kg-dosed chimpanzees both became infected by day 14 [30]. The antibody is being currently evaluated in a phase 1 trial (Table 17.1).

Another approach to prevent HCV infection is therapeutic vaccination. T- and B-cell epitopes are administered to elicit a humoral or cellular immune response. The therapeutic vaccine GI 5005 expressing NS3 and core antigens was administered in combination with peginterferon alfa-2a and ribavirin in treatment-naïve and non-responder genotype 1 patients. Triple therapy with GI-5005 showed superior end of treatment response rates when compared with standard therapy (63% vs. 45%) [31]. However, the sustained virologic response (SVR) rates were not improved. Other therapeutic vaccines are in development (Table 17.1).

Entry Inhibitors Against Host Structures

Promising targets are the HCV receptors CD81, SR-B1, occludin, and claudin-1. CD81 is highly conserved and essential for HCV infection. The problem of CD81 is the ubiquitous distribution

that may be associated with adverse effects. Monoclonal anti-CD81 antibodies have been shown to block HCV infection *in vivo*. Furthermore, potential small molecule inhibitors have been reported. However, clinical data on DAA targeting CD81 are not yet available.

The entry inhibitor ITX-5061 is directed against SR-B1. In preclinical trials the compound has shown a potency in the picomolar range, and was equally potent against both genotype 1 and genotype 2 viruses. The ability of ITX-5061 to reduce viral load in treatment-naïve and previously treated patients with HCV infection is currently being evaluated in a placebo-controlled, randomized trial (Table 17.1).

Occludin and claudin-1 are major components of bicellular tight junctions that are located in the apical part of lateral membranes and comprise an elaborate network of paired strands, which form kissing points that eliminate extracellular space. It is the question if tight junctions are potential targets for drug therapy. The toxins produced by *vibrio cholerae* and *clostridium perfringens* exert their toxic effect by altering the tight junction indicating that tight junction proteins are potential targets for therapy. However, targeting the tight junction might be associated with severe adverse reactions. Recently, it was shown that anti-claudin antibodies have the potential to inhibit infection of cultured hepatocytes confirming that claudin-1 is a potential drug target despite its location in tight junctions [32]. Clinical trials and potential adverse events are not yet available.

Inhibition of Post-translational Processing (NS2, NS3/4A)

NS2

Due to our limited knowledge about the function of NS2, this protein has been neglected as a drug target until recently. However, recent advances have identified NS2 as an essential HCV protein with multiple functions. The structure of the NS2 cysteine protease shares no obvious similarities to any known protease in eukaryotes and would therefore make an attractive target for antiviral therapy. Inhibitors have not been described so far.

NS3/4A

The three-dimensional structure of NS3/4A revealed an unusually shallow substrate-binding pocket (Fig. 17.4). This structure of the binding pocket made the design of specific inhibitors difficult because long interaction surfaces are required. Nevertheless, NS3/4A is the best studied target for antiviral therapy to date. As shown in Table 17.2, multiple NS3/4A protease inhibitors have been developed and have entered clinical evaluation. The clinical development of the most advanced NS3/4A protease inhibitors telaprevir and boceprevir is described below.

The heterogeneity of HCV has a strong impact on antiviral activity of direct antiviral drugs and the development of resistance. The current NS3/4A protease inhibitors have been developed for HCV genotype 1-infected patients (Table 17.2). Compared with HCV genotype 1-infected patients, the protease inhibitor telaprevir, e.g., has a markedly reduced antiviral activity in HCV genotype 3-infected patients [33].

A major problem in the use of current NS3/4A protease inhibitors is the development of drug-resistant HCV strains. Several mutations associated with resistance were identified *in vitro* and in patients during treatment with protease inhibitors. The currently known mutations associated with resistance are shown in Fig. 17.6. NS3/4A inhibitors can be distinguished into linear and macrocyclic inhibitors. As shown in Fig. 17.6, the resistance profile differs slightly among the NS3/4A inhibitors of the two classes. The resistance mutation at position 168 is associated with macrocyclic inhibitors. For several protease inhibitors, escape was shown to be less frequent in genotype 1b-infected patients than in genotype 1a-infected patients which is most likely related to the requirement of two mutations for resistance development in genotype 1b compared to one mutation in genotype 1a isolates [34].

Another strategy to overcome NS3/4A protease inhibitor resistance is to target the NS4A cofactor. GS9132/ACH-806 was the first NS4A antagonist which showed promising results in a phase I trial but was not further developed due to renal toxicity. ACH-1095 is another NS4A antagonist for which phase I trials are planned.

Table 17.2 Post-translational processing inhibitors

NS3/4A protease inhibitors in registered clinical trials	Company	Target	Study phase (identifier)
Telaprevir (VX-950)	Vertex	NS3	Phase 3, naïve/treatment-experienced GT1 pts (NCT00627926/ NCT00703118) Triple 8–12 weeks, followed by SOC 12–36 weeks
Boceprevir (SCH 503034)	Schering-Plough	NS3	Phase 3, naïve/treatment-experienced GT1 pts. (NCT00705432/ NCT00708500) SOC lead-in 4 weeks, followed by Triple 24–44 weeks
Danoprevir (RG7227)	Roche	NS3	Phase 2, naïve GT1 pts (NCT00963885) Triple 12–24 weeks, followed by SOC 12–36 weeks
TMC435350	Tibotec/Medivir	NS3	Phase 2, naïve/treatment-experienced GT1 pts (NCT00561353) Triple 4 weeks, followed by SOC 24–44 weeks
Vaniprevir (MK-7009)	Merck	NS3	Phase 2, naïve/treatment-experienced GT1 pts (NCT00704184/NCT00704405) Triple 4/24–48 weeks, followed by SOC 44/0–24 weeks
BI201335	Boehringer Ingelheim	NS3	Phase 2, naïve/treatment-experienced GT1 pts (NCT00984620/NCT00774397) Triple 12/24–48 weeks, followed by SOC 12/0 weeks
BMS-650032	Bristol-Myers-Squibb	NS3	Phase 2, naïve GT1 pts (NCT01030432) Triple 24–48 weeks
ABT-450	Abbott	NS3	Phase 2, naïve GT1 pts (NCT01074008) Triple 12 weeks, followed by SOC 36 weeks
GS-9256	Gilead	NS3	Phase 2, naïve/treatment-experienced GT1 pts (NCT01072695)
ACH-0141625	Achillion	NS3	Phase 2, naïve GT1 pts (NCT01180790)
MK-5172	Merck	NS3	Phase 1 (NCT00998985), monotherapy
IDX320	Idenix	NS3	Phase 1 (NCT01157104), single dose
VX-985	Vertex	NS3	Phase 1 (NCT01144936), monotherapy
Discontinued			
Ciluprevir (BILN 2061)	Boehringer	NS3	Stopped
GS-9132/ACH806	Gilead/Achillion	NS4	Stopped
Narlaprevir (SCH900518)	Schering-Plough/ Merck	NS3	On hold

Inhibition of Viral Replication

Viral Targets

NS5A

In 2010, the first small molecule NS5A inhibitor BMS-790052 was reported [35]. BMS-790052 was effective in replicons expressing a broad range of HCV genotypes and in the cell culture infectious HCV system. The half-maximum effective concentration (EC₅₀) of the inhibitor was in the picomolar range. In a phase 1 clinical trial a single 100-mg dose of BMS-790052 reduced

HCV RNA by 3.3 log₁₀ within 24 h. BMS-790052 is currently being evaluated in a phase 2 trial in combination with peginterferon alfa-2a/ribavirin. As shown in Table 17.3, other NS5A inhibitors are in clinical and preclinical development.

NS5B

The RNA-dependent RNA polymerase plays a central role in the HCV replication cycle and is therefore an ideal target for drug therapy. Two classes of NS5B polymerase inhibitors, nucleoside

	V36A/M	T54S/A	V55A	Q80R/K	R155K/T/Q	A156S	A156T/V	D168A/V/T/H	V170A/T
Telaprevir (linear)			*						*
Boceprevir (linear)							*		
SCH900518 (linear)									
Ciluprevir** (macrocyclic)									
Danoprevir (R7227) (macrocyclic)						*	*		
MK-7009 (macrocyclic)									
TMC435350 (macrocyclic)									
BI-201335 (macrocyclic)									

Fig. 17.6 Cross-resistance table of different NS3 protease inhibitors based on mutations selected in patients from clinical studies and/or from in vitro studies. **Mutations associated with resistance in vitro but were not described in patients. *Mutations associated with

resistance in vitro. Resistance mutations of linear NS3 protease inhibitors are shown in *dark blue*, and resistance mutations described for macrocyclic NS3 protease inhibitors are shown in *light blue* (based on data from Sarrazin and Zeuzem [68])

Table 17.3 Replication inhibitors

Polymerase inhibitors	Company	Target	Study phase (identifier)
Nuc			
R7128	Roche/Pharmasset	Active site	Phase 2, naïve GT 1/4 pts (NCT00869661) Triple 8–12 weeks followed by SOC 16–40 weeks
PSI-7977	Pharmasset		phase 2, naïve GT 1, 2/3 pts (NCT01188772) GT1: Triple 12 weeks, followed by 12–36 weeks SOC, GT2/3: Mono for 12 weeks
IDX184	Idenix	Active site	Phase 1 (NCT00807001), monotherapy
Non-Nuc			
Filibuvir (PF868554)	Pfizer	Site 2/thumb 2	Phase 2, naïve GT1 pts (NCT00987337) Triple for 24 weeks, followed by SOC 24 weeks
GS-9190	Gilead	Site 4/palm 2	Phase 2, naïve GT1 pts (NCT00743795) Triple 48 weeks Phase 2, naïve/non-naïve GT1 pts (NCT01072695) GS-9256+GS-9190±ribavirin or SOC for 4 weeks
ANA598	Anadys	n.a.	Phase 2, naïve GT1 pts (NCT00978497) Triple 12 weeks, followed by SOC 12–36 weeks
ABT-333	Abbott	Site 4/palm 2	Phase 2, naïve GT1 pts (NCT00851890) Triple for 4 weeks
ABT-072	Abbott		Phase 2, naïve GT 1 pts (NCT01074008) Triple for 12 weeks, SOC for 36 weeks
VX-222 (VCH222)	Vertex (ViroChem Pharma)	Site 2/thumb 2	Phase 2, naïve GT1 pts (NCT00911963) Triple for 12 weeks, SOC for 36 weeks Phase 2, naïve GT1 pts (NCT01080222) VX-222 plus telaprevir±SOC for 12 weeks
BI207127	Boehringer Ingelheim	Site 1/thumb 1	Phase 1, naïve GT1 pts (NCT01132313) BI 207127+BI201335±ribavirin 4, 24, 48 weeks

(continued)

Table 17.3 (continued)

Polymerase inhibitors	Company	Target	Study phase (identifier)
Discontinued			
Valopicitabine (NM283)	Idenix/Novartis	Active site	Stopped
R1626	Roche	Active site	Stopped
HCV-796	ViroPharma/ Wyeth	Site 4/palm 2	Stopped
MK-3281	Merck	Site 1/thumb 1	Stopped
XTL-2125	XTL		Stopped
NS5A-Inhibitor			
BMS-790052	Bristol-Myers-Squibb	NS5A domain 1 inhibitor	Phase 2, naïve/treatment experienced GT1 pts (NCT00874770/ NCT01170962) Triple for 48/24 weeks
AZD7295	Arrow		Phase 1 (NCT00781976), monotherapy
PPI-1301	Presidio		Preclinical

and non-nucleoside polymerase inhibitors, have been developed. Nucleoside analogue polymerase inhibitors are converted into triphosphates by cellular kinases and incorporated into the elongating RNA strand as chain terminators. Generally, they show similar efficacy against all HCV genotypes and have a high genetic barrier.

Several structurally distinct non-nucleoside inhibitors of the HCV RNA-dependent RNA-polymerase NS5B have been reported to date, including benzimidazole, benzothiadiazine, and disubstituted phenylalanine/thiophene or dihydropyranone derivatives (Table 17.3). These agents target different sites within the polymerase and compared to nucleoside inhibitors have a lower genetic barrier. Different resistance profiles due to distinct target sites can be expected for the class of non-nucleoside inhibitors. In contrast to nucleoside polymerase inhibitors, a restricted spectrum of activity of non-nucleoside polymerase inhibitors against different HCV genotypes and subtypes has been described.

Host Targets

Cyclophilin

Cyclophilins are ubiquitous proteins in human cells that are involved in protein folding. Moreover, cyclophilins participate in HCV replication. It was shown that cyclophilin B binds to the HCV NS5B polymerase and stimulates its RNA-binding activity. Cyclophilin inhibitors

show strong antiviral activity in vitro and in vivo. The cyclophilin inhibitor alisporivir (previously DEBIO 025) showed a 3.6 \log_{10} mean decline of HCV RNA after a 14-day oral treatment with an effect against different genotypes (HCV 1, 3, and 4) [36]. For the combination of alisporivir with peginterferon alfa-2a, a decline of HCV RNA of 4.61–4.75 \log_{10} IU/ml and 5.89–5.91 \log_{10} IU/ml was reported for HCV genotype 1, 4 and genotype 2-, 3-infected patients, respectively [37]. Other cyclophilin inhibitors such as SCY-635 are in clinical development.

Inhibition of Translation (UTR, IRES)

Several strategies have been developed for the inhibition of translation (Table 17.4). The most advanced strategies are antisense DNA or RNA oligonucleotides and small molecule IRES inhibitors. Antisense oligonucleotides have a complement sequence of the target mRNA and can prevent translation of viral proteins. The antisense compounds AVI-4065 and ISIS-14803 progressed to phase 2 clinical trials. However, the development of both compounds was stopped due to lack of antiviral efficacy and/or potential hepatotoxicity.

Ribozymes are RNA molecules that catalyze cleavage of a target RNA molecule based on sequence-specific recognition. Ribozymes show antiviral activity in vitro. Heptazyme, an IRES-specific ribozyme was investigated in

Table 17.4 Translation inhibitors

Translation inhibitors	Company	Target	Study phase (identifier)
TT 033	Tacere Therapeutics	siRNA	Preclinical
mi-R-122	Alnylam	RNA interference	Preclinical
RNAi	CombiMatrix	RNA interference	Preclinical
siRNA-034	Sirna Therapeutics/Merck	RNA interference	Preclinical
AVI-4065	AVI BioPharma	Antisense	Phase 2, stopped (NCT00229749)
ISIS-14803	Isis Pharmaceuticals	Antisense	Phase 2, stopped (NCT00035945)

phase 2 trials, however, the studies were halted due to cardiotoxicity in monkey animal studies.

RNA interference is a sequence-specific RNA degradation process induced by double-stranded RNA. RNA interference can be initiated by siRNA or short hairpin RNA (shRNA) that associate with various proteins to form an RNA-inducing silencing complex with nuclease and helicase activity. The siRNA or shRNA guides this complex to the complementary target RNA and the nuclease component cleaves the target RNA in a sequence-specific manner. This approach appears reasonable also in HCV treatment and in vitro experiments achieved promising results. Several compounds have been developed (Table 17.4), however, are yet at preclinical stage. The major limitation of this strategy is in vivo delivery; several approaches for in vivo delivery are currently explored.

The liver-specific micro RNA-122 has recently been shown to be required for HCV replication. SPC3649 is a micro RNA-122 inhibitor that is currently evaluated in phase 1 trials. In HCV-infected chimpanzees, SPC3649 treatment was associated with reduction in HCV RNA without severe adverse events [38].

Assembly and Release Viral Target (Core)

HCV core proteins play a major role in the formation of viral particles. Furthermore, HCV core interacts with several cellular proteins. It is the most conserved of all HCV proteins. Organization of HCV assembly requires the oligomerization of the core protein. Recently, peptides and small molecules were identified which inhibit core-core interaction and block viral production in cell culture. Targeting the core protein is a promising

anti-HCV strategy, however the development of inhibitors is still at the preclinical stage [39].

Host Target (Glycosylation)

Proper glycosylation of HCV structural proteins is required for maturation, assembly, and secretion of infective particles. Inhibition of glycosylation is another potential approach for HCV antiviral therapy. Celgosivir is a potent inhibitor of alpha-glucosidase which affects the early stages of glycoprotein processing [40]. Alpha-glucosidase is a host enzyme. A potential advantage of celgosivir is the low probability to develop drug-resistant viral mutants. On the other hand, development of adverse reactions are likely. Celgosivir showed promising results in preclinical trials and was investigated in phase 2 trials [41]. In non-responders, celgosivir in combination with peginterferon alfa-2b plus ribavirin showed a stronger decline of HCV-RNA than peginterferon alfa-2b plus ribavirin alone ($-1.63 \log_{10} \text{ IU/l}$ vs. $-0.92 \log_{10} \text{ IU/l}$). Celgosivir development, however, is currently on hold.

Historical Development of the Most Advanced Direct Antiviral Agents Against the NS3/4 Protease and NS5B Polymerase

Viral Targets

NS3/4A Inhibitors Ciluprevir

In 2003, the first randomized placebo-controlled study with the NS3/4A protease inhibitor ciluprevir in patients with chronic hepatitis C was presented [42]. Oral administration of ciluprevir to

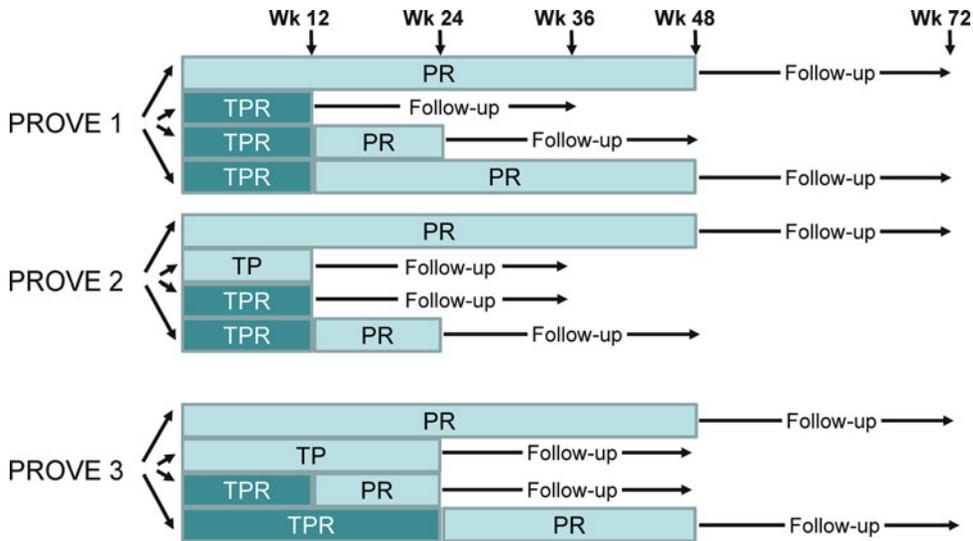


Fig. 17.7 Telaprevir phase 2 trials PROVE 1 and PROVE 2 were performed with treatment-naïve HCV genotype 1-infected patients in USA and in Europe, respectively. PROVE 3 was performed with HCV genotype 1-infected

patients with prior non-response or relapse to SOC. TPR, telaprevir plus peginterferon alfa-2a, Ribavirin; TP, telaprevir plus peginterferon alfa-2a; PR, peginterferon alfa-2a plus ribavirin

patients with chronic HCV genotype 1 infection for 2 days was associated with 2–3 \log_{10} IU/ml decline of HCV RNA in most of the patients. The study provided proof-of-concept that HCV NS3/4A protease inhibitors are able to block HCV replication in patients with chronic hepatitis C. Due to potential cardiotoxicity, clinical development of ciluprevir was stopped.

Telaprevir

Phase 1: Monotherapy

The first monotherapy trial of telaprevir in HCV genotype 1-infected patients was started in 2004 [43]. During treatment with telaprevir, all patients (naïve and non-responders to previous SOC therapy) showed a $\geq 2 \log_{10}$ IU/ml decline of HCV RNA. Despite strong antiviral efficacy, telaprevir monotherapy was associated with the rapid emergence of drug-resistant HCV strains occurring in 75% of patients during telaprevir monotherapy [44]. Overall, this landmark study showed that due to rapid resistance development, only a minority of patients with chronic hepatitis C have the likelihood to be cured with telaprevir monotherapy and that combination of compounds with different resistance profiles are necessary.

Phase 1: Telaprevir in Combination with Peginterferon Alfa-2a

Subsequently, telaprevir in combination with pegylated interferon-alfa-2a was investigated in treatment-naïve HCV genotype 1-infected patients [45, 46]. The study showed a stronger decline of HCV RNA after 2 weeks of treatment in the combination arm than in the respective monotherapy arms ($-5.49 \log_{10}$ IU/ml vs. $-1.09 \log_{10}$ IU/ml and $-3.99 \log_{10}$ IU/ml for peginterferon alfa-2a/telaprevir combination therapy vs. peginterferon alfa-2a and telaprevir monotherapy, respectively). The study provided proof-of-concept that telaprevir has at least additive antiviral effects in combination with pegylated interferon-alfa-2a.

Phase 2: Telaprevir in Combination with Peginterferon Alfa-2a with and Without Ribavirin Treatment-Naïve Patients (PROVE 1/2)

The PROVE1/2 studies (PROVE 1 was conducted in USA, PROVE 2 was conducted in Europe) were the first studies to explore whether a direct antiviral compound has the potential to increase SVR rates in treatment-naïve patients with chronic hepatitis C [47, 48].

Both studies had a similar design (Fig. 17.7). In both trials telaprevir was administered for

12 weeks (T12) in combination with peginterferon alfa-2a and ribavirin for 12 weeks (PR12) or 24 weeks (PR24), respectively. In addition, PROVE 1 contained a T12/PR48 arm that was replaced by a T12/P12 without ribavirin arm in the PROVE 2 trial. The results of the PROVE trials provided the first SVR rates of a DAA with SOC in patients with chronic hepatitis C. The highest SVR rates were obtained in the T12/PR48 (PROVE 1) and in the T12/PR24 arm. The SVR rates in these telaprevir arms were significantly higher compared with the SVR rates in the control arm (67%/69% vs. 41%/46% for triple therapy vs. SOC in PROVE1/PROVE2, respectively).

In PROVE 2, the SVR rate in patients treated with telaprevir/peginterferon alfa-2a without ribavirin for 12 weeks was lower than in patients treated with telaprevir/peginterferon alfa-2a plus ribavirin for 12 weeks (36% vs. 60%). The lower rate of SVR in the group without ribavirin was due to a higher relapse rate compared to the groups with ribavirin (48% vs. 14–29%).

Three major conclusions were drawn from the PROVE 1/2 studies: (1) protease inhibitors are able to increase SVR rates in treatment-naïve patients with HCV genotype 1 infection, (2) improved SVR rates may be achieved with shorter treatment duration, and (3) ribavirin has additive antiviral activity to telaprevir and is required to optimize SVR rates.

Phase 3: Telaprevir in Combination with Peginterferon Alfa-2a with and Without Ribavirin
The ADVANCE and the ILLUMINATE trials are phase 3 studies to investigate the efficacy and safety of telaprevir in combination with peginterferon alfa-2a and ribavirin in treatment-naïve HCV genotype 1 patients [49, 50]. Overall, the SVR rates in the telaprevir arms were superior to standard therapy (69–75% vs. 44%). The studies are discussed in detail in Chap. 19.

Retreatment of Previous SOC

Non-responders

The PROVE3 trial was a randomized, placebo-controlled phase 2 study assessing safety and efficacy of telaprevir plus peginterferon alfa-

2a±ribavirin in HCV genotype 1 patients who previously failed peginterferon/ribavirin treatment [51]. The overall SVR rates were significantly higher in the telaprevir arms (peginterferon alfa-2a/ribavirin/telaprevir for 12 or 24 weeks followed by peginterferon alfa-2a/ribavirin for 12 and 24 weeks, respectively) compared with the control arm (51%, 52% vs. 14%). Subgroup analysis of previous peginterferon/ribavirin non-responders showed superior SVR rates in the triple therapy arms compared with the SOC control arm or the peginterferon/telaprevir arm without ribavirin (38–39% vs. 9–10%). Overall, the study confirmed that protease inhibitors in combination with SOC will also be a treatment option for patients who failed previous antiviral therapy.

The REALIZE trial is a phase 3 trial investigating efficacy and safety of telaprevir in combination with peginterferon alfa-2a/ribavirin in patients with prior treatment failure to SOC. The overall SVR rates following telaprevir-based retreatment were superior to retreatment with standard therapy (64–66% vs. 17%). The study is described in detail in Chap. 19.

Resistance Against Telaprevir

Mutations associated with resistance to telaprevir were identified using a highly sensitive sequencing method [52, 53]. Several mutations associated with resistance to telaprevir were identified in the HCV NS3 protease catalytic domain (Fig. 17.6). Mutations occurred either as single mutations (V36A/M, T54A, R155K/T, A156S/T/V) or as double mutations (at positions 36+155 or 36+156). The rapid occurrence of these mutations during treatment indicates that mutations are present before treatment and are selected during treatment with telaprevir. The resistance mutations can be distinguished into low-level resistance and high-level resistance mutations. Furthermore, it was shown that resistance is associated with a reduced ability of the virus to replicate (lower viral fitness).

Safety of Telaprevir

Overall, telaprevir has a good safety profile, however, premature discontinuation rates were higher in the telaprevir arms compared with the

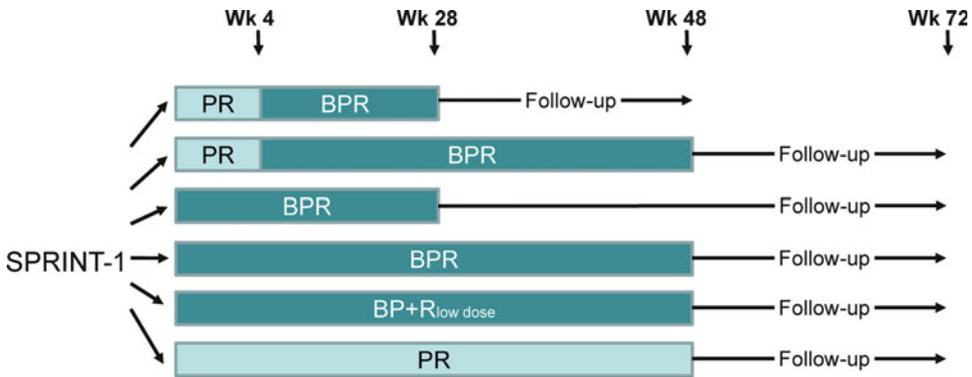


Fig. 17.8 Boceprevir phase 2 SPRINT-1 trial BPR, boceprevir, peginterferon alfa-2b, ribavirin (800–1,400 mg); PR, peginterferon alfa-2b, ribavirin; low doseR, ribavirin (400–1,000 mg)

SOC arms (12–21% vs. 4–11%). The most common adverse events, such as fatigue and influenza-like symptoms, were consistent with typical interferon-related systemic symptoms, while macropapular rash and pruritus occurred more frequently in the telaprevir study arms compared with the SOC control arms (41–60% vs. 20–41%). The skin symptoms typically occurred within 1–4 weeks after initiation of telaprevir dosage. Furthermore, a stronger decrease of hemoglobin in the telaprevir arms compared with the SOC arm was reported.

Boceprevir

Boceprevir is the second N3/4A protease inhibitor that has advanced in clinical development. Boceprevir binds reversibly to the NS3 protease active site.

Phase 1: Boceprevir Monotherapy

Boceprevir monotherapy for 2 weeks in HCV genotype 1 patients with prior failure to SOC was associated with a mean $-2.06 \log_{10}$ IU/ml reduction in HCV RNA. Boceprevir was well tolerated at all doses. Similar to telaprevir, viral breakthrough with selection of resistant variants occurred during boceprevir dosage.

Phase 1: Boceprevir in Combination with Peginterferon Alfa-2b

Subsequently, a randomized, double-blind crossover study investigated boceprevir in combination

with peginterferon alfa-2b in SOC non-responders. In this study, boceprevir was administered either alone for 7 days or in combination with peginterferon alfa-2b for 14 days in comparison to 14 days of peginterferon alfa-2b monotherapy. In this study, double combination therapy showed a -2.45 to $2.88 \log_{10}$ IU/ml decline of HCV-RNA compared with -1.08 to $1.26 \log_{10}$ IU/ml in patients re-treated with pegylated interferon alfa-2b alone [54].

Phase 2: Boceprevir in Combination with Peginterferon Alfa-2b with and Without Ribavirin SPRINT-1 was a phase 2 trial in which safety, tolerability, and antiviral efficacy of boceprevir in combination with pegylated interferon alfa-2b and ribavirin was investigated in treatment-naïve patients with chronic hepatitis C [55]. Treatment with boceprevir in combination with pegylated interferon alfa-2b and ribavirin was either performed continuously for 28 or 48 weeks or after a previous 4-week lead-in phase of pegylated interferon alfa-2b and ribavirin alone (Fig. 17.8). The lead-in phase was chosen to determine a potential benefit of pre-treatment with pegylated interferon alfa-2b and ribavirin to avoid resistance development. The control group was treated with pegylated interferon alfa-2b and ribavirin for 48 weeks. SVR rates after 28 weeks of triple treatment were 54 and 56% after 24 weeks with an additional 4 weeks of pre-treatment lead in with pegylated interferon alfa-2b and ribavirin. SVR rates after 48 weeks of triple treatment were

67 and 75% after 44 weeks with an additional 4 weeks of pre-treatment lead-in with pegylated interferon alfa-2b and ribavirin. Patients in the pegylated interferon alfa-2b/ribavirin control group achieved a SVR of 38%.

Phase 3: Boceprevir in Combination with Peginterferon Alfa-2b with Ribavirin

SPRINT-2 and RESPOND-2 are phase 3 studies to investigate efficacy and safety of boceprevir in combination with peginterferon alfa-2b and ribavirin in treatment-naïve or treatment-experienced patients, respectively, with chronic HCV genotype 1 infection [56, 57]. In brief, the overall SVR rates in both trials were superior in the boceprevir arms compared with the control arm (63–66% vs. 38% and 59–67% vs. 21%, respectively). The studies are discussed in detail in Chap. 19.

Resistance

To analyze development of resistance against boceprevir, a detailed clonal analysis of mutations selected during treatment with boceprevir monotherapy was performed [54]. As shown in Fig. 17.6, mutations associated with lower susceptibility to boceprevir were similar to mutations associated with resistance against telaprevir.

Safety

Boceprevir has a good safety and tolerability profile. The most common adverse events were anemia, nausea, vomiting, and dysgeusia.

NS5B Inhibitors

Nucleoside Analogues

Valopicitabine

Valopicitabine was the first nucleoside (nuc) analogue polymerase inhibitors tested in patients with chronic hepatitis C [58]. Valopicitabine showed antiviral activity in monotherapy (mean HCV-RNA decline 0.15–1.21 log₁₀ IU/ml after 14 days in patients infected with HCV genotype 1 and prior non-response to interferon-based antiviral treatment) and in combination therapy with interferon alfa (mean HCV-RNA decline 3.75–4.41 log₁₀

IU/ml after 36 weeks in treatment-naïve patients infected with HCV genotype 1). The development of valopicitabine was stopped due to gastrointestinal adverse events which were severe in some patients.

R1626

The nuc analogue R1626, a prodrug of R1479, was investigated in treatment-naïve patients with HCV genotype 1 infection in combination with peginterferon alfa-2a and ribavirin. After 48 weeks (4 weeks R1626 plus peginterferon alfa-2a with or without ribavirin followed by 44 weeks of peginterferon plus ribavirin) the virologic response rates were 52–84% in the R1626 treatment arms and 65% in the control arm with peginterferon alfa-2a/ribavirin [59]. Despite promising results the clinical development of R1626 was stopped due to severe lymphopenia.

RG7128

HCV genotype 3-infected patients who did not respond to SOC were retreated with peginterferon alfa-2a/ribavirin for 24 or 48 weeks in combination with RG7128 for 4 weeks or placebo. SVR rates in the R7128 group were higher than in the placebo group indicating that nuc-polymerase inhibitors have the potential to increase SVR rates in patients with chronic hepatitis C [60]. RG7128 is currently being evaluated in a phase 2 study assessing efficacy and safety in combination with pegylated interferon alfa-2a and ribavirin in treatment-naïve HCV genotype 1- or 4-infected patients. Further nuc-polymerase inhibitors are shown in Table 17.4.

Non-nucleoside Analogues

HCV-796 was the first non-nuc polymerase inhibitor that demonstrated substantial antiviral activity in patients with chronic hepatitis C [61]. Monotherapy showed a maximum antiviral effect after 4 days of treatment with a mean HCV RNA reduction of –1.4 log₁₀ IU/ml. Similar to monotherapy with protease inhibitors, resistant variants rapidly occurred during HCV-796 monotherapy. The combination of HCV-796 and peginterferon

		S96T	S282 T	C316 Y/N	S365 T/A	M414 T/L	L419 M/V	M423 T/I/V	Y448 C/H	I482 L/V/I	V494 I/A	P495 S/L/A/ T	P496 A/S	V499 A	G554 D	D559 G
Nuc	Valopicitabine															
	R7128															
	R1626															
NNI site 1	BI207127															
	GS9190															
NNI site 2	Filibuvir															
	VCH-759															
	VCH-916															
NNI site 3	ANA598															
NNI site 4	HCV796															
	ABT-333															

Fig. 17.9 Cross-resistance table for resistance-associated mutation in NS5B polymerase inhibitors Nuc, nucleoside analogue polymerase inhibitors; NNI, non-nuc polymerase inhibitors (based on data from Sarrazin and Zeuzem [68])

alfa-2b produced a mean viral reduction of -3.3 to $3.5 \log_{10}$ IU/ml after 14 days of treatment compared to $-1.6 \log_{10}$ IU/ml with peginterferon alfa-2b alone. Due to clinically significant elevations of liver enzymes, the clinical development of HCV-796 was discontinued [62].

Several other non-nuc polymerase inhibitors are currently in clinical trials and are currently investigated in phase 2 clinical trials (Table 17.3). Larger trials showing that non-nuc polymerase inhibitors increase SVR rates are pending.

Resistance

Several resistance-associated mutations within the NS5B gene have been identified in vitro and in vivo (Fig. 17.9). In general, resistance-associated mutations are different between nuc and non-nuc analogue NS5B polymerase inhibitors. Furthermore, the resistance-associated mutations differ between non-nuc inhibitors targeted against different sites of the NS5B polymerase. Resistance-associated mutations against nuc-inhibitors have been identified only in vitro but not yet in vivo (most likely due to a low viral replication fitness).

Current Understanding of DAA Treatment

Among all approaches for direct antiviral treatment of patients with chronic hepatitis C, two NS3/4A protease inhibitors have progressed into phase 3 development (telaprevir and boceprevir). Both compounds show a good safety profile and high antiviral efficacy. Assuming approval by regulatory agencies, these drugs will become the new standard treatment for HCV genotype 1 patients in combination with peginterferon alfa and ribavirin. The results of the phase 3 clinical trials will be discussed in Chap. 19.

Future of DAA Treatment

Combination Therapy Without Interferon

With the introduction of the protease inhibitors telaprevir and boceprevir for treatment of chronic HCV genotype 1 infection, the major goal of recent

years to improve SVR rates in this difficult-to-treat population has been achieved. Triple therapy, however, continues to be unsatisfactory for two reasons: (1) Anti-HCV therapy still depends on peginterferon alfa and ribavirin and therefore SOC null responders are less likely to achieve SVR, (2) Triple therapy is associated with more adverse events than previous SOC. Therefore, the next goal of anti-HCV therapy will be to develop an interferon alfa-free regimen with better tolerability.

This goal can be achieved with combination of two or more direct antivirals with non-overlapping resistance profiles. The most promising drug combinations to date are protease inhibitor plus nucleoside or non-nucleoside analogue polymerase inhibitors or the combination of a protease inhibitor with a NS5A inhibitor.

Combination of NS3/4A Inhibitor Plus NS5B Polymerase Inhibitor

The INFORM-1 trial was the first study that investigated an interferon alfa-free approach for treatment of chronic hepatitis C. The nucleoside polymerase inhibitor RG7128 and the protease inhibitor RG7227 were administered to treatment-naïve and treatment-experienced HCV genotype 1-infected patients for 2 weeks. All patients who received combination therapy achieved profound reduction in HCV RNA without evidence of treatment emergent resistance (HCV drop after 2 weeks $-4.8 \log_{10}$ IU/ml, $-4.0 \log_{10}$ IU/ml, and $-4.9 \log_{10}$ IU/ml in treatment-naïve, relapse, and null responders to SOC) [63].

Another phase 1 trial also investigated an interferon-free combination of the NS3/4A protease inhibitor BI201335 and the NS5B polymerase inhibitor BI207127 plus ribavirin for 4 weeks in treatment-naïve HCV genotype 1-infected patients [64]. The combination therapy showed a rapid sharp decline of HCV RNA followed by second-phase decline in most of the patients. The majority of patients (11/15) and all patients (17/17) in the 400 mg TID low and the 600 mg TID high-dose arm of BI207127 had a HCV RNA level lower than 25 IU/ml at week 4.

The combination will be investigated in a phase 2 trial testing different dose regimens and longer treatment with SVR as primary endpoint.

GS-9256 and GS-9190 are a NS3/4A protease inhibitor and a non-nucleoside NS5B inhibitor, respectively, that have demonstrated antiviral activity in HCV genotype 1-infected patients during monotherapy studies. GS-9256- and GS-9190-associated mutations were introduced into 1b replicons and antiviral susceptibility was tested in transient replication assays. GS-9256 maintained antiviral activity in replicons bearing mutations associated with lower susceptibility to GS-9190 and vice versa. GS-9190 maintained antiviral activity in replicons bearing mutations associated with lower susceptibility to GS-9256. The combination is now in active clinical development [65].

Combination of NS3/4A Inhibitor Plus NS5A Inhibitor

A phase 2 study investigated the efficacy and safety of the NS5A inhibitor BMS-790052 in combination with the NS3/4A inhibitor BMS-650032 with and without peginterferon alfa-2a/ribavirin in HCV genotype 1 patients with null response to prior SOC [66]. An interim analysis reported RVR rates of 63 and 60% in the arm without interferon/ribavirin and the arm with interferon/ribavirin, respectively. However, between week 4 and 12, 6 of 11 patients in the interferon-free combination arm showed a viral breakthrough, while all patients in the interferon/ribavirin containing arm maintained viral suppression.

Individualized Treatment

Virologic Response

In the phase 3 trials with telaprevir and boceprevir, it was shown that response-guided therapy is associated with similar SVR rates as fixed duration therapy for 48 weeks treatment. These trials indicate the rapidity of virologic response will remain important to guide treatment duration.

Genotype

Both protease inhibitors telaprevir and boceprevir have been developed for treatment of HCV genotype 1-infected patients. For telaprevir, the antiviral activity was shown to be lower in HCV genotype 2 and markedly reduced in HCV genotype 3- and 4-infected patients compared with HCV genotype 1 patients [33, 67]. In addition, boceprevir was reported to have lower antiviral activity in HCV genotype 2- and 3-infected patients [68]. The HCV genotype will therefore remain an important factor for individualized therapy. New NS3/4A protease inhibitors such as ACH-2684 and MK-5172 currently at preclinical or in early clinical development have activity against genotypes 1–6 and genotypes 1, 3, respectively, and may overcome this restriction [69, 70]. Nuc polymerase inhibitors targeted against the active center of the NS5B polymerase have the highest potential for genotype non-1 direct antiviral therapy. NS5A inhibitors such as PPI-461 also have been shown to possess the potential for pan-genotype activity in a recently presented phase 1 study [71].

IL28B Genotype

Genome-wide association studies have recently identified single-nucleotide polymorphisms in the region of the IL28B gene on chromosome 19, coding for the interferon- λ -3 or IL28B gene which are strongly associated with treatment response to SOC treatment in patients infected with HCV genotype 1 [72–75]. The good response variant was associated with a twofold increase in the rate of cure. Allele frequencies differ between ethnic groups, largely explaining the observed differences in response rates between Caucasians, African Americans, and Asians.

The IL28B C/C genotype has been shown to be associated with improved early viral kinetics and greater likelihood of RVR and cEVR in HCV genotype 1 patients [75]. The IL28B genotype is likely to aid in clinical decision making with SOC regimens. Future studies will investigate

the possibility of individualizing treatment duration and novel regimens according to IL28B type [76].

In an ongoing phase 2 trial with the non-nuc polymerase inhibitor ANA598, it was shown that ANA598 is able to improve RVR and EVR rates in IL28B CT and TT patients where SOC alone is less efficacious (23% vs. 0% and 69% vs. 50% for ANA598 plus SOC vs. SOC alone) [77]. The study shows that the IL28B genotype distribution may be also important for DAA treatment. Furthermore, the knowledge of the IL28B genotype may be relevant for designing early phase clinical trials with small patient numbers (stratification according to IL28B genotype). A modeling study showed that the probability of 10% imbalance is 31%, 18%, and 6% in trials including 60, 120, or 240 patients [78].

Treatment Beyond NS3/4A Protease-, NS5B Polymerase-, and NS5A- Inhibitors

Future Viral Targets

The recent development of DAA focused on the non-structural proteins NS3, NS5A, NS5B. NS4 inhibitors are in development and appear as an attractive approach. The only remaining non-structural protein against which no inhibitors have been presented so far is the NS2 protein. Recent studies better characterized the function and structure of NS2 making it also an attractive anti-HCV drug target.

The entry mechanism of HCV is also a promising target for anti-HCV therapy. While all other targets aim at inhibition of replication of an established infection, entry inhibitors may enable the prevention of de novo infection, i.e., to prevent HCV infection following liver transplantation or accidental needle stick injury. As chronic HCV is the result of continuous de novo infection and turnover of infected cells, entry inhibition could also strongly support the antiviral effect of replication inhibitors.

New Interferons and Immunomodulators

Albinterferon is a long-acting interferon alfa-2b which has to be administered every 2–4 weeks. The phase 3 trials showed that albinterferon has similar SVR rates compared with peginterferon alfa-2a [79, 80]. Due to unresolved safety issues the clinical development of albinterferon has been discontinued.

Interferon lambda targets a different receptor than interferon alfa and may be particularly interesting due to the recent discovery of the association between single nucleotide polymorphisms in the IL28B gene with response to interferon-based therapy. A pegylated interferon lambda recently demonstrated superior RVR rates compared with peginterferon alfa-2a (75% vs. 50%) [81]. Most importantly, interferon lambda has an improved tolerability profile compared with interferon alfa [81].

Further potential combinations are DAA with immunomodulators such as toll-like receptor agonists and/or DAA with cyclophilin inhibitors (e.g., alisporivir).

Summary

Tremendous progress has been made in the understanding of pathogenesis and replication of the HCV since its discovery in 1989. For more than one decade peginterferon alfa/ribavirin is the standard treatment for HCV and no new compounds have been approved. Due to the development of cell culture HCV models and structure determination of HCV proteins, many antiviral targets have been identified. The most promising and clinically advanced direct anti-HCV compounds are inhibitors against the NS3/4A protease and the NS5B polymerase. Further promising targets in the future are entry inhibitors against the envelope proteins E1, E2, assembly inhibitors targeted against core or p7, NS2 protease inhibitors and NS5A inhibitors. Due to the risk of selection for resistant strains, the current protease inhibitors telaprevir and boceprevir

have to be administered in combination with peginterferon alfa and ribavirin. The phase 3 trials have shown SVR rates in the order of 69–75% in HCV genotype 1 patients. The future of anti-HCV therapy will focus on the development of interferon-free treatment regimens.

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