World Journal of Virology

Online Submissions: http://www.wjgnet.com/esps/
wjv@wjgnet.com
doi:10.5501/wjv.v2.i1.6

World J Virol 2013 February 12; 2(1): 6-15
ISSN 2220-3249 (online)

MINIREVIEW

Hepatitis C virus resistance to new specifically-targeted antiviral therapy: A public health perspective

Karina Salvatierra, Sabrina Fareleski, Alicia Forcada, F Xavier López-Labrador

Karina Salvatierra, Sabrina Fareleski, F Xavier López-Labrador, Joint Unit in Genomics and Health, Centre for Public Health Research, Public Health Department, Generalitat Valenciana/Institut Cavanilles, University of Valencia, 46020 Valencia, Spain
Alicia Forcada, Instituto de Biomedicina de Valencia-Consejo Superior de Investigaciones Científicas, 46010 Valencia, Spain
F Xavier López-Labrador, CIBER in Epidemiology and Public Health (CIBER-ESP), Instituto de Salud Carlos III, 46020 Valencia, Spain
F Xavier López-Labrador, Department of Microbiology, Medical School, University of Valencia, 46010 Valencia, Spain
Author contributions: Salvatierra K and Fareleski S contributed equally to this work; all authors wrote the review article.
Supported by The Fondo de Investigación Sanitaria, Instituto de Salud Carlos III, Spanish Ministry of Economy, Directorate of Science (PI10/00512 and CIBER-ESP); López-Labrador FX Holds a P.I. Position supported by the Fondo de Investigación Sanitaria, Instituto de Salud Carlos III, Spain
Correspondence to: Dr. F Xavier López-Labrador, Joint Unit in Genomics and Health, Centre for Public Health Research, Public Health Department, Generalitat Valenciana/Institut Cavanilles, University of Valencia, Av. Catalunya 21, 46020 Valencia, Spain. f.xavier.lopez@uv.es
Telephone: +34-96-1985839 Fax: +34-96-1925703
Received: April 7, 2012 Revised: December 30, 2012
Accepted: January 17, 2013
Published online: February 12, 2013

Abstract

Until very recently, treatment for chronic hepatitis C virus (HCV) infection has been based on the combination of two non-viral specific drugs: pegylated interferon-α and ribavirin, which is effective in, overall, about 40%-50% of cases. To improve the response to treatment, novel drugs have been designed to specifically block viral proteins. Multiple compounds are under development, and the approval for clinical use of the first of such direct-acting antivirals in 2011 (Telaprevir and Boceprevir), represents a milestone in HCV treatment. HCV therapeutics is entering a new expanding era, and a highly-effective cure is envisioned for the first time since the discovery of the virus in 1989. However, any antiviral treatment may be limited by the capacity of the virus to overcome the selective pressure of new drugs, generating antiviral resistance. Here, we try to provide a basic overview of new treatments, HCV resistance to new antivirals and some considerations derived from a Public Health perspective, using HCV resistance to protease and polymerase inhibitors as examples.

Key words: Specifically-targeted antiviral therapy; Direct-acting antiviral; Protease inhibitors; Polymerase inhibitors; Viral resistance

Salvatierra K, Fareleski S, Forcada A, López-Labrador FX. Hepatitis C virus resistance to new specifically-targeted antiviral therapy: A public health perspective. *World J Virol* 2013; 2(1): 6-15 Available from: URL: http://www.wjgnet.com/2220-3249/full/v2/i1/6.htm DOI: http://dx.doi.org/10.5501/wjv.v2.i1.6

INTRODUCTION

Hepatitis C virus (HCV) infects an estimated 170 million people worldwide, which represents around 2%-3% of the global population[1]. Chronic HCV infection causes a progressive liver disease associated with increased risk of liver cirrhosis and hepatocellular carcinoma[2]. When end-stage liver disease is established, the only reliable therapeutic intervention, liver transplantation, is limited by the fact that a new chronic hepatitis is established in the graft, which can be lost in the early years post-transplantation[3]. The burden of HCV disease varies throughout the world, with country-specific prevalence ranging from < 1% to > 10%. The epidemiology of HCV infection is changing, and the transmission routes, demographics of infected individuals, and HCV genotype distribution varies between countries[4,5]. Public Health policies will likely need

to be adapted to these differences. With the new availability of highly effective therapies, there is now a time of increasing opportunities to significantly reduce HCV-related morbidity and mortality. Until very recently the standard treatment for chronic HCV infection was the combination of pegylated interferon-α (Peg-IFNα) and ribavirin (RBV)[6]. Rather than targeting the virus directly, these drugs are immunomodulators, although RBV may also increase the mutation rate of the HCV genome[7]. The majority of responder patients remain virus-free after 5 years of follow-up and are considered to be cured[8]. However, the efficacy of this therapy is variable, ranging from 40% to 80% depending on: viral genotype, stage of liver fibrosis, viral load, side effects and treatment discontinuation, body-mass index, age, race, and host genetics[9]. Genome-wide association studies revealed single nucleotide polymorphisms in the promoter region of the *IL28B* gene, encoding interferon lambda-3, as the strongest predictors for treatment response[10]. Increasing response rates are expected due to the development of numerous new direct-acting antivirals (DAAs) active against HCV (STAT-C: Specifically-Targeted Antiviral-Treatment for hepatitis C). Some of these compounds are in advanced clinical trials to be used either as an adjunct to Peg-IFNα + RBV, and/or combined with other DAAs. STAT-C drugs include inhibitors of the viral proteins NS3/4A, NS4B, NS5A, and NS5B[11,12]. The well-defined virus-specific enzymatic functions of the NS3/4A serine protease and the NS5B RNA-dependent RNA-polymerase (RdRp) made them the initial focus for drug development and represent the most advanced STAT-C drugs, showing potent antiviral efficacy *in vitro* and *in vivo*. In 2011, the NS3/4A protease inhibitors (PIs) Telaprevir (Vertex Pharmaceuticals) and Boceprevir (Schering-Merck) became the first STAT-C compounds approved for clinical use; and NS5A inhibitors and several NS5B polymerase inhibitors entered phase Ⅱ of development[11]. In addition, STAT-C drugs targeting other HCV proteins and drugs directed to host proteins that interact with the virus have also entered clinical development. There is a glimpse of optimism in the field, hoping that new STAT-C medications will allow shorter treatment durations and increase the rates of patients responding to antiviral treatment[13].

From a Public Health perspective, the extension of new, more effective, treatments including STAT-C compounds will: (1) eventually reduce the disease burden of chronic hepatitis C in the near future; (2) reduce the long-term costs of delayed care by increasing efforts to screen undiagnosed cases, with the aim of giving access to treatment and preventing progression of the disease; and (3) require a reinforcement of Public Health surveillance[14].

However, this optimism may be tempered by evidence demonstrating that HCV variants resistant to STAT-C compounds are rapidly selected *in vitro* and *in vivo*[15-17]. Eventually all classes of STAT-C, including NS3/4A protease and NS5B polymerase inhibitors, select for HCV resistant variants, although some nucleosidic inhibitors exhibit a higher barrier to resistance[18]. This is not surprising, given the high error rate of HCV replication and the rapid turnover of circulating virions. In fact, resistant variants arise from preexisting subpopulations of viral genomes already circulating in the infected individual, before therapy is started[19]. Selective drug pressure changes the balance between the different intra-individual HCV quasispecies; and resistant genomes dominate the circulating viruses in patients with treatment failure or suboptimal treatment response, as evidenced in patients treated with NS3/4 PIs[20-22]. Fortunately, resistant variants remain sensitive to Peg-IFN + RBV, which still makes their elimination possible with the current Peg-IFN + RBV treatment[16]. However, these therapy regimens are complex, and simplification is eagerly pursued. Finally, most of the first-generation STAT-C compounds have been designed using HCV proteins and replicon assays based in HCV subtype 1b, and the efficacy in other viral subtypes may be suboptimal (lower genetic barrier), given the diversity of this virus and the high number of genotypes and subtypes[23]. Due to the low genetic barrier (the number of nucleotide substitutions required for the virus to acquire resistance to a given drug) of some compounds, there are concerns that high-level resistance will develop quickly, and the possibility of transmission of resistant strains among intravenous drug users[24]. From a Public Health perspective, it seems necessary: (1) to determine the prevalence of major HCV resistant variants in the infected population; (2) to determine the efficacy of STAT-C compounds on HCV subtypes other than 1b; and (3) to establish virology laboratories for HCV genotypic resistance testing and surveillance. Finally, because the distribution of HCV subtypes varies in different geographical regions, policies may have to be refined locally to give access to treatment with optimized STAT-C regimes.

HCV BIOLOGY AND THE BASIS FOR RESISTANCE

HCV is an enveloped virus, the only member of the genus *Hepacivirus* within the family *Flaviviridae*, with a positive-sense, single-stranded RNA genome of about 9600 bases flanked by two highly-conserved non-coding regions[25]. The genome encodes a single polyprotein of around 3000 amino acids, processed by both host and viral proteases into the mature three structural and seven non-structural proteins, including the components of the capsid and envelope (core, E1 and E2) and the viral enzymes needed for replication and virion assembly (p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B), respectively (Figure 1)[26]. Like other RNA viruses, the high error rate of the RdRp makes HCV evolution very rapid, with a mutation rate estimated at 10^{-3} to 10^{-5} nucleotides per site[27].

Evolution of the virus has led to the distinction of six major genotypes and more than 40 subtypes. Genotypes 1, 2 and 3 are more common in Western countries, genotypes 1, 4 and 5 in Africa, and genotype 6 in Asia[23]. The distribution of HCV genotypes and subtypes varies be-

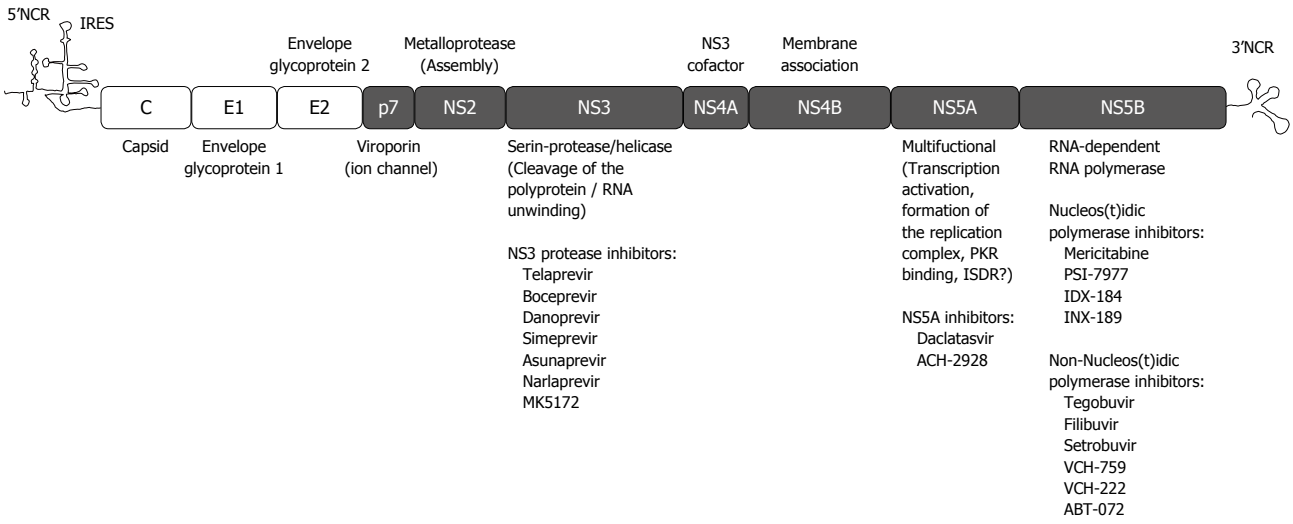

Figure 1 Diagram of the hepatitis C virus genes and the viral polyprotein, with two non-coding regions in the 5' and 3' ends of the viral genome, structural (with) and non-structural (grey) proteins. The targets for the most-developed Specifically-Targeted Antiviral-Treatment for hepatitis C compounds are indicated, together with drugs in advanced development.

tween countries probably because of the spread through different routes of transmission, such as blood-transfusion and healthcare-related practices (subtype 1b) or intravenous drug use (subtype 1a and genotype 3) which is now the main route of infection in most countries[4]. For instance, the prevalence of HCV subtype 1b varies from more than 50% of infected individuals in Italy, Poland, Romania, Turkey, or Russia to less than 25% in Canada. Similarly, the prevalence of non-1 genotypes is also very variable, accounting from less than 25% of the infections in Romania, Turkey, or the Czech Republic to more than 50% in Sweden, Norway, or the United Kingdom[4]. Local HCV genotype/subtype epidemiology may be relevant to the design of optimal treatment strategies because the cross-genotype efficacy of most STAT-C compounds is very limited (see below).

HCV displays another level of genetic variability: intraindividual variation. The combination of a high mutation rate with the production of around 10^{12} virions per day[28] results in every infected individual carrying a pool of slightly variant viral genomes which can eventually contain every possible single (and maybe double) mutants: a cloud of variants common in RNA viruses, so-called "quasispecies"[21,29].

This extensive genetic variability gives HCV the capacity to generate drug resistance. Mutations that change amino acids of STAT-C target proteins, including NS3 and NS5B, do occur in the absence of the drugs, and can cause conformational changes that may interfere with drug-target interaction. If DAA-resistant variants are already present before the start of treatment, they will be rapidly selected and become dominant in the viral quasispecies once the drug is administered, because they will be under positive selective pressure. In addition, unless the replication of the virus is fully suppressed, even if resistance is not present prior to therapy, adaptive mutations can eventually emerge during STAT-C administration, that reduce the susceptibility of the virus to the drug.

Not surprisingly, several mutations were soon identified *in vitro* to be associated with reduced susceptibility to NS3 and NS5B inhibitors[17], some of these are present in natural isolates from naive individuals[30-35], and were later related to STAT-C treatment failure in clinical trials[18].

The role of naturally-occurring variations in resistance to STAT-C inhibitors is therefore a focus of intensive research. An example is the study of HCV resistance to NS3/4A protease and NS5B polymerase inhibitors. Table 1 shows a summary, extracted from several reviews, of the most important amino acid variations in HCV NS3/4A protease, NS5A protein and NS5B polymerase associated with resistance to DAAs.

HCV resistance to NS3/4A PIs

The viral *NS3* gene encodes amino acids 1027-1657 of the polyprotein (numbering on HCV-H77-1a strain), including a serine protease located in the N-terminal domain (amino acids NS3 1-181) and an NTPase/RNA helicase in the C-terminal part (amino acids NS3 182-623)[36]. The chymotrypsin-like protease requires a cofactor, the NS4A protein, and is responsible for critical steps in the virus lifecycle: (1) the cleavage of the viral polyprotein in the NS3-NS4A, NS4A-NS4B, NS4B-NS5A and NS5A-NS5B junctions; and (2) the modification of the cellular response, interfering with the interferon pathway[37-39]. Thus, blocking NS3/4A protease activity may inhibit both processing of the viral polyprotein and viral down-regulation of the innate immune response.

Clinical development of the first-in-class HCV PI (Ciluprevir, BILN-2061) showed exceptional antiviral activity both *in vitro* and *in vivo* in phase I Studies, but further development was halted because cardiotoxicity was detected in animals[40]. These promising results were reproduced with other PIs, such as Telaprevir (VX-950), Boceprevir

Table 1 Most important variations related to hepatitis C virus viral resistance to NS3 protease and NS5b polymerase *in vitro* and/or *in vivo*

Inhibitor class	Amino acid variations implicated in resistance
First generation NS3/A PI	
Ciluprevir	(NS3) R155K/T/Q, A156V/T, D168A/V/T/H
Telaprevir	(NS3) V36M/A, T54A, R155K/T, A156V/T/S, V36M/A+R155K/T, V36M/A+A156V/T
Boceprevir	(NS3) V36M/A/L, T54S/A, R155K, V55A, R155T, A156S, V158I, V170A, I170T
Second generation NS3/A PI	
Danoprevir	(NS3) R155K, D168E
Simeprevir	(NS3) Q80R/Q, R155K/T/Q, A156S/V/T, D168A/V/T/H
Asunaprevir	(NS3) R155K, A156V/T, D168A/E/T/V/Y
Narlaprevir	(NS3) V36A/M, R155K/T/Q, A156S/V/T, V170A
MK5172	(NS3) A156V/T, D168A/V/T/H
NS5A inhibitors	
Daclatasvir	(NS5A) Q30R, L31 M/V, Y93C/N
NA polymerase inhibitors	
Mericitabine	(NS5B) S282T *in vitro*, not reported *in vivo*
PSI-7977	Not reported *in vivo*
IDX-184	Not reported *in vivo*
INX-189	Not reported
NNI polymerase inhibitors	
Tegobuvir	(NS5B) C316N, Y448H
Filibuvir	(NS5B) M423T/I/V, M426T, I482T
Setrobuvir	(NS5B) M414T/L, G554D, D559G
VCH-759	(NS5B) L419M/V, M423T/I/V, I482L/V/T, V494I/A
VCH-222	(NS5B) L419M, M423T, I482L
ABT-072	(NS5B) C316Y, M414T, Y448H/C, S556G

Summary from[11,17,18,77-80]. PI: Protease inhibitors; NA: Nucleos(t)ide analogs; NNI: Non-nucleos(t)idic (allosteric) inhibitors.

(SCH-503034), and Danoprevir (ITMN-191). Several other PIs belonging to two inhibitor classes are in clinical trials: linear ketoamids, and macrocyclic compounds, including Simeprevir (TMC435, Tibotec/Medivir), Asunaprevir (BMS-650032, Bristol-Myers Squibb), Danoprevir (RG7227, Roche/InterMune), BI201335 (Boehringer-Ingelheim), ACH-1625 and ACH-2684 (Achillion), Vaniprevir and MK-5172 (Merck and Co.)[11,12].

Two of these have been approved recently for clinical use: Telaprevir and Boceprevir. HCV resistance to both linear and macrocyclic PIs by amino acid substitutions is well documented *in vitro* and *in vivo*[41]. The selection of resistant mutants is rapid (during the first weeks of exposure to the DAA), and compound-specific, although some resistant strains however may show a reduced fitness, which allows viral control using the standard Peg-IFN + RBV treatment[16]. A major concern of HCV resistance to first-generation PIs is cross-resistance. Substitutions NS3-R155K/T and NS3-A156S/T/V confer a high level of resistance to both Boceprevir and Telaprevir and cross-resistance to most NS3 PIs. Substitutions NS3-V36A/M and NS3-T54A/S confer a low level of resistance to both Telaprevir and Boceprevir, and NS3-V170A/T to Boceprevir. There is also some cross-resistance of mutations in positions 36, 54 and 170 with other compounds, while changes in positions NS3-80, NS3-155 and NS3-168 are implicated in resistance to macrocyclic inhibitors[17,42]. Double mutants may also be selected by STAT-C treatments, combining two resistance mutations for the same or different PI class, with a potential for broad resistance to both linear and macrocyclic inhibitors[43]. However, given the available experience with human immunodeficiency virus, the selection of double or triple HCV mutants resistant to different drugs targeting different viral genes (*i.e.*, protease and polymerase) seems unlikely. Selected resistant variants in the protease have been implicated in late relapse after cessation of treatment, and may decline or remain detectable for years after treatment failure[44-46]. These resistance mutations may also revert to wild-type virus with time, but still some resistant viruses revert very slowly[47]. In addition, resistant variants exist, at different levels, before treatment. First, the NS3/4 protease is polymorphic in sites associated with resistance between HCV genotypes 1-6, and between some subtypes. For instance, variations in NS3-V170 are present in most HCV genotype 1 isolates, and polymorphism in NS3-D168 is characteristic of HCV genotype 3[32]. As the development of NS3/4A PIs was based in HCV genotype 1, subtype 1b, their antiviral activity with non-1b genotypes, may be not as effective, although some PIs inhibit more than one HCV genotype[22,42,48-50]. Currently neither Boceprevir nor Telaprevir should be used in patients infected with HCV genotypes other than 1. First-generation PIs have some activity in HCV genotypes 2 and 4, but very limited activity in genotype 3-infected patients[51]. Among PIs in clinical development, Simeprevir showed potent activity against HCV genotype 1, but lesser activity against genotypes 2, 4, 5, and 6[50].

Second, even within HCV genotype 1, large studies in several countries have found resistance in STAT-C naive patients (prevalence up to 5.5%)[33-35]. Thus, naturally-occurring polymorphisms can modify the treatment

response to STAT-C. In addition, there are differences in the genetic barrier to resistance between viral subtypes. HCV subtype 1a has a low genetic barrier for approved PIs, and this is the reason for higher viral breakthrough rates and selection of resistant variants observed in patients infected with subtype 1a during treatment with Boceprevir and Telaprevir. The resistance mutation R155K emerges from a single nucleotide substitution in subtype 1a viruses; whereas two different substitutions are needed in the subtype 1b viruses[17,18]. Viral breakthrough and relapse after treatment with Simeprevir is usually associated with signature resistance mutations at NS3 positions 80, 122, 155, and/or 168 (positions 80 and 168 are polymorphic between subtypes), but the distribution of mutations also varies significantly between subtypes 1a and 1b[52]. For Asunaprevir, the primary NS3 protease substitutions associated with high-level resistance identified *in vitro* occur predominately at the polymorphic amino acid residue D168 (D168A/G/H/V/Y). In addition, in single- and 3-d multiple-ascending-dose studies in HCV genotype 1a- or 1b-infected patients, a predominant pre-existing NS3 baseline polymorphism (NS3-Q80K) had ambiguous effects, but no clinically-relevant resistance-associated variants emerged in these clinical studies[53]. Finally, the large turnover and population size, together with the high mutation rate of the virus implicates that HCV variants resistant to new DAAs may be present as minority species (not detectable by direct population sequencing) within the complex pool of viral genomes circulating in a single patient[33,54,55]. Pre-existing variants resistant to Boceprevir or Telaprevir may impair virologic response before treatment is initiated[56,57]. The role of naturally-occurring polymorphisms and minority variants in treatment failure is just being elucidated, and clinical development of STAT-C compounds with pan-genotypic activity is needed.

HCV resistance to NS5A inhibitors

The HCV NS5A genomic region encodes a serine phosphoprotein of 448 amino acids (a.a. 2421-3011 of the polyprotein, numbering on HCV-H77-1a strain), which seems to have a role in transcriptional activation and participates in enhancing viral replication. NS5A has been linked to interferon sensitivity, and includes a variable region (V3), and PKR and zinc binding domains[26]. NS5A replication complex inhibitors undergoing clinical trials include Daclatasvir (BMS-790052, Bristol-Myers Squibb) and ACH-2928 (Achillion)[12]. Daclastavir is a potent oral NS5A inhibitor, studied in combination with the NS3 PI Asunaprevir alone (n = 11), or plus Peg-IFNα + RBV (n = 10) for 24 wk in genotype-1 infected patients[58]. Double and quad combination therapy produced SVR in 36% and 90% of patients respectively. In the double therapy group, viral relapse occurred in one patient (HCV subtype 1a). An analysis of baseline samples revealed a preexisting NS3 variant (R155K) conferring resistance to Asunaprevir at the time of viral relapse, whereas the NS5A resistance variant Q30E was detected only at relapse. All patients with viral breakthrough (n = 6, 55%) were infected with HCV subtype 1a. There was no resistance variants at baseline and resistance variants to both Daclatasvir and Asunaprevir had emerged in all cases by the time of viral breakthrough. Viral variants in the NS5A domain included Q30R, L31 M/V, and Y93C/N; and variants in the NS3 protease included R155K and D168A/E/T/V/Y[58]. Another phase Ⅱa study in Japan examined the combination of Daclastavir with Asunaprevir, without Peg-IFN and RBV (n = 10, HCV genotype 1b). All the nine patients who completed the full course of treatment, achieved SVR (HCV-RNA was negative at weeks 12 and 24), and there as no viral breakthrough[59]. In a survey in Japan, resistance mutations to Daclatasvir NS5A-L31M and/or Y93H were detected in 11.2% of 307 untreated patients with HCV subtype 1b infection, and Y93H (8.2%) was more prevalent than L31M (2.7%)[60]. Fifteen patients (4.9%) were infected with NS3-protease variants harboring V36A, T54S, Q80R or D168E, resistance mutations. While mutations conferring resistance to Daclatasvir or to NS3 inhibitors were frequent in this treatment-naive study population, double mutants with possible resistance to both drugs were rare. In addition, the cross-genotypic activity of Daclatasvir is under investigation. Thus, there is a rationale for expanding these double or quad therapy regimes including Dacaltasvir, but potential differences in efficacy between subtype 1a and 1b viruses should be further explored.

HCV resistance to NS5B polymerase inhibitors

The HCV NS5B genomic region encodes a 66 kDa protein composed of 591 amino acids (a.a. 2421-3011 of the polyprotein, numbering on HCV-H77-1a strain): an RdRp. The HCV RdRp resembles other viral polymerases, with a GDD motif and a right hand structure with palm, fingers and thumb domains[61]. The polymerase replicates the viral genome, in the catalytic sites located at the palm domain, from a negative strand RNA template intermediate, using an active triphosphate (NTP) as primer[62]. Depending on their chemical structure and mechanism of action, specific NS5B inhibitors can be divided into two groups: nucleoside/nucleotide analogues (NA) and non-nucleoside inhibitors (NNI). NA are alternative NTP substrates for the polymerase, forming a structure in the catalytic site that prevents the addition of new NTPs, resulting in premature chain-termination of nascent RNA[61]. The NNI inhibitors, rather than competing with NTPs, act by blocking the enzyme in the initiation of replication, preventing the conformational change necessary to elongate the nascent new copy of the RNA viral genome[63]. Four different allosteric NNI binding sites have been identified: NNI-Site A/thumb 1, NNI-Site B/thumb 2, NNI-Site C/palm 1, and NNI-Site D/palm 2; which are targets for benzimidazoles, dihydropyrones and thiophenes, benzothiazidines, and benzofurans, respectively[61]. Although the *NS5B* gene is less variable than other parts of the HCV genome, viral genetic polymorphism and mutation may also limit the

efficacy of NS5B-specific inhibitors[19,35,64,65].

The nucleos(t)ide inhibitors usually act at the catalytic site of the RdRp, where the GDD motif is located. In the replicon model, 2'-C-methyl-nucleosides select for the NS5B-S282T change, and some nucleotide inhibitors select for several other changes (S15G, R222Q, C223Y, C223H, L320I, V321I)[61,66,67]. However, a combination of at least three changes (S15G/C223H/V321I) was required to confer a high level of resistance[68]. NA inhibitors show, in fact, a high genetic barrier for the emergence of resistance[69], and no resistance mutations have been observed in NA monotherapy[70], or in combination with a PI[71]. In addition, in combination with Peg-IFNα + RBV, no viral breakthroughs due to resistance mutations could be identified[70]. NA inhibitors are therefore the most promising drugs due to the limited number of resistance mutations described[11], and the low frequency of resistant viruses circulating in the population[19,72]. Unfortunately, drugs in development in this class are few, compared to NNI.

NNI exhibit a different range of resistance profiles depending on the target site in the NS5B RdRp. Numerous substitutions associated with resistance to NNI were found mainly in the four allosteric sites A, B, C and D. Some particular changes seem extremely important for resistance, such as NS5B-M423V/I, which increases 31 times the resistance to the drug AG-02154[65]. In addition, secondary mutations may increase resistance profiles. When combined with the substitutions T19P, M71V, M423V or A442T, the change NS5B-A338V causes an increase of up to 17 times the resistance to dihydropyrones and Thiophene[73]. Variable responses to NNI may be due to natural variation in baseline susceptibility[74]. The NS5B-C316N change is frequent in HCV subtype 1b[32], and together with NS5B-Y448H, NS5B-D559G or NS5B-Y555C can increase resistance to benzofurans 30 fold.

NS5B polymerase inhibitors undergoing clinical trials include Mericitabine (RG-7128, Roche), PSI-7977 and Tegobuvir (Gilead), INX-189 (Inhibitex), Filibuvir (PF-00868554, Pfizer), VCH-222 (Vertex), ABT-072 (Abbott), and Setrobuvir (ANA-598, Anadys)[12]. Among these, nucleos(t)ide analogs such as RG-7128, PSI-7977 and INX-189 display a high genetic barrier to resistance and can be efficacious against several HCV genotypes, while non-nucleosidic inhibitors (Filibuvir, VCH-222, ABT-072, Setrobuvir, Tegobuvir) normally display a lower genetic barrier and a genotype-dependent antiviral effect (Table 1)[70,75].

CONCLUSION

Specifically-targeted antiviral therapies for HCV are entering the clinics, and more than 50 compounds are in development. The first major targets are NS3/A protease and NS5B polymerase, but other viral and host targets are in of drug development, such as the viral NS3 helicase, NS4A and NS5A proteins, or cyclophyllin inhibitors and modulators of the innate immune response. In the near future, the rate of sustained virological response will be much greater than with Peg-IFNα + RBV treatment. Due to the selective pressure of DAAs, resistant viruses circulating in the infected population can lead to treatment failure. A single mutation in the coding sequence of the viral enzyme may be sufficient to confer different levels of resistance to a particular drug. The emergence of these resistant variants has been generally observed soon after starting treatment, especially in monotherapy, as a result of the rapid decline of wild-type virus and the dominance of preexisting minority variants. Therefore, the selection of resistant viral strains can compromise new STAT-C regimens, and clinicians must take into account this problem.

In clinical trials with a single STAT-C drug, viral isolates from patients with treatment failure have exhibited one, or more than one, resistance mutation and some of them lasted for years. These results indicate that the success of new treatments using a single STAT-C may be compromised, and will require a high genetic barrier to resistance, optimal drug exposure and strict adherence. From a Public Health perspective, the allocation of resources needs to be maximized to treatment regimens ensuring success; this will depend on the effectiveness of drugs inhibiting all viral variants, minimizing the emergence of mutations. Maximum effectiveness to prevent the emergence of resistance will probably rely on a combination of DAAs with a high genetic barrier to inhibit different viral targets simultaneously, ideally with each inhibitor linked to a different set of resistance mutations.

Several combinations of DAAs, with or without concomitant Peg-IFNα + RBV administration, have obtained different efficacies in controlling HCV replication, with those regimens including compounds with a higher barrier to resistance [like nucleos(t)ide analogues, or NS5A inhibitors] being the more promising in minimizing the emergence of resistance[58,71,76]. To ensure the success of future treatments, it will be important to evaluate the true frequencies of naturally-occurring substitutions that may confer resistance to new DAA's in HCV isolates circulating in the infected population. At the Public Health level, it may be interesting to reinforce epidemiological surveillance to obtain specific data on the geographical distribution of HCV genotypes/subtypes and their prevalence in different cohorts of infected patients, because of the differential response to currently approved and new STAT-C treatment regimes in development. Strategies for increasing (or limiting) access to new treatments may require different approaches in different geographical regions. In addition, active HCV resistance surveillance is needed. Genotype sequencing on viral breakthrough (and also prior to treatment initiation) of the HCV genomic regions targeted by DAAs may be useful to identify resistance pathways, particularly in those patients in whom Peg-IFNα + RBV therapy has failed; the first candidates for newer STAT-C regimens.

Finally, the future availability of highly potent STAT-C combinations can potentially decrease the global burden

of HCV disease, and pave the way for HCV eradication. However, the high cost of STAT-C drugs, their limited efficacy in non-1 HCV genotypes, the emergence of resistance, and the need for sophisticated monitoring of new treatments makes them unreliable for resource-limited countries where the highest prevalence of chronic infection is concentrated. Clinical development of simple and affordable all-oral combination therapeutic regimes with antiviral activity to all HCV genotypes is required, and the development of an effective vaccine is still a relevant unmet goal.

REFERENCES

1 **Lavanchy D**. Chronic viral hepatitis as a public health issue in the world. *Best Pract Res Clin Gastroenterol* 2008; **22**: 991-1008 [PMID: 19187863 DOI: 10.1016/j.bpg.2008.11.002]

2 **Ghany MG**, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; **49**: 1335-1374 [PMID: 19330875 DOI: 10.1002/hep.22759]

3 **Berenguer M**, Prieto M, Rayón JM, Mora J, Pastor M, Ortiz V, Carrasco D, San Juan F, Burgueño MD, Mir J, Berenguer J. Natural history of clinically compensated hepatitis C virus-related graft cirrhosis after liver transplantation. *Hepatology* 2000; **32**: 852-858 [PMID: 11003634 DOI: 10.1053/jhep.2000.17924]

4 **Cornberg M**, Razavi HA, Alberti A, Bernasconi E, Buti M, Cooper C, Dalgard O, Dillion JF, Flisiak R, Forns X, Frankova S, Goldis A, Goulis I, Halota W, Hunyady B, Lagging M, Largen A, Makara M, Manolakopoulos S, Marcellin P, Marinho RT, Pol S, Poynard T, Puoti M, Sagalova O, Sibbel S, Simon K, Wallace C, Young K, Yurdaydin C, Zuckerman E, Negro F, Zeuzem S. A systematic review of hepatitis C virus epidemiology in Europe, Canada and Israel. *Liver Int* 2011; **31** Suppl 2: 30-60 [PMID: 21651702 DOI: 10.1111/j.1478-3231.2011.02539.x]

5 **Averhoff FM**, Glass N, Holtzman D. Global burden of hepatitis C: considerations for healthcare providers in the United States. *Clin Infect Dis* 2012; **55** Suppl 1: S10-S15 [PMID: 22715208 DOI: 10.1093/cid/cis361]

6 **Zeuzem S**. Interferon-based therapy for chronic hepatitis C: current and future perspectives. *Nat Clin Pract Gastroenterol Hepatol* 2008; **5**: 610-622 [PMID: 18838975 DOI: 10.1038/ncpgasthep1274]

7 **Cuevas JM**, González-Candelas F, Moya A, Sanjuán R. Effect of ribavirin on the mutation rate and spectrum of hepatitis C virus in vivo. *J Virol* 2009; **83**: 5760-5764 [PMID: 19321623 DOI: 10.1128/JVI.00201-09]

8 **Maylin S**, Martinot-Peignoux M, Moucari R, Boyer N, Ripault MP, Cazals-Hatem D, Giuily N, Castelnau C, Cardoso AC, Asselah T, Féray C, Nicolas-Chanoine MH, Bedossa P, Marcellin P. Eradication of hepatitis C virus in patients successfully treated for chronic hepatitis C. *Gastroenterology* 2008; **135**: 821-829 [PMID: 18593587 DOI: 10.1053/j.gastro.2008.05.044]

9 **Asselah T**, Estrabaud E, Bieche I, Lapalus M, De Muynck S, Vidaud M, Saadoun D, Soumelis V, Marcellin P. Hepatitis C: viral and host factors associated with non-response to pegylated interferon plus ribavirin. *Liver Int* 2010; **30**: 1259-1269 [PMID: 20633102 DOI: 10.1111/j.1478-3231.2010.02283.x]

10 **Suppiah V**, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Müller T, Bahlo M, Stewart GJ, Booth DR, George J. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 2009; **41**: 1100-1104 [PMID: 19749758 DOI: 10.1038/ng.447]

11 **Gelman MA**, Glenn JS. Mixing the right hepatitis C inhibitor cocktail. *Trends Mol Med* 2010; Epub ahead of print [PMID: 21106440 DOI: 10.1016/j.molmed.2010.10.005]

12 **Poordad F**, Dieterich D. Treating hepatitis C: current standard of care and emerging direct-acting antiviral agents. *J Viral Hepat* 2012; **19**: 449-464 [PMID: 22676357 DOI: 10.1111/j.1365-2893.2012.01617.x]

13 **Enserink M**. Infectious diseases. First specific drugs raise hopes for hepatitis C. *Science* 2011; **332**: 159-160 [PMID: 21474720 DOI: 10.1126/science.332.6026.159]

14 **Hatzakis A**, Wait S, Bruix J, Buti M, Carballo M, Cavaleri M, Colombo M, Delarocque-Astagneau E, Dusheiko G, Esmat G, Esteban R, Goldberg D, Gore C, Lok AS, Manns M, Marcellin P, Papatheodoridis G, Peterle A, Prati D, Piorkowsky N, Rizzetto M, Roudot-Thoraval F, Soriano V, Thomas HC, Thursz M, Valla D, van Damme P, Veldhuijzen IK, Wedemeyer H, Wiessing L, Zanetti AR, Janssen HL. The state of hepatitis B and C in Europe: report from the hepatitis B and C summit conference*. *J Viral Hepat* 2011; **18** Suppl 1: 1-16 [PMID: 21824223 DOI: 10.1111/j.1365-2893.2011.01499.x]

15 **Trozzi C**, Bartholomew L, Ceccacci A, Biasiol G, Pacini L, Altamura S, Narjes F, Muraglia E, Paonessa G, Koch U, De Francesco R, Steinkuhler C, Migliaccio G. In vitro selection and characterization of hepatitis C virus serine protease variants resistant to an active-site peptide inhibitor. *J Virol* 2003; **77**: 3669-3679 [PMID: 12610142 DOI: 10.1128/JVI.77.6.3669-3679.2003]

16 **Kieffer TL**, Sarrazin C, Miller JS, Welker MW, Forestier N, Reesink HW, Kwong AD, Zeuzem S. Telaprevir and pegylated interferon-alpha-2a inhibit wild-type and resistant genotype 1 hepatitis C virus replication in patients. *Hepatology* 2007; **46**: 631-639 [PMID: 17680654 DOI: 10.1002/hep.21781]

17 **Sarrazin C**, Zeuzem S. Resistance to direct antiviral agents in patients with hepatitis C virus infection. *Gastroenterology* 2010; **138**: 447-462 [PMID: 20006612 DOI: 10.1053/j.gastro.2009.11.055]

18 **Halfon P**, Locarnini S. Hepatitis C virus resistance to protease inhibitors. *J Hepatol* 2011; **55**: 192-206 [PMID: 21284949 DOI: 10.1016/j.jhep.2011.01.011]

19 **Le Pogam S**, Seshaadri A, Kosaka A, Chiu S, Kang H, Hu S, Rajyaguru S, Symons J, Cammack N, Nájera I. Existence of hepatitis C virus NS5B variants naturally resistant to non-nucleoside, but not to nucleoside, polymerase inhibitors among untreated patients. *J Antimicrob Chemother* 2008; **61**: 1205-1216 [PMID: 18343801 DOI: 10.1093/jac/dkn085]

20 **Susser S**, Welsch C, Wang Y, Zettler M, Domingues FS, Karey U, Hughes E, Ralston R, Tong X, Herrmann E, Zeuzem S, Sarrazin C. Characterization of resistance to the protease inhibitor boceprevir in hepatitis C virus-infected patients. *Hepatology* 2009; **50**: 1709-1718 [PMID: 19787809 DOI: 10.1002/hep.23192]

21 **Rong L**, Dahari H, Ribeiro RM, Perelson AS. Rapid emergence of protease inhibitor resistance in hepatitis C virus. *Sci Transl Med* 2010; **2**: 30ra32 [PMID: 20445200 DOI: 10.1126/scitranslmed.3000544]

22 **Bae A**, Sun SC, Qi X, Chen X, Ku K, Worth A, Wong KA, Harris J, Miller MD, Mo H. Susceptibility of treatment-naive hepatitis C virus (HCV) clinical isolates to HCV protease inhibitors. *Antimicrob Agents Chemother* 2010; **54**: 5288-5297 [PMID: 20855726 DOI: 10.1128/AAC.00777-10]

23 **Simmonds P**. Genetic diversity and evolution of hepatitis C virus--15 years on. *J Gen Virol* 2004; **85**: 3173-3188 [PMID: 15483230 DOI: 10.1099/vir.0.80401-0]

24 **Tong CY**, Cane PA, Pillay D. ARHAI: antiviral resistance. *J Antimicrob Chemother* 2012; **67** Suppl 1: i65-i68 [PMID: 22855880]

25 **Miller RH**, Purcell RH. Hepatitis C virus shares amino acid sequence similarity with pestiviruses and flaviviruses as well as members of two plant virus supergroups. *Proc Natl*

Acad Sci USA 1990; **87**: 2057-2061 [PMID: 2156259]
26 **Moradpour D**, Penin F, Rice CM. Replication of hepatitis C virus. *Nat Rev Microbiol* 2007; **5**: 453-463 [PMID: 17487147 DOI: 10.1038/nrmicro1645]
27 **Brown RJ**, Juttla VS, Tarr AW, Finnis R, Irving WL, Hemsley S, Flower DR, Borrow P, Ball JK. Evolutionary dynamics of hepatitis C virus envelope genes during chronic infection. *J Gen Virol* 2005; **86**: 1931-1942 [PMID: 15958671 DOI: 10.1099/vir.0.80957-0]
28 **Neumann AU**, Lam NP, Dahari H, Gretch DR, Wiley TE, Layden TJ, Perelson AS. Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-alpha therapy. *Science* 1998; **282**: 103-107 [PMID: 9756471 DOI: 10.1126/science.282.5386.103]
29 **Domingo E**, Menéndez-Arias L, Quiñones-Mateu ME, Holguín A, Gutiérrez-Rivas M, Martínez MA, Quer J, Novella IS, Holland JJ. Viral quasispecies and the problem of vaccine-escape and drug-resistant mutants. *Prog Drug Res* 1997; **48**: 99-128 [PMID: 9204684]
30 **Holland-Staley CA**, Kovari LC, Golenberg EM, Pobursky KJ, Mayers DL. Genetic diversity and response to IFN of the NS3 protease gene from clinical strains of the hepatitis C virus. *Arch Virol* 2002; **147**: 1385-1406 [PMID: 12111414 DOI: 10.1007/s00705-002-0807-5]
31 **Vallet S**, Gouriou S, Nousbaum JB, Legrand-Quillien MC, Goudeau A, Picard B. Genetic heterogeneity of the NS3 protease gene in hepatitis C virus genotype 1 from untreated infected patients. *J Med Virol* 2005; **75**: 528-537 [PMID: 15714495 DOI: 10.1002/jmv.20302]
32 **López-Labrador FX**, Moya A, Gonzàlez-Candelas F. Mapping natural polymorphisms of hepatitis C virus NS3/4A protease and antiviral resistance to inhibitors in worldwide isolates. *Antivir Ther* 2008; **13**: 481-494 [PMID: 18672527]
33 **Bartels DJ**, Zhou Y, Zhang EZ, Marcial M, Byrn RA, Pfeiffer T, Tigges AM, Adiwijaya BS, Lin C, Kwong AD, Kieffer TL. Natural prevalence of hepatitis C virus variants with decreased sensitivity to NS3.4A protease inhibitors in treatment-naive subjects. *J Infect Dis* 2008; **198**: 800-807 [PMID: 18637752 DOI: 10.1086/591141]
34 **Kuntzen T**, Timm J, Berical A, Lennon N, Berlin AM, Young SK, Lee B, Heckerman D, Carlson J, Reyor LL, Kleyman M, McMahon CM, Birch C, Schulze Zur Wiesch J, Ledlie T, Koehrsen M, Kodira C, Roberts AD, Lauer GM, Rosen HR, Bihl F, Cerny A, Spengler U, Liu Z, Kim AY, Xing Y, Schneidewind A, Madey MA, Fleckenstein JF, Park VM, Galagan JE, Nusbaum C, Walker BD, Lake-Bakaar GV, Daar ES, Jacobson IM, Gomperts ED, Edlin BR, Donfield SM, Chung RT, Talal AH, Marion T, Birren BW, Henn MR, Allen TM. Naturally occurring dominant resistance mutations to hepatitis C virus protease and polymerase inhibitors in treatment-naïve patients. *Hepatology* 2008; **48**: 1769-1778 [PMID: 19026009 DOI: 10.1002/hep.22549]
35 **Gaudieri S**, Rauch A, Pfafferott K, Barnes E, Cheng W, McCaughan G, Shackel N, Jeffrey GP, Mollison L, Baker R, Furrer H, Günthard HF, Freitas E, Humphreys I, Klenerman P, Mallal S, James I, Roberts S, Nolan D, Lucas M. Hepatitis C virus drug resistance and immune-driven adaptations: relevance to new antiviral therapy. *Hepatology* 2009; **49**: 1069-1082 [PMID: 19263475 DOI: 10.1002/hep.22773]
36 **Grakoui A**, McCourt DW, Wychowski C, Feinstone SM, Rice CM. Characterization of the hepatitis C virus-encoded serine proteinase: determination of proteinase-dependent polyprotein cleavage sites. *J Virol* 1993; **67**: 2832-2843 [PMID: 8386278]
37 **Meylan E**, Curran J, Hofmann K, Moradpour D, Binder M, Bartenschlager R, Tschopp J. Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. *Nature* 2005; **437**: 1167-1172 [PMID: 16177806 DOI: 10.1038/nature04193]
38 **Li XD**, Sun L, Seth RB, Pineda G, Chen ZJ. Hepatitis C virus protease NS3/4A cleaves mitochondrial antiviral signaling protein off the mitochondria to evade innate immunity. *Proc Natl Acad Sci USA* 2005; **102**: 17717-17722 [PMID: 16301520 DOI: 10.1073/pnas.0508531102]
39 **Foy E**, Li K, Sumpter R, Loo YM, Johnson CL, Wang C, Fish PM, Yoneyama M, Fujita T, Lemon SM, Gale M. Control of antiviral defenses through hepatitis C virus disruption of retinoic acid-inducible gene-I signaling. *Proc Natl Acad Sci USA* 2005; **102**: 2986-2991 [PMID: 15710892 DOI: 10.1073/pnas.0408707102]
40 **Lamarre D**, Anderson PC, Bailey M, Beaulieu P, Bolger G, Bonneau P, Bös M, Cameron DR, Cartier M, Cordingley MG, Faucher AM, Goudreau N, Kawai SH, Kukolj G, Lagacé L, LaPlante SR, Narjes H, Poupart MA, Rancourt J, Sentjens RE, St George R, Simoneau B, Steinmann G, Thibeault D, Tsantrizos YS, Weldon SM, Yong CL, Llinàs-Brunet M. An NS3 protease inhibitor with antiviral effects in humans infected with hepatitis C virus. *Nature* 2003; **426**: 186-189 [PMID: 14578911 DOI: 10.1038/nature02099]
41 **Kieffer TL**, Kwong AD, Picchio GR. Viral resistance to specifically targeted antiviral therapies for hepatitis C (STAT-Cs). *J Antimicrob Chemother* 2010; **65**: 202-212 [PMID: 19903720 DOI: 10.1093/jac/dkp388]
42 **Lim SR**, Qin X, Susser S, Nicholas JB, Lange C, Herrmann E, Hong J, Arfsten A, Hooi L, Bradford W, Nájera I, Smith P, Zeuzem S, Kossen K, Sarrazin C, Seiwert SD. Virologic escape during danoprevir (ITMN-191/RG7227) monotherapy is hepatitis C virus subtype dependent and associated with R155K substitution. *Antimicrob Agents Chemother* 2012; **56**: 271-279 [PMID: 22064535 DOI: 10.1128/AAC.05636-11]
43 **Sarrazin C**, Kieffer TL, Bartels D, Hanzelka B, Müh U, Welker M, Wincheringer D, Zhou Y, Chu HM, Lin C, Weegink C, Reesink H, Zeuzem S, Kwong AD. Dynamic hepatitis C virus genotypic and phenotypic changes in patients treated with the protease inhibitor telaprevir. *Gastroenterology* 2007; **132**: 1767-1777 [PMID: 17484874 DOI: 10.1053/j.gastro.2007.02.037]
44 **Zeuzem S**, Hezode C, Ferenci P, Dusheiko GM, Alves K, Bengtsson L, Gharakhanian S, Kauffman R, Alam J, Pawlotsky J. Telaprevir in combination with peginterferon-alfa-2a with or without ribavirin in the treatment of chronic hepatitis C: Final results of the PROVE 2 study. *Hepatology* 2008; **48**: 418A-419A
45 **Susser S**, Welker MW, Zettler M, Wohnsland A, Hughes E, Ralston R, Tong X, Zeuzem S, Sarrazin C. Clonal analysis of mutations selected in the HCV NS3 protease domain of genotype 1 non-responders treated with boceprevir (SCH503034). *J Hepatol* 2008; **48**: S29
46 **Susser S**, Forestier N, Vermehren J, Grigorian N, Karey U, Zeuzem S, Sarrazin C. Decline of detectable resistance mutations within the NS3 protease quasispecies during long-term follow-up after treatment with telaprevir. *J Hepatol* 2010; **52**: S300
47 **Vierling JM**, Ralston R, Lawitz EJ, McCone J, Gordon S, Pound D, Davis M, Galati J, Jacobson I, Rossaro L, Anderson FH, King J, Cassidy W, Bourliere M, Esteban-Mur R, Ravendhran N, Galler G, Mendez P, Brass CA, Albrecht JK. Long-term outcomes following combination treatment with boceprevir plus pegintron/ribavirin (P/R) in patients with chronic hepatitis C, genotype 1 (CHC-G1). *J Hepatol* 2010; **52**: S470-S471
48 **Imhof I**, Simmonds P. Genotype differences in susceptibility and resistance development of hepatitis C virus to protease inhibitors telaprevir (VX-950) and danoprevir (ITMN-191). *Hepatology* 2011; **53**: 1090-1099 [PMID: 21480315 DOI: 10.1002/hep.24172]
49 **Gottwein JM**, Scheel TK, Jensen TB, Ghanem L, Bukh J. Differential efficacy of protease inhibitors against HCV genotypes 2a, 3a, 5a, and 6a NS3/4A protease recombinant viruses. *Gastroenterology* 2011; **141**: 1067-1079 [PMID: 21699793 DOI: 10.1053/j.gastro.2011.06.004]

50 **Moreno C**, Berg T, Tanwandee T, Thongsawat S, Van Vlierberghe H, Zeuzem S, Lenz O, Peeters M, Sekar V, De Smedt G. Antiviral activity of TMC435 monotherapy in patients infected with HCV genotypes 2-6: TMC435-C202, a phase IIa, open-label study. *J Hepatol* 2012; **56**: 1247-1253 [PMID: 22326470 DOI: 10.1016/j.jhep.2011.12.033]

51 **Mangia A**, Mottola L. Treatment of non-genotype 1 hepatitis C virus patients. *Curr Gastroenterol Rep* 2012; **14**: 87-93 [PMID: 22113745 DOI: 10.1007/s11894-011-0235-3]

52 **Lenz O**, Fevery B, Vijgen L, Verbeeck J, Peeters M, Beumont-Mauviel M, Zeuzem S, Picchio G. TMC435 in patients infected with HCV genotype 1 who have failed previous pegylated interferon / ribavirin treatment: Virologic analyses of the ASPIRE trial. *J Hepatol* 2012; **56** Suppl 2: S5

53 **McPhee F**, Friborg J, Levine S, Chen C, Falk P, Yu F, Hernandez D, Lee MS, Chaniewski S, Sheaffer AK, Pasquinelli C. Resistance analysis of the hepatitis C virus NS3 protease inhibitor asunaprevir. *Antimicrob Agents Chemother* 2012; **56**: 3670-3681 [PMID: 22508297 DOI: 10.1128/AAC.00308-12]

54 **Nasu A**, Marusawa H, Ueda Y, Nishijima N, Takahashi K, Osaki Y, Yamashita Y, Inokuma T, Tamada T, Fujiwara T, Sato F, Shimizu K, Chiba T. Genetic heterogeneity of hepatitis C virus in association with antiviral therapy determined by ultra-deep sequencing. *PLoS One* 2011; **6**: e24907 [PMID: 21966381 DOI: 10.1371/journal.pone.0024907]

55 **Fonseca-Coronado S**, Escobar-Gutiérrez A, Ruiz-Tovar K, Cruz-Rivera MY, Rivera-Osorio P, Vazquez-Pichardo M, Carpio-Pedroza JC, Ruíz-Pacheco JA, Cazares F, Vaughan G. Specific detection of naturally occurring hepatitis C virus mutants with resistance to telaprevir and boceprevir (protease inhibitors) among treatment-naïve infected individuals. *J Clin Microbiol* 2012; **50**: 281-287 [PMID: 22116161 DOI: 10.1128/JCM.05842-11]

56 **Hiraga N**, Imamura M, Abe H, Hayes CN, Kono T, Onishi M, Tsuge M, Takahashi S, Ochi H, Iwao E, Kamiya N, Yamada I, Tateno C, Yoshizato K, Matsui H, Kanai A, Inaba T, Tanaka S, Chayama K. Rapid emergence of telaprevir resistant hepatitis C virus strain from wildtype clone in vivo. *Hepatology* 2011; **54**: 781-788 [PMID: 21626527 DOI: 10.1002/hep.24460]

57 **Vermehren J**, Susser S, Lange CM, Forestier N, Karey U, Hughes E, Ralston R, Tong X, Zeuzem S, Sarrazin C. Mutations selected in the hepatitis C virus NS3 protease domain during sequential treatment with boceprevir with and without pegylated interferon alfa-2b. *J Viral Hepat* 2012; **19**: 120-127 [PMID: 22239501 DOI: 10.1111/j.1365-2893.2011.01449.x]

58 **Lok AS**, Gardiner DF, Lawitz E, Martorell C, Everson GT, Ghalib R, Reindollar R, Rustgi V, McPhee F, Wind-Rotolo M, Persson A, Zhu K, Dimitrova DI, Eley T, Guo T, Grasela DM, Pasquinelli C. Preliminary study of two antiviral agents for hepatitis C genotype 1. *N Engl J Med* 2012; **366**: 216-224 [PMID: 22256805 DOI: 10.1056/NEJMoa1104430]

59 **Chayama K**, Takahashi S, Toyota J, Karino Y, Ikeda K, Ishikawa H, Watanabe H, McPhee F, Hughes E, Kumada H. Dual therapy with the nonstructural protein 5A inhibitor, daclatasvir, and the nonstructural protein 3 protease inhibitor, asunaprevir, in hepatitis C virus genotype 1b-infected null responders. *Hepatology* 2012; **55**: 742-748 [PMID: 21987462 DOI: 10.1002/hep.24724]

60 **Suzuki F**, Sezaki H, Akuta N, Suzuki Y, Seko Y, Kawamura Y, Hosaka T, Kobayashi M, Saito S, Arase Y, Ikeda K, Kobayashi M, Mineta R, Watahiki S, Miyakawa Y, Kumada H. Prevalence of hepatitis C virus variants resistant to NS3 protease inhibitors or the NS5A inhibitor (BMS-790052) in hepatitis patients with genotype 1b. *J Clin Virol* 2012; **54**: 352-354 [PMID: 22658798 DOI: 10.1016/j.jcv.2012.04.024]

61 **De Francesco R**, Carfí A. Advances in the development of new therapeutic agents targeting the NS3-4A serine protease or the NS5B RNA-dependent RNA polymerase of the hepatitis C virus. *Adv Drug Deliv Rev* 2007; **59**: 1242-1262 [PMID: 17869377 DOI: 10.1016/j.addr.2007.04.016]

62 **Behrens SE**, Tomei L, De Francesco R. Identification and properties of the RNA-dependent RNA polymerase of hepatitis C virus. *EMBO J* 1996; **15**: 12-22 [PMID: 8598194]

63 **Reich S**, Golbik RP, Geissler R, Lilie H, Behrens SE. Mechanisms of activity and inhibition of the hepatitis C virus RNA-dependent RNA polymerase. *J Biol Chem* 2010; **285**: 13685-13693 [PMID: 20194503 DOI: 10.1074/jbc.M109.082206]

64 **Beaulieu PL**. Recent advances in the development of NS5B polymerase inhibitors for the treatment of hepatitis C virus infection. *Expert Opin Ther Pat* 2009; **19**: 145-164 [PMID: 19441916 DOI: 10.1517/13543770802672598]

65 **Shi ST**, Herlihy KJ, Graham JP, Fuhrman SA, Doan C, Parge H, Hickey M, Gao J, Yu X, Chau F, Gonzalez J, Li H, Lewis C, Patick AK, Duggal R. In vitro resistance study of AG-021541, a novel nonnucleoside inhibitor of the hepatitis C virus RNA-dependent RNA polymerase. *Antimicrob Agents Chemother* 2008; **52**: 675-683 [PMID: 18070954 DOI: 10.1128/AAC.00834-07]

66 **Thompson A**, Patel K, Tillman H, McHutchison JG. Directly acting antivirals for the treatment of patients with hepatitis C infection: a clinical development update addressing key future challenges. *J Hepatol* 2009; **50**: 184-194 [PMID: 19022518 DOI: 10.1016/j.jhep.2008.10.011]

67 **Lam AM**, Espiritu C, Bansal S, Micolochick Steuer HM, Zennou V, Otto MJ, Furman PA. Hepatitis C virus nucleotide inhibitors PSI-352938 and PSI-353661 exhibit a novel mechanism of resistance requiring multiple mutations within replicon RNA. *J Virol* 2011; **85**: 12334-12342 [PMID: 21957306 DOI: 10.1128/JVI.05639-11]

68 **Sofia MJ**. Nucleotide prodrugs for HCV therapy. *Antivir Chem Chemother* 2011; **22**: 23-49 [PMID: 21860070 DOI: 10.3851/IMP1797]

69 **McCown MF**, Rajyaguru S, Le Pogam S, Ali S, Jiang WR, Kang H, Symons J, Cammack N, Najera I. The hepatitis C virus replicon presents a higher barrier to resistance to nucleoside analogs than to nonnucleoside polymerase or protease inhibitors. *Antimicrob Agents Chemother* 2008; **52**: 1604-1612 [PMID: 18285474 DOI: 10.1128/AAC.01317-07]

70 **Le Pogam S**, Seshaadri A, Ewing A, Kang H, Kosaka A, Yan JM, Berrey M, Symonds B, De La Rosa A, Cammack N, Nájera I. RG7128 alone or in combination with pegylated interferon-α2a and ribavirin prevents hepatitis C virus (HCV) Replication and selection of resistant variants in HCV-infected patients. *J Infect Dis* 2010; **202**: 1510-1519 [PMID: 20942646 DOI: 10.1086/656774]

71 **Gane EJ**, Roberts SK, Stedman CA, Angus PW, Ritchie B, Elston R, Ipe D, Morcos PN, Baher L, Najera I, Chu T, Lopatin U, Berrey MM, Bradford W, Laughlin M, Shulman NS, Smith PF. Oral combination therapy with a nucleoside polymerase inhibitor (RG7128) and danoprevir for chronic hepatitis C genotype 1 infection (INFORM-1): a randomised, double-blind, placebo-controlled, dose-escalation trial. *Lancet* 2010; **376**: 1467-1475 [PMID: 20951424 DOI: 10.1016/S0140-6736(10)61384-0]

72 **Sentandreu V**, González-Candelas F, Moya A, López-Labrador FX. Hepatitis C virus NS3/4A protease and NS5B polymerase polymorphism in worldwide isolates: anticipated problems for STAT-C therapies in non-1 genotypes. In: Fourth European Congress of Virology 2010; Cernobbio, Italy; 2010 Apr 7-11

73 **Howe AY**, Cheng H, Thompson I, Chunduru SK, Herrmann S, O'Connell J, Agarwal A, Chopra R, Del Vecchio AM. Molecular mechanism of a thumb domain hepatitis C virus nonnucleoside RNA-dependent RNA polymerase inhibitor. *Antimicrob Agents Chemother* 2006; **50**: 4103-4113 [PMID: 16940072 DOI: 10.1128/AAC.00365-06]

74 **Sun SC**, Bae A, Qi X, Harris J, Wong KA, Miller MD, Mo H. Natural variation in drug susceptibility to HCV polymerase inhibitors in treatment-naïve HCV patient isolates. *J Viral*

Hepat 2011; **18**: 861-870 [PMID: 21108698 DOI: 10.1111/j.1365-2893.2010.01396.x]

75 **Lam AM**, Espiritu C, Bansal S, Micolochick Steuer HM, Niu C, Zennou V, Keilman M, Zhu Y, Lan S, Otto MJ, Furman PA. Genotype and subtype profiling of PSI-7977 as a nucleotide inhibitor of hepatitis C virus. *Antimicrob Agents Chemother* 2012; **56**: 3359-3368 [PMID: 22430955 DOI: 10.1128/AAC.00054-12]

76 **Zeuzem S**, Buggisch P, Agarwal K, Marcellin P, Sereni D, Klinker H, Moreno C, Zarski JP, Horsmans Y, Mo H, Arterburn S, Knox S, Oldach D, McHutchison JG, Manns MP, Foster GR. The protease inhibitor, GS-9256, and non-nucleoside polymerase inhibitor tegobuvir alone, with ribavirin, or pegylated interferon plus ribavirin in hepatitis C. *Hepatology* 2012; **55**: 749-758 [PMID: 22006408 DOI: 10.1002/hep.24744]

77 **Kwo PY**, Vinayek R. The therapeutic approaches for hepatitis C virus: protease inhibitors and polymerase inhibitors. *Gut Liver* 2011; **5**: 406-417 [PMID: 22195237 DOI: 10.5009/gnl.2011.5.4.406]

78 **Chevaliez S**, Asselah T. Mechanisms of non-response to antiviral treatment in chronic hepatitis C. *Clin Res Hepatol Gastroenterol* 2011; **35** Suppl 1: S31-S41 [PMID: 21742299 DOI: 10.1016/S2210-7401(11)70005-5]

79 **Thompson AJ**, Locarnini SA, Beard MR. Resistance to anti-HCV protease inhibitors. *Curr Opin Virol* 2011; **1**: 599-606 [PMID: 22440917 DOI: 10.1016/j.coviro.2011.10.001]

80 **Soriano V**, Vispo E, Poveda E, Labarga P, Martin-Carbonero L, Fernandez-Montero JV, Barreiro P. Directly acting antivirals against hepatitis C virus. *J Antimicrob Chemother* 2011; **66**: 1673-1686 [PMID: 21652618 DOI: 10.1093/jac/dkr215]

P-Reviewers Hong JR, Kodama EN, Margulies B
S- Editor Song XX **L- Editor** Hughes D **E- Editor** Zheng XM