The treatment of HEPATITIS C
Hepatitis C virus infection is a major health problem worldwide and no vaccine is yet available against this virus. It is estimated that in Europe 5-10 million people are chronically infected and most of them are not aware of their infections. About 150,000 new cases occur annually in Europe and 80% of them progress to chronic infections and liver disease. Every year 4-5% of chronic infected patients develop hepatocellular carcinoma (HCC). About 300,000 people die annually of HCV cirrhosis and related complications worldwide.

The goal of antiviral treatment in patients with chronic hepatitis C (HCV) infection is a persistent viral clearance. The achievement of this short-term outcome is the only way to ensure that, in patients with chronic liver damage, the underlying liver disease is going to stabilize and liver-related mortality to decrease. Interferon and ribavirin have represented the approved pharmacotherapies for HCV infection for many years. They were associated with suboptimal cure rates and had side effects that result in non-compliance and premature treatment discontinuation.

After dominating both Infectious Diseases and Gastroenterologist and Hepatologists’ interests for over 25 years, Hepatitis C is nowadays a curable disease and its treatment represents one of the most important successes in the history of Medicine. The introduction of simple, short, all oral regimens based on direct acting antivirals (DAA) able to directly target different regions of HCV genome and also associated with excellent tolerability has completely changed the approach to HCV chronic infection and related complications. We can now treat patients with advanced cirrhosis, transplanted patients and subjects with renal failure. On the other hand, new problems emerged such as, for example, how to manage and prioritize patients with recent HCV infection diagnosis and how to discover subjects not yet diagnosed. Moreover, some questions related to DAA failures or drug-to-drug interactions remain to be solved.

With the enormous number of publications, guidelines and expert opinions currently available, sometimes it is difficult to stay up-to-date. Guidelines are continuously changing in accordance with the availability of new drugs combinations. That is why we asked an outstanding panel of Experts to help us make the best choices for the patient.
Professor De Francesco, Professor Aghemo, Professor Ciancio, Professor Cecherini-Silberstin, Professor Martini, Professor Naggie and Professor Pol were asked to provide an overview on the current state of the art of antiviral treatments in terms of drugs, regimens, side effects, resistance risk and drug-to-drug interactions and to highlight both the most relevant advantages and the few limitations of HCV treatments available in the vast majority of European countries. The situation in Europe is still variegated and, in some countries, Italy for instance, the access to new pangenotypic regimes is expected in the coming months.

Looking ahead Professor Hezode and Professor Buti set the scene on both future combination regimens and near future scenarios. For a number of years, until HCV becomes a “rare” disease, physicians involved in the HCV treatment will face problems related to access to cure, screening and drug costs reductions rather than treatment failures or side effects.

It was a privilege to have the support of this outstanding group of Experts contributing to this volume. This publication would not have been possible without their critical, dutiful and elegant interpretation of each specific topic.

In less than 10 years, Hepatitis C will probably be a disease of only historical relevance but until then there are subgroups of patients who still represent a challenge.

I hope the readers will receive this volume with enthusiasm and will refer to it when having to make difficult decisions for their patients.

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INTRODUCTION

Hepatitis C virus (HCV) is a leading cause of chronic liver disease worldwide. It is currently estimated that 2-3% of the world’s population are chronically infected with HCV and more than 350,000 people die each year because of HCV-related liver disease. Moreover, three to four million new infections occur in the world each year. A significant number of chronically infected individuals will develop progressive liver disease, ultimately leading to cirrhosis and cancer of the liver. Although a vaccine to prevent HCV infection is not available, astonishing progress has been made in terms of developing highly effective curative drug regimens. Until recently, therapy for chronic hepatitis C consisted of a combination of pegylated interferon-α (PEG-IFN) and ribavirin (RBV). This approach eradicates the virus in less than 50% of treated patients and is characterized by several serious side effects and numerous contraindications. In recent years, the treatment paradigm for chronic HCV infection has undergone a revolution: insight into the viral life cycle has enabled the development of new treatment regimens using combinations of direct-acting antiviral agents (DAA), now leading to cure rates of >90% in virtually all treated patients, including those with advanced liver disease and/or those who failed prior interferon-based therapy.

The first molecular clone of the HCV genome was isolated in 1989. Based on the analysis of the genome sequence, HCV was recognized to be an enveloped, single-stranded RNA virus and was classified as a member of the the Flaviviridae family. Subsequent work in several laboratories elucidated that the 9.6 kb positive-sense RNA genome encodes a polyprotein of about 3000 amino acids, that is proteolytically cleaved, by the action of host and viral proteases, into 10 different products (Figure 1.1). Already by the mid-90s, it had become clear that the HCV gene products included some highly attractive drug targets, such as the NS3/4A serine protease and the NS5B RNA-dependent RNA polymerase, and the methodology to assay the activity of these viral enzymes in a test tube had started to become available to the scientific community. However, the inability to efficiently propagate the virus in cell culture represented a major obstacle toward drug discovery and development. A breakthrough in this sense came in 1999, when Lohmann et al. reported selection of the first functional subgenomic replicons in cell culture. These replicons consisted of a genotype
Hepatitis C: Current and new drugs

1b HCV RNA engineered to express an antibiotic-selectable marker gene in place of the structural protein region. After RNA transfection into a human hepatoma cell line followed by antibiotic selection, only cells that contained replicating HCV RNAs were able to form colonies. This led to the isolation and characterization of functional HCV subgenomic replicons. The availability of the replicon system paved the way to a detailed understanding of the different stages of the HCV life-cycle and greatly facilitated the identification and validation of new drug targets. Moreover, the replicon system soon became a critically-needed cell-based system for evaluating potential antiviral compounds as well as for studying in vitro the evolution of viral resistance to new antiviral candidates. The discovery of the replicon system was awarded the 2016 Lasker-DeBakey clinical medical research award in recognition of the critical impact that system had in the discovery and development of the new antiviral agents that have eventually revolutionized the treatment of chronic hepatitis C.

Historically, the development of HCV DAAs has focused on three major viral targets: the NS3/4A protease, the NS5B RNA dependent RNA polymerase, and the NS5A replication complex protein (Figure 1.1). This chapter summarizes the progress in the development of DAA-based drugs and therapies, with particular emphasis on those compound classes and combinations that have led to the most effective clinical results in terms of providing a sustained virological cure.

In Table 1.1 the general characteristics of current DAA classes are summarized.

INHIBITORS OF THE NS3/4A SERINE PROTEASE

NS3 is a multifunctional enzyme that harbors a serine protease domain in its 180 N-terminal amino acids and an RNA helicase/ATPase domain in the C-terminal remainder of the protein. The NS3 serine protease domain is in fact one subunit of a heterodimeric
The treatment of Hepatitis C

enzyme complex requiring also another viral protein, NS4A, for full catalytic activity and for anchoring the protease to the replication complex membranes. For this reason, this viral enzyme is currently referred to as the NS3/4A protease (Figure 1.2A). The activity of the NS3/4A protease is responsible for 4 cleavages, within the HCV polyprotein, required for the proteolytic maturation of the non-structural proteins, NS3 to NS5B (Figure 1.1). Hence, the inhibition of this enzyme can block viral replication by preventing the biogenesis of a mature RNA replication complex, including a functional RNA-dependent RNA polymerase. Beyond the viral substrates in the viral polyprotein, the NS4/4A protease also cleaves the cellular proteins TRIV and MAVS, which are critical adaptor molecules in the Toll-like receptor 3 and other innate immunity pathways. Thus, in addition to direct inhibition of viral replication, agents targeting the NS3/4A protease might have indirect effects on restoring innate antiviral response to HCV infection.

The analysis of the three-dimensional crystal structure of the NS3/4A protease has revealed that a relatively flat and featureless substrate-binding site. Because of the absence of well-defined pockets in the active site, the substrate is recognized via a series of weak molecular interactions distributed along a rather large surface. This mechanism of substrate recognition, reminiscent of a protein-protein interaction, has represented a very difficult starting point for the development of competitive inhibitors of the enzyme. In spite of this formidable challenge, a number of highly potent and efficacious active-site protease inhibitors have been designed and some of them have reached the market as approved anti-HCV drugs.

From a chemical point of view, NS3/4 protease inhibitors (PIs) can be divided into three main categories: covalent linear PIs, non-covalent linear PIs, and non-covalent macrocyclic PIs. The terms “first-generation” or “second-generation” NS3/4A inhibitors are also used to define subsequent PIs generations (Figure 1.2B). First generation NS3/4A PIs are defined as agents that are effective mainly on genotype 1 HCV and for which the virus has a low genetic barrier to the development of resistant variants. First-generation NS3/4A PIs are in turn distinct in “first-wave” (covalent linear inhibitors) and “second wave” (non-covalent linear or mac-

| TABLE 1.1 - HCV genotype coverage of approved and forthcoming DAA-based regimens. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Approved combination regimens   | Genotype 1     | Genotype 2     | Genotype 3     | Genotype 4     | Genotypes 5/6  |
| Sofosbuvir+ribavirin             | Blue           | Yellow         | Red            | Red            | Red            |
| Sofosbuvir/ledipasvir+RBV        | Black          | Red            | Yellow         | Red            | Red            |
| Sofosbuvir/simeprevir+RBV        | Red            | Red            | Yellow         | Red            | Red            |
| Sofosbuvir/daclatasvir+RBV       | Red            | Red            | Yellow         | Red            | Red            |
| Sofosbuvir/velpatasvir+RBV       | Red            | Red            | Red            | Red            | Red            |
| Ombitasvir/paritaprevir/ritonavir+dasabuvir+RBV | Yellow         | Red            | Yellow         | Red            | Red            |
| Ombitasvir/paritaprevir/ritonavir±RBV | Red            | Red            | Yellow         | Red            | Red            |
| Grazoprevir/elbasvir+RBV         | Red            | Red            | Red            | Red            | Red            |

Experimental combination regimens

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<tr>
<th>Genotype 1</th>
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<th>Genotype 3</th>
<th>Genotype 4</th>
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<tr>
<td>Sofosbuvir/velpatasvir/voxilaprevir</td>
<td>Red</td>
<td>Red</td>
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<tr>
<td>Uprifosbuvir/grazoprevir/ruzasvir</td>
<td>Red</td>
<td>Red</td>
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<tr>
<td>Glecaprevir/pibrentavir</td>
<td>Red</td>
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Blue: optimal; yellow: suboptimal; red: not indicated.
Hepatitis C: Current and new drugs

Second-generation NS3/4A PIs are defined as agents for which the virus has a high barrier to the development of viral resistance, retain activity against the viral variants that are resistant to first-generation compounds and are substantially active across all HCV genotypes. All currently approved second-generation PIs have macrocyclic peptidomimetic structures (Figure 1.2B).

FIRST-GENERATION NS3/4A HCV PROTEASE INHIBITORS

The first clinical proof-of-concept for NS3/4A PIs was achieved with ciluprevir (BILN 2001), a macrocyclic protease inhibitor that, for the first time, showed that a very fast drop in viremia could be achieved in patients, establishing a new paradigm for how to conduct early, proof-of-concept DAA clinical trials. Unfortunately, development of ciluprevir had to be halted because of cardiotoxicity issues in non-human primates. The first NS3/4A inhibitors to gain approval to the market, for use in combination with RBV and PEF-IFN, were boceprevir and telaprevir in 2011. These are both linear peptidomimetics containing a chemical warhead (α-ketoamide) that forms a covalent bond with the protease catalytic serine (Figure 1.2B). Although PIs of the first wave represented a major advance in the treatment of chronic hepatitis C, they had several limitations in terms of adverse effects,
very frequent dosing, narrow genotype spectrum, and exceedingly low barrier to resistance. In fact, resistance-associated substitutions (RASs) that conferred in vitro as well as clinical resistance to these drugs were readily generated at several positions close to the NS3/NS4A protease active site, including Val36, Thr54, Arg155, Ala156 and Ala156 (Figure 1.2A). In addition, these agents have an efficacy spectrum essentially limited to genotype 1 HCV (Table 1.I).

Compounds belonging to the second wave of first-generation PIs include macrocyclic peptidomimetic compounds such as simeprevir (Figure 1.2B) and paritaprevir. Unlike their first-wave counterpart, first generation second-wave PIs do not have the chemical reactivity needed to covalently attack their target, generally leading to fewer and less severe side effects. In addition, these agents have pharmacokinetic profiles compatible with once or twice daily dosing (low-dose ritonavir boosting is used with paritaprevir in order to decrease dosing frequency). Both of these agents are now approved for use in IFN-free combination regimens against chronic infection with genotype 1 and genotype 4 HCV (Table 1.II). Although the spectrum of action of second-wave NS3/4A PIs on the different HCV genotypes is somewhat broader compared to their predecessors, these agents still have limited spectrum of action and are invariably ineffective on genotype 3.

Along with the restricted genotype coverage, the genetic barrier to resistance observed with first generation NS3/4 PIs is very low, with extensive cross-resistance observed between the different compound classes. In particular, mutations of Arg155 have shown to confer broad cross-resistance to all first-generation inhibitors. Conversely, mutations of Val36 or Thr54 have been exclusively observed in association with covalent linear inhibitors of the first wave, and mutations of Asp168 are specifically found to confer broad resistance to non-covalent peptidomimetic inhibitors of the second wave, either linear or macrocyclic.9

SECOND-GENERATION ACTIVE SITE AND ALLOSTERIC NS3/4A PROTEASE INHIBITORS

Grazoprevir (Figure 1.2B) is an approved second-generation NS3/4A PI (Table 1.I), with broad genotype coverage and a higher barrier to resistance compared to first-generation PIs.10 This property is combined with significant activity against common viral variants associated with resistance to first-generation PIs, such as those containing multi-drug RASs at position Arg155 and Asp168. A recent crystallographic study analyzing the molecular basis of drug resistance against NS3/4A PIs indicated that first-generation PIs make a direct interaction with Arg155 and Asp168, whereas grazoprevir interacts in a unique conformation with the catalytic triad, thus avoiding direct contact with the residues responsible for broad PI resistance.11 In line with a higher barrier to resistance compared to

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<td>Barrier to resistance</td>
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<td>: minimal; ++: intermediate; +++: good.</td>
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first-generation inhibitors, no viral breakthrough has been observed in genotype 1 HCV patients who received this drug as monotherapy for up to 7 days. Moreover, genotype 3 patients responded with a robust decline in viral RNA, albeit only at the higher drug doses. 

Voxilaprevir (GS-9857)\(^\text{13}\) and glecaprevir (ABT-493)\(^\text{14}\) are additional second-generation PIs with broad genotype coverage and high barrier to resistance, currently in late stage development for possible use as a component of a combination DAA regimen that could be active across all viral genotypes (Table 1.1).

### NS5B POLYMERASE INHIBITORS

The NS5B RNA-dependent RNA polymerase (RdRp) is the enzyme directly responsible for the synthesis of the HCV RNA genome and is another highly pursued therapeutic target. In analogy to other nucleic acid polymerases, NS5B has the typical right-hand structure, consisting of a thumb-domain and a fingers-domain encircling the enzyme active site located within the palm-domain (Figure 1.3A). Clinically relevant inhibitors of the NS5B RdRp are classified into nucleotide (NI) and non-nucleotide (NNI) inhibitors, which act at different sites and distinct stages of RNA synthesis (Figure 1.3B).

### NON-NUCLEOTIDE INHIBITORS

NNI scaffolds have been discovered primarily in screening campaigns aimed at inhibiting the \textit{in-vitro} enzymatic activity of the HCV RdRp NS5B. Although chemically very diverse, virtually all HCV NNIs described so far are non-competitive with NTP substrates and inhibit the polymerase by inhibiting initiation of RNA synthesis. NNIs are generally believed to act by interfering with dynamic conformational changes that take place during the transition from initiation to processive RNA synthesis. In keeping with this notion, NNI-binding sites are all “allosteric” sites, almost invariably found on the relatively little conserved regions on the enzyme surface (Figure 1.3A). Because of this reason, NNIs have a restricted spectrum of activity against the various HCV genotypes and present a very low barrier to emergence of resistance. Different NNI binding sites are illustrated in Figure 1.3A. These include the so-called “thumb site I”, “thumb site II”, “palm site I”, and “palm site II”. Significant variability in the amino acid sequence is observed at these sites, making it difficult to achieve antiviral efficacy against different genotypes or even HCV isolates within the same genotype. As a result, most reported NNIs are rather specific for genotype 1 – if not only for genotype 1b.

Several structurally related NNIs have been shown to bind to the thumb I site (Figure 1.3A). This class of inhibitors interfere with the intramolecular contacts between the thumb and the finger tips, thus preventing the formation of a productive enzyme/RNA complex.\(^\text{15}\) These agents are also known as “finger-loop” inhibitors and are characterized by having a common benzimidazole or indole chemical core. HCV variants resistant to these agents are characterized by RASs at positions Pro495, Pro496 and Thr389 (reviewed in Delang \textit{et al.}).\(^\text{16}\) Clinically, agents belonging to this class of NNIs display reduced activity against genotype 1a HCV compared to genotype 1b.\(^\text{17}\) Several thumb I NNIs, including TMC647055\(^\text{18}\) and Beclabuvir\(^\text{19}\) have reached phase IIa clinical trials and Beleovir\(^\text{20}\) has advanced to Phase IIb, but none of them, thus
far, has gained regulatory approval.

Thumb II NNIs bind to a cavity located at the base of the thumb domain of NS5B (Figure 1.3A). Signature RASs at positions Leu419, Met423, and Ile482 in the viral polymerase have been shown to confer broad resistance to this class of compounds. Lomibuvir, a tiophene carboxylic acid, and filibuvir, a dihydropyranone derivative, proceeded to Phase II clinical trials but their development was eventually discontinued.

Palm I NNI-binding site is located at the junction of the palm and the thumb domain of NS5B, in proximity to the catalytic site (Figure 1.3A). Benzothiazidazine compounds, such as setrobuvir, bind to this NNI site. The most frequently selected RASs are Cys316Tyr, Met414Thr, Tyr448His/Cys, or Ser556Gly. Setrobuvir proceeded to Phase II clinical trials. Acylpyrrolidines are another class of palm I-binding compounds. In this class, GSK625433 was advanced into phase I clinical trials, but this agent was discontinued because positive in pre-clinical carcinogenicity studies.
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The palm II NNI-binding site partially overlaps with the palm I site and is located proximal to the junction between the palm and thumb domain (Figure 1.3A). A class of benzofurans was originally identified as an NNI class binding to this site. Signature RASs for these molecules map at residues Leu314, Cys316, Ile363, Ser365 and Met414. HCV-796 showed significant activity in early stage clinical trials, but was discontinued because of adverse side effects. HCV variants resistant to imidazopyridines, another HCV NNI chemotype, carry substitutions in the same site (Cys316Tyr) as well as mutations in a β-hairpin loop located in close proximity of the catalytic active site (Cys445Phe, Tyr448His, Tyr452His). Recent data revealed that imidazopyridine NNIs require metabolic activation for activity. The resulting metabolite, after forming a conjugate with glutathione, directly and specifically interacts with NS5B. Within the class of imidazopyridine NNIs, tegobuvir/GS-9190 proceeded in Phase II clinical trials, but is not being pursued further. Finally, Dasabuvir (Figure 1.3B) is a palm site II site inhibitor and – thus far – is the only approved NNI (Table 1.I). Dasabuvir is based on a benzothiadiazine scaffold and is only active on genotype 1 HCV. As for the other members of this NNI class, signature RASs are found at position Cys316, Met414, Tyr448 and Ser556.

NUCLEOTIDE ANALOGUES

Unlike NNIs, NIs target HCV the catalytic site of the NS5B enzyme, compete with the incoming nucleoside triphosphate for binding and incorporation. NIs are mimics of the natural polymerase substrates and are incorporated by the polymerase in the nascent RNA, leading to premature chain termination. Nucleoside inhibitors need three sequential phosphorylation steps by cellular kinases to be converted to the active NTP form. Conversely, nucleotide polymerase inhibitors are administered as prodrugs of nucleoside 5'-monophosphates, thus bypassing the rate-limiting step represented by the first phosphorylation step. Because the active site of the polymerase is highly conserved, NIs have similar efficacy across all HCV genotypes/isolates. For the same reason, HCV NIs are associated with a high barrier to development of drug-resistance and may also be active on viruses related to HCV, such as for example Zika virus.

Currently, the only approved NI drug is sofosbuvir (Table 1.I). The discovery of sofosbuvir was awarded the 2016 Lasker-DeBakey clinical medical research award for revolutionizing the treatment of chronic hepatitis C. This agent is a prodrug of uridine analogue monophosphate, containing a 2'-fluoro-C-methyl substitution on the sugar ring (beta-D-2'-deoxy-2'-fluoro-2'-C-methyluridine monophosphate; Figure 1.3B). Sofosbuvir is readily metabolized to the monophosphate form upon intracellular hydrolysis and further modified to the active NTP in hepatocytes.

Ser282Thr substitution has been identified as the hallmark mutation associated to resistance to 2'-C-methyl NIs. Emergence of this RAS is hardly ever observed in the clinical practice, however, partly because a serine to threonine change needs a transversion mutation (interchanges of purine for pyrimidine bases), which is much less frequently generated compared to transitions mutations (interchanges within purine or pyrimidines). Additionally, the Ser282Thr substitution severely reduces HCV replication capacity, explaining the high-barrier to resistance observed with 2'-modified NIs. In line with this notion, relevant viral resistance has been hardly observed in any sofosbuvir-based clinical study, regardless of the viral genotype. For all the reasons discussed above, sofosbuvir has become the backbone of many DAA combination therapeutic regimens (see below).

Importantly, 2'-C-methyl is the hallmark of NIs active on the polymerase of HCV and other viruses of the Flaviridae family. Structural studies have indeed indicated that
the essential 2'-C-methyl modification of the ribose ring causes a steric conflict with the incoming NI substrate, resulting in effective chain termination.\textsuperscript{34} Other NI in late stage development include a number of 2'-C-methyl ribonucleotide prodrugs, such as uprifosbuvir (MK-3682), ACH-3422 and AL-335.\textsuperscript{35}

**NS5A INHIBITORS**

HCV NS5A is a multifunctional, dimeric protein that does not possess any known enzymatic activity, but is critically implicated in HCV RNA replication and virion assembly.\textsuperscript{36} The NS5A protein structure consists of three domains: domain I (amino acids 1-213), domain II (amino acids 250-342) and domain III (amino acids 356-447). The three-dimensional crystallographic structure of domain I has revealed a dimeric form containing zinc- and an RNA-binding motif (Figure 1.4A).\textsuperscript{37} Not being associated to any measurable enzymatic activity, NS5A has been considered “not druggable” for a very long time. In recent years, however, compounds acting on NS5A have emerged as the most potent inhibitors of HCV replication, with specific examples displaying anti-HCV activity in the low pM range. NS5A inhibitors were initially discovered by replicon screening. Medicinal chemistry efforts subsequent to the initial hit identification led to the synthesis of extremely potent compounds, characterized by a characteristic dimer-like structure (Figure 1.4B). The prototype of this “palindromic” NS5A inhibitor class is daclatasvir, a compound with potent activity against a broad range of HCV genotypes,\textsuperscript{38} now approved for clinical use (Table 1.1).

![Fig. 1.4. - Structure of the NS5A protein and amino acid positions implicated in resistance resistance to NS5A inhibitors. A) Three-dimensional structure of NS5A protein Domain I. The structural Zn\textsuperscript{2+} ion is in gray. The amino acids corresponding to main resistance mutations are evidenced and numbered. The amino acids corresponding to the main RASs for different inhibitor classes are evidenced and numbered. Other positions that are mutated in NS5A-resistant HCV variants are not visible in the Domain I crystallographic structure; B) chemical structures of selected approved NS5A inhibitors.](image-url)
The precise mechanism of action of NS5A-inhibitors is not yet completely understood. They were initially claimed to be NS5A inhibitors mainly based on the selection of resistant HCV replicon variants with RASs that mapped in NS5A Domain I. Changes corresponding to substitutions of NS5A Tyr93 were found by different groups to be signature mutations conferring broad resistance to this class of antivirals. Interestingly, Tyr93 is found near the protein dimer interface (Figure 1.4B), suggesting a binding mode in which the inhibitor interacts across the NS5A dimer interface, making simultaneous contacts to both protein monomers. This would be in line with the extraordinary potency observed for these highly symmetric inhibitors. Recently, NS5A inhibitors were shown to interfere with the accumulation of phosphatidylinositol 4-phosphate in the membranous HCV replication compartment. In the HCV replication complex membranes, phosphatidylinositol 4-phosphate is produced by PI4KIIIα, a specific kinase that is specifically recruited and activated by the interaction with NS5A. These new data indirectly suggest that interaction of these antiviral agents with NS5A might interfere with the recruitment and/or activation of PI4KIIIα by HCV NS5A.

Today, with five NS5A inhibitors currently used in clinical practice for HCV treatment, NS5A inhibitors have become an integral part of several DAA regimens. Currently approved NS5A inhibitors include daclatasvir, ledipasvir, ombitasvir, elbasvir and velpatasvir. Conversely, odalasvir, pibrentasvir, and ruzasvir are NS5A inhibitors in late stage clinical development (Table 1.1).

In spite of the potent antiviral activity, combined with a broad spectrum of action of different genotypes, the genetic barrier to resistance for NS5A inhibitors is typically low, especially for genotype 1a. For example, with daclatasvir, resistant variants emerge readily, with the more relevant RASs found at NS5A residues 28, 30, 31, and 93 for genotype 1a and residues 31 and 93 for genotype 1b. Unlike for other DAA classes, HCV variants harboring NS5A RASs show high replication fitness. As a result, they can be frequently found circulating in patients prior to exposure to an NS5A-targeted drug. For the same reason, once a resistant variant carrying an NS5A RAS emerges under therapy, it can persist for a very long time.

Notably, agents like ruzasvir, elbasvir, velpatasvir, odalasvir (ACH-3102) and pibrentasvir (ABT-530) have been reported to be endowed with potent activity against all genotypes as well as with a minimal potency shift from the wild-type virus to viruses carrying RASs selected by early NS5A inhibitors. In analogy to what discussed for PIs, these agents can be viewed as “second-generation” NS5A inhibitors.

**TREATMENT STRATEGIES**

A major challenge in treating hepatitis C originates from the diversity inherent to the virus. HCV is in fact classified into seven major genotypes, with more than known 50 subtypes. Interferon (IFN)-based regimens, eventually in pegylated formulations and with the addition of RBV, represented the standard of care for chronic hepatitis C for >20 years after the discovery of HCV. Treatment outcomes varied greatly between genotypes, with cure rates that peaked at around 40% for the “hard-to-treat” genotypes 1 and 4. These poor outcomes – along with the many severe adverse side effects associated to lengthy IFN regimens – were major drivers of the search for newer, more tolerated and effective treatments.