Original article

Resistance mutations are rare among protease inhibitor treatment-naive hepatitis C genotype-1 patients with or without HIV coinfection

Gaspar Lisboa-Neto[1], Caroline F Noble[2], João R Rebello Pinho[2,3], Fernanda M Malta[2], Michele S Gomes-Gouvêa[2], Mónica V Alvarado-Mora[2], Mariliza H da Silva[4,5], Andrea GB Leite[6], Leonora Z Piccoli[6], Flaviane K Rodrigues[7], Flair J Carrilho[2], Maria C Mendes-Correa[1,7]*

[1]Department of Infectious Diseases, University of São Paulo School of Medicine, São Paulo, SP, Brazil
[2]Laboratory of Tropical Gastroenterology and Hepatology 'João Alves de Queiroz and Castorina Bittencourt Alves', LIM-07, Institute of Tropical Medicine, Department of Gastroenterology, University of São Paulo School of Medicine, São Paulo, SP, Brazil
[3]Albert Einstein Medicina Diagnóstica, Hospital Israelita Albert Einstein, São Paulo, SP, Brazil
[4]Centro de Referência e Treinamento em DST/AIDS do Estado de São Paulo, São Paulo, SP, Brazil
[5]Clínicas de Especialidades, São Bernardo do Campo, SP, Brazil
[6]Centro Especializado em Saúde, Caxias do Sul, RS, Brazil
[7]Infectious Diseases Research Unit, ABC Foundation Medical School, Santo André, SP, Brazil

*Corresponding author e-mail: jrrpinho@usp.br

Background: HCV has a high replication rate and a lack of proofreading activity, leading to a greatly diverse viral population. This diversity may lead to emergence of resistant strains in direct-acting antiviral therapy. The frequency of naturally occurring HCV protease inhibitor (PI) mutations has been addressed in many countries, but there are few data on the prevalence of these mutations in Brazilian patients.

Methods: We evaluated the sequence of HCV NS3 protease gene in 247 patients (135 HCV-monoinfected and 112 HIV–HCV-coinfected patients). HCV RNA was extracted from plasma and a fragment of 765 base pairs from the NS3 region was amplified and sequenced with Sanger-based technology.

Results: HIV–HCV-coinfected patients were more likely to be older than 40 years and have an HCV subtype-1a infection. Overall, 21.9% of patients had at least one amino acid substitution in the NS3 region; 14 patients (5.7%) harboured at least one resistance mutation (T54S, V55A, Q80R) and the Q80K mutation was not found in our case series. There was no difference between monoinfected and coinfected patients regarding the frequency of natural polymorphisms and resistance mutations.

Conclusions: Baseline HCV NS3 amino acid substitutions identified herein are considered mostly natural polymorphisms with no clinical impact on PI-based therapy. The identified resistance mutations may be associated with low-level resistance to PIs *in vitro*. Q80K substitution seems to be a rare event in Brazil. HIV coinfection was not associated with a greater frequency of such substitutions in the studied sample.

Introduction

HCV infection is a leading cause of chronic liver disease worldwide [1,2]. There are currently about 160 million infected individuals, corresponding to approximately 3% of the world's population [3]. A cross-sectional study conducted in all Brazilian macro-regions from 2005 to 2009 using a sample of 19,503 inhabitants aged between 10 and 69 years determined an overall weighted prevalence of hepatitis C antibodies in 1.38%, corresponding to about 2.8 million infected individuals in Brazil [4].

Due to shared routes of transmission, chronic hepatitis C infection is a common comorbidity among patients infected with HIV. HIV coinfection modifies the natural history of hepatitis C, promoting a rapidly progressive liver disease in this population [5]. Until recently, the only available treatment for HIV–HCV patients has been pegylated interferon plus ribavirin. However, this therapy provides clearance in fewer than one-third of patients with HCV genotype-1, which is the most

©2015 International Medical Press 1359-6535 (print) 2040-2058 (online)

prevalent HCV genotype worldwide [6]. Therefore, more effective therapeutic alternatives are needed to treat this group of patients.

In recent years, treatment of HCV infection has undergone a remarkable evolution with the advent of antiviral drugs targeting non-structural HCV proteins capable of blocking the viral cell cycle at different sites. These drugs are known as direct-acting antivirals (DAAs) [7]. The first class of DAAs available for hepatitis C treatment include telaprevir and boceprevir. These two drugs belong to the HCV NS3 protease inhibitor (PI) group [8].

HCV's high replication rate, together with the low fidelity of HCV polymerase result in the rapid emergence of viral variants, leading to viral nucleotide polymorphisms but also to variants harbouring resistance mutations, which may decrease susceptibility to a future antiviral therapy [9,10]. There are a few studies from different areas of the world describing the prevalence of DAA-naive patients with viral populations predominantly resistant to DAAs. This prevalence may vary from 0 to 7%, depending on the group of patients included or the sequencing methodology employed [11–14].

In viruses such as HIV, a consistent correlation has been shown between some polymorphisms and antiviral therapy failure. Nevertheless, the clinical impact of baseline mutations and their implication for DAA-based therapy for HCV remains unknown [8].

Previous work carried out with Brazilian populations has reported prevalences of 4.1% to 18.9% of NS3 mutations in HCV-monoinfected patients [15–18]. However, the presence of HCV NS3 PI resistant mutations have yet to be studied in Brazilian HCV patients coinfected with HIV.

The aim of this study was to describe the frequency of natural polymorphisms and resistance mutations in HCV protease from HCV and HCV–HIV-infected patients in Brazil and to analyse the possible association between amino acid substitutions and clinical and demographic variables.

Methods

Patients

A total of 300 HCV-infected patients were enrolled in this study. This cohort included 150 HCV-monoinfected patients and 150 HCV–HIV-1-coinfected patients. Patients were followed-up in five different infectious disease reference centres in Brazil: Infectious Diseases Research Unit (ABC Foundation Medical School), Clínicas de Especialidades de São Bernardo do Campo, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Centro de Referência e Treinamento DST/AIDS-SP (all located in the São Paulo Metropolitan area) and the outpatient clinic for HIV patients in Caxias do Sul, Rio Grande do Sul.

All patients diagnosed with chronic hepatitis C who were referred to these reference centres between 2011 and 2013 were invited to participate in the study. Patients were included in the study if they had an HCV genotype-1 infection, had detectable levels of viral RNA in the serum, were naive to PIs or other investigational anti-HCV drugs and signed an informed consent form to participate in the study. The exclusion criteria consisted of HBV coinfection or the presence of other hepatic diseases.

Ethics statement

This study was approved by the Ethics Committee of the ABC School of Medicine (Faculdade de Medicina do ABC, São Paulo, Brazil) under protocol number 011/2010. Written informed consent was obtained from each participant prior to enrolment in the study.

Analysed variables

Medical records were retrospectively reviewed to ascertain demographic (age and gender) and clinical characteristics as well as laboratory data. HCV-associated variables included liver fibrosis score (based on the METAVIR classification), HCV subtype and information on viral response to prior treatment against HCV (with pegylated interferon and ribavirin). We also recorded type of virus exposure: blood transfusion, intravenous or inhaled drug use, tattooing/piercing, high-risk sexual behaviour, occupational exposure and non-sexual HCV household contact.

For HIV patients, we also evaluated antiretroviral therapy use, HIV viral load and $CD4^+$ T-cell count at the time the blood sample was collected and during the previous 12 months.

Determination of HCV viral load and genotyping

HCV RNA was detected by real time PCR using a commercially available kit (Cobas AmpliPrep/Cobas TaqMan HCV test, version 2.0; Roche Diagnostics, Branchburg, NJ, USA) [19] and extracted from plasma samples using the QIAamp® Ultrasens Virus kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. HCV genotyping was conducted according to the methodology described by Sandres-Sauné *et al*. [20].

Amplification and sequencing of the NS3 protease gene

A fragment of 765 base pairs from the NS3 region of the HCV genome was amplified using primers, as previously described [15]. Initially, the extracted viral RNA was transcribed to cDNA and amplified by PCR using SuperScript® III One-Step RT-PCR System with

©2015 International Medical Press

Platinum® Taq High Fidelity DNA Polymerase kit (Life Technologies, Carlsbad, CA, USA). Cycling conditions included an initial cDNA synthesis step at 50°C for 30 min followed by a denaturation step at 94°C for 2 min, 35 cycles of PCR amplification (94°C for 30 s, 56°C for 30 s, 68°C for 1 min and 30 s) and a final 5 min extension step at 68°C.

The initial reverse transcription PCR product was subjected to a second round of PCR by using Platinum Taq DNA polymerase (Life Technologies). Cycling conditions for the second round consisted of a denaturation step at 94°C for 3 min, followed by 30 cycles at 94°C for 30 s, 60°C for 30 s, 72°C for 1 min and 30 s, and a final 5 min extension step at 72°C. PCR products from the second amplification were detected by agarose gel (2%).

Amplified PCR products were purified using an ExoSAP-IT PCR Clean-up kit (Affymetrix, Cleveland, OH, USA), then sequenced with a BigDye® Terminator v3.1 Cycle Sequencing kit (Life Technologies, Foster City, CA, USA) and electrophoresed in the ABI3500 DNA sequencer (Life Technologies).

Analysis of mutations associated with resistance to PIs

The consensus sequences of each sample were obtained by aligning both sequenced strands (sense and antisense) using CodonCode Aligner software (CodonCode Corporation, Dedham, MA, USA). Assembled contigs were aligned using the CLUSTALW program integrated with the BioEdit v.7.0.8 software (Ibis Bioscience, Carlsbad, CA, USA). Nucleotides were translated into amino acids and analysed for the presence of previously-identified substitutions conferring resistance to NS3 PIs: V36A/M, T54A/S, V55A, Q80K/R, R155K/T, A156S/T/V, D168A/V/E/T and for polymorphisms previously found in these patients in amino acid residues V36, F43, T54, V55, Q80, R109, S122, I132, S138, R155, A156, V158, D168, V170 and M175 [10,21].

Statistical analyses

We first conducted a frequency distribution analysis of the previously-described polymorphisms of the HCV NS3 protease and of the mutations associated with resistance to PIs in Group 1 (HCV–HIV-coinfected patients) and Group 2 (monoinfected patients). We also conducted a frequency distribution analysis of patient demographic and clinical variables.

To determine whether amino acid substitutions were associated with each collected clinical or demographic variable, we used the Student's *t*-test. For categorical variables, we used the Pearson's χ^2 or Fisher's exact tests if the group was small. Two-tailed *P*-values were calculated and considered statistically significant if $P \leq 0.05$. Analyses were conducted using SPSS software, version 15.0 (SPSS Inc., Chicago, IL, USA).

Results

From the 300 patients initially enrolled in this study, 15 monoinfected and 38 coinfected patients were excluded due to poor quality NS3 amplification and sequencing (30 samples) or low viraemia level (23 samples). The remaining 247 HCV-infected patients included 135 HCV monoinfected patients and 112 HIV-1-coinfected patients.

Demographic and clinical characteristics are summarized in Table 1. A total of 63% of patients were male and the mean age was 44.86 years (±9.66). In the coinfected group, most patients were older than 40 years old (P=0.001). This group was also more frequently infected with HCV subtype-1a (P=0.0001) and mostly acquired their viral infection through intravenous drug use or high-risk sexual behaviour (P=0.0001). A history of blood transfusion was more frequent among HCV-monoinfected patients (P=0.0001). Two patients in the coinfected group were haemophiliacs.

Mutations associated with resistance to PIs

A total of 54 out of 247 (21.86%) sequences from HCV genotype-1 patients showed at least one NS3 amino acid substitution. The monoinfected and coinfected groups did not differ significantly in terms of the frequency of viruses with at least one of these substitutions – neither in the frequency of NS3 natural polymorphisms at positions 36, 55, 80, 122 or 175 nor in the known NS3 PI resistance mutations sites, isolated or in combination (Table 2).

Among HCV–HIV-coinfected patients, the NS3 protease variants detected included: M175L (1/23), Q80L (4/23), Q80R (2/23), S122G (7/23), S122N (1/23), S122T (1/23), T54S (3/23), V55I (3/23), V36L (1/23) and V55A (4/23). In the monoinfected group, NS3 substitutions included: M175L (2/31), S122G (21/31), S122N (2/31), S122T (2/31), T54S (2/31), V55A (3/21) and V55I (1/31). Among patients with at least one amino acid substitution, only 14 were related to a low-level resistance to PIs (T54S/V55A/Q80R). Most patients had amino acid substitutions considered to be natural polymorphisms (M175L, Q80L, S122G/N/T, V55I and V36L). S122G/N/T was the most frequent substitution and was more common among monoinfected patients (25 monoinfected versus 9 coinfected; P=0.004). Q80K was not detected in any patient.

In total, six individuals (four coinfected and two monoinfected patients) had more than one amino acid substitution in a single sequence, and these included double (T54S+V55I; V55A+Q80R; T54S+S122G; V55A+S122G) or triple (T54S+V55I+Q80L) substitution combinations.

The comparison between patients with any NS3 amino acid substitution (54 patients) and patients without amino acids substitutions (193 patients) did not

Table 1. Demographics, clinical characteristics and risk factors for HCV infection among HCV-infected individuals according to HIV infection

	All patients	HCV–HIV-coinfected patients	HCV-monoinfected patients	OR (95% CI)	*P*-value
Overall characteristics					
Patients	247 (100)	112 (100)	135 (100)	–	–
Gender, male	156 (63.15)	76 (67.80)	80 (59.25)	1.45 (0.85, 2.45)	0.163
Mean age, years (SD)	44.86 (9.66)	44.7 (7.51)	44.90 (11.3)	–	0.939
Age ≥40 years	**162 (65.60)**	**86 (76.78)**	**76 (59.30)**	**2.56 (1.47, 4.47)**	**0.001**
HCV characteristics					
Subtype-1a	**141 (57.08)**	**95 (84.82)**	**46 (34.07)**	**10.81 (5.77, 20.23)**	**0.0001**
Mean HCV viral load, IU/ml (SD)	2,959,047 (3,819,330)	3,432,810 (4,096,501)	2,573,018 (3,546,414)	–	0.084
Previous HCV treatment with PEG-IFN/ribavirin	104 (42.10)	51 (46.43)	53 (6.60)	1.29 (0.77, 2.14)	0.320
Non-responders	79 (75.96)	43 (84.31)	36 (67.90)	2.53 (0.98, 6.56)	0.051
Relapsers	9 (8.60)	5 (9.80)	4 (7.54)	1.33 (0.33, 5.26)	0.682
Response to treatment unknown	16 (14.40)	3 (5.80)	13 (24.52)	0.19 (0.05, 0.72)	0.014
HIV characteristics					
Mean CD4+ T-cells, cells/mm^3 (SD; *n*=106)	–	567 (288.30)	–	–	–
HIV viral load (detectable; *n*=96)	–	16 (16.67)	–	–	–
AIDS-defining illness (*n*=96)	–	46 (47.91)	–	–	–
HIV patients on HAART	–	106 (94.64)	–	–	–
Liver biopsy	**87 (35.20)**	**61 (54.46)**	**26 (19.25)**	–	**0.0001**
No fibrosis or mild fibrosis (METAVIR F0 and F1)	44 (50.50)	26 (42.62)	18 (69.23)	–	–
Moderate-to-severe fibrosis (METAVIR F2 and F3)	33 (37.90)	26 (42.62)	7 (26.92)	–	–
Liver cirrhosis	10 (11.50)	9 (14.75)	1 (3.84)	–	–
Risk factors					
Blood transfusion before 1993	**48 (19.40)**	**8 (7.10)**	**40 (29.60)**	**0.18 (0.08, 0.41)**	**0.0001**
Intravenous drug use	**84 (34.00)**	**73 (65.10)**	**11 (8.14)**	**21.10 (10.17, 43.73)**	**0.0001**
High-risk sexual behaviour[a]	**77 (31.20)**	**50 (44.65)**	**27 (20)**	**3.22 (1.83, 5.66)**	**0.0001**

Values are *n* (%) unless otherwise indicated. Significant values are in bold. All statistical analyses were carried out with Pearson's χ^2 test or Fisher's exact test except for categorical variables when Student's *t*-test was applied. [a]High-risk sexual behaviour (sexual intercourse with HIV-positive or HCV-positive partners or intravenous drug users; sexual intercourse without use of condom or more than three sexual partners per year).

Table 2. Natural polymorphisms and resistance mutations in selected sites along the HCV NS3 protease sequence in both groups of patients

	All patients (*n*=247)	HCV–HIV-coinfected patients (*n*=112)	HCV-monoinfected patients (*n*=135)	*P*-value
HCV NS3 amino acid substitutions	54 (21.86)	23 (20.53)	31 (22.96)	0.646
HCV NS3 natural polymorphisms[a]	40 (16.19)	14 (12.50)	26 (19.26)	0.151
HCV NS3 PI resistance mutations (T54 S/V55 A/Q80 R)	14 (5.66)	9 (8.03)	5 (3.70)	0.143
T54S	6 (2.42)	4 (3.57)	2 (1.48)	0.415
V55A	6 (2.42)	3 (2.68)	3 (2.22)	1.000
Q80R	1 (0.40)	1 (0.89)	0 (0)	0.453
V55A+Q80R	1 (0.40)	1 (0.89)	0 (0)	0.453

Values are *n* (%) unless otherwise indicated. All statistical analyses were carried out with Pearson's χ^2 test or Fisher's exact test. [a]V36L, V55I, Q80L, S122 G/N/T and M175L. PI, protease inhibitor.

reveal any difference regarding most of the analysed variables (gender, age, HIV coinfection, HCV–HIV transmission risk factor, HCV RNA level, liver fibrosis score and previous treatment for HCV). HCV subtype-1b was the only variable associated with any HCV protease amino acid substitution (38 versus 68; *P*=0.0001).

In the HIV-coinfected group, no statistical difference in CD4+ T-cell count was observed when stratified according to the presence or absence of HCV protease variants: 543 cells/mm^3 (±266) versus 586 cells/mm^3 (±295), respectively, *P*=0.559. Furthermore, only nine patients had CD4+ T-cell counts below 200 cells/mm^3 and none

©2015 International Medical Press

of them presented NS3 protease polymorphisms in the analysed sites or resistance mutations to DAAs.

Discussion

The aim of the present study was to evaluate the prevalence of natural polymorphisms in the NS3 protease related to resistance to PIs in patients with chronic hepatitis C, with or without HIV coinfection. Overall, 21.86% of patients showed at least one NS3 amino acid substitution in the analysed sites. These substitutions were found in 20.53% of HCV–HIV-coinfected patients and in 22.96% of HCV-monoinfected patients. The frequency of natural polymorphisms and mutations associated with resistance to PIs was similar between the coinfected and monoinfected groups. This result is in line with several published studies [22–26], but contradicts Morsica *et al.* [27], who reported a higher prevalence of mutations in 31 HIV–HCV-coinfected patients compared to 250 HCV sequences retrieved from GenBank (16.2% versus 0.8%; $P \leq 0.05$). In the current study, polymorphism S122G was the most common substitution in HCV genotype-1b samples. This finding possibly indicates a greater variability at NS3 position 122 in HCV Brazilian samples. However, *in vitro* studies have not shown any influence of S122G substitution on PI inhibitor activity [21,28].

As a group, coinfected patients were older (>40 years old) than monoinfected individuals and the great majority of them harboured genotype-1a (84.82%). This finding may have had some impact on HCV diversity, which could eventually modify some results observed in this study. Older age among HCV–HIV-coinfected patients was also reported in a large study involving databases from the Brazilian Ministry of Health [29], and other studies described genotype-1a as more prevalent among different sets of coinfected patients [30,31]. Genotype-1a has been associated with a lower sustained virological response (SVR) and a greater chance of selection of mutants resistant to PIs (that is, V36M, R155K and Q80K) [5]. On the other hand, the proportion of monoinfected patients harbouring genotype-1b was high (65.9%), a finding which has previously been reported in Brazilian patients [32–34].

Previous studies in different parts of the world (for example, Italy and France) have reported a prevalence of resistance-associated mutations of 7.5% to 26% in coinfected patients and 0.8% to 19.3% in monoinfected patients [23–25,27]. Prevalence of NS3 mutations in HCV-monoinfected subjects reported to date in Brazil range from 4.1% to 18.9% [15–18].

It is important to emphasize that mutations linked to high-level PI resistance were not found among patients included in this cohort. In fact, only three variants associated with low-to-moderate level resistance were identified: Q80R, V55A and T54S. These mutations lead to a low-to-moderate fold-change in drug median effective concentration (that is, 5–20-folds in EC_{50}). V55A substitution may decrease susceptibility to boceprevir in HCV genotype-1b-infected patients, while T54S may trigger resistance to both first generation PIs (telaprevir and boceprevir) and Q80R may result in almost sixfold resistance to simeprevir [10,35,36].

Worldwide, few case series have identified primary mutations associated with medium or high levels of resistance. Actually, they are generally found in less than 3% of screened samples [13]. The clinical impact of baseline mutations in the NS3 region (associated with high or low levels of resistance) is not yet understood for all PIs. Some case series including more than 2,000 patients undergoing telaprevir- or boceprevir-based therapy showed no impact of primary mutations in SVR after use [13,14]. On the contrary, the natural occurrence of the Q80K mutation has been associated with lower chances of achieving SVR after simeprevir use [35]. We did not find any patient harbouring this mutation among the 247 patients analysed in this series.

When all patients (monoinfected and coinfected) were evaluated together, we observed an association between polymorphisms/mutations in the NS3 region and HCV subtype-1b (P=0.0001). This finding may reflect the fact that subtype distribution was not homogeneous across groups.

Regarding HIV profile, immunosuppression defined by $CD4^+$ T-cell depletion is associated with a diminished $CD8^+$ T-cell response specific to HCV, which may prompt an increased pool of viral variants eventually harbouring resistance mutations [37]. There was no difference in the frequency of $CD4^+$ T-cell count and HCV polymorphisms/mutations in the studied group, as the great majority of HIV-coinfected patients received antiretroviral therapy and thus had a good immune profile.

In summary, this study shows that 22.96% of HCV-monoinfected patients and 20.53% of HIV-coinfected patients harboured at least one amino acid substitution in the HCV NS3 protease. Overall, these variants were considered natural polymorphisms, being eventually associated with low-level resistance to PIs (that is, T54S/V55A/Q80R). The Q80K mutation was not observed in this series of patients. HIV coinfection was not associated with a greater frequency of polymorphisms or resistance mutations in the HCV NS3 protease region. Understanding HCV genetic variability can influence the clinical decision-making policies for DAA therapy in Brazil and this would allow for a safer use of PIs (first and second wave) in interferon-based or interferon-free therapy regimens. Further local protocols enrolling larger samples are needed to confirm these results.

Acknowledgements

This Project was supported by grant 2011/50633-4 from the São Paulo Research Foundation (FAPESP) and the Alves de Queiroz Family Fund for Research. GL-N is a graduate student in the Department of Infectious Diseases at the University of São Paulo. FMM, MSG-G and MVA-M were graduate students from the Department of Gastroenterology at the University of São Paulo and received fellowships from FAPESP (2010/50081-9, 2009/53306-4 and 2008/50461-6) and from Fundação Faculdade de Medicina de São Paulo. CFN is a graduate student from the Department of Gastroenterology at the University of São Paulo who received a fellowship from CAPES. JRRP and MCM-C received fellowships from CNPq (Bolsista de Produtividade em Pesquisa do CNPq - Nível 2).

Disclosure statement

JRRP is an employee at Albert Einstein Medicina Diagnóstica, São Paulo, Brazil. MVA-M is currently employed at Janssen-Cilag Farmacêutica Ltda., São Paulo, Brazil. All other authors who took part in this study declare that they do not have anything to disclose regarding funding or conflicts of interest with respect to the manuscript.

References

1. Guirao AA, Yuste SR, Regueiro BJ. [Epidemiology and clinical manifestations of viral hepatitis]. *Enferm Infecc Microbiol Clin* 2006; **24:**264–276. Spanish.
2. Lin L, Verslype C, van Pelt JF, van Ranst M, Fevery J. Viral interaction and clinical implications of coinfection of hepatitis C virus with other hepatitis viruses. *Eur J Gastroenterol Hepatol* 2006; **18:**1311–1319.
3. Maasoumy B, Wedemeyer H. Natural history of acute and chronic hepatitis C. *Best Pract Res Clin Gastroenterol* 2012; **26:**401–412.
4. Pereira LM, Martelli CM, Moreira RC, *et al*. Prevalence and risk factors of hepatitis C virus infection in Brazil, 2005 through 2009: a cross-sectional study. *BMC Infect Dis* 2013; **13:**60.
5. Soriano V, Labarga P. Treatment of hepatitis C in patients infected with human immunodeficiency virus in the direct-acting antiviral era. *Infect Dis Clin North Am* 2012; **26:**931–948.
6. Wegzyn CM, Wyles D. Antiviral drug advances in the treatment of human immunodeficiency virus (HIV) and chronic hepatitis C virus (HCV). *Curr Opin Pharmacol* 2012; **12:**556–561.
7. Welsch C, Jesudian A, Zeuzem S, Jacobson I. New direct-acting antiviral agents for the treatment of hepatitis C virus infection and perspectives. *Gut* 2012; **61 Suppl 1:**i36–i46.
8. Sarrazin C, Hézode C, Zeuzem S, Pawlotsky JM. Antiviral strategies in hepatitis C virus infection. *J Hepatol* 2012; **56:**S88–S100.
9. Vermehren J, Sarrazin C. The role of resistance in HCV treatment. *Best Pract Res Clin Gastroenterol* 2012; **26:**487–503.
10. Wyles DL. Antiviral resistance and the future landscape of hepatitis C virus infection therapy. *J Infect Dis* 2013; **207 Suppl 1:**S33–S39.
11. Kuntzen T, Timm J, Berical A, *et al*. Naturally occurring dominant resistance mutations to hepatitis C virus protease and polymerase inhibitors in treatment-naive patients. *Hepatology* 2008; **48:**1769–1778.
12. Paolucci S, Fiorina L, Piralla A, *et al*. Naturally occurring mutations to HCV protease inhibitors in treatment-naive patients. *Virol J* 2012; **9:**245.
13. Bartels DJ, Sullivan JC, Zhang EZ, *et al*. Hepatitis C virus variants with decreased sensitivity to direct-acting antivirals (DAAs) were rarely observed in DAA-naive patients prior to treatment. *J Virol* 2013; **87:**1544–1553.
14. Barnard RJ, Howe JA, Ogert RA, *et al*. Analysis of boceprevir resistance associated amino acid variants (RAVs) in two Phase 3 boceprevir clinical studies. *Virology* 2013; **444:**329–336.
15. Peres-da-Silva A, de Almeida AJ, Lampe E. Mutations in hepatitis C virus NS3 protease domain associated with resistance to specific protease inhibitors in antiviral therapy naive patients. *Arch Virol* 2010; **155:**807–811.
16. Hoffmann L, Ramos JA, Souza EV, *et al*. Dynamics of resistance mutations to NS3 protease inhibitors in a cohort of Brazilian patients chronically infected with hepatitis C virus (genotype 1) treated with pegylated interferon and ribavirin: a prospective longitudinal study. *Virol J* 2013; **10:**57.
17. Zeminian LB, Padovani JL, Corvino SM, Silva GF, Pardini MI, Grotto RM. Variability and resistance mutations in the hepatitis C virus NS3 protease in patients not treated with protease inhibitors. *Mem Inst Oswaldo Cruz* 2013; **108:**13–17.
18. Nishiya AS, de Almeida-Neto C, Ferreira SC, *et al*. HCV genotypes, characterization of mutations conferring drug resistance to protease inhibitors, and risk factors among blood donors in São Paulo, Brazil. *PLoS ONE* 2014; **9:**e86413.
19. Chevaliez S, Bouvier-Alias M, Rodriguez C, Soulier A, Poveda JD, Pawlotsky JM. The Cobas AmpliPrep/Cobas TaqMan HCV test, version 2.0, real-time PCR assay accurately quantifies hepatitis C virus genotype 4 RNA. *J Clin Microbiol* 2013; **51:**1078–1082.
20. Sandres-Sauné K, Deny P, Pasquier C, *et al*. Determining hepatitis C genotype by analyzing the sequence of the NS5b region. *J Virol Methods* 2003; **109:**187–193.
21. HCV Phenotype Working Group, HCV Drug Development Advisory Group. Clinically relevant HCV drug resistance mutations figure and tables. *Ann Forum Collab HIV Res* 2012; **14:**1–10.
22. Halfon P, Bourlière M, Khiri H, *et al*. Mutation rate in hepatitis C virus NS3 protease is not influenced by HIV-1 protease inhibitor therapy. *AIDS* 2008; **22:**1694–1696.
23. Trimoulet P, Belzunce C, Faure M, *et al*. Hepatitis C virus (HCV) protease variability and anti-HCV protease inhibitor resistance in HIV/HCV-co-infected patients. *HIV Med* 2011; **12:**506–509.
24. Gaudieri S, Rauch A, Pfafferott K, *et al*. Hepatitis C virus drug resistance and immune-driven adaptations: relevance to new antiviral therapy. *Hepatology* 2009; **49:**1069–1082.
25. Vicenti I, Rosi A, Saladini F, *et al*. Naturally occurring hepatitis C virus (HCV) NS3/4A protease inhibitor resistance-related mutations in HCV genotype 1-infected subjects in Italy. *J Antimicrob Chemother* 2012; **67:**984–987.
26. Treviño A, de Mendoza C, Parra P, *et al*. Natural polymorphisms associated with resistance to new antivirals against hepatitis C virus (HCV) in newly diagnosed HIV/HCV-co-infected patients in Spain. *Antivir Ther* 2011; **16:**413–416.
27. Morsica G, Bagaglio S, Uberti-Foppa C, Galli L, Lazzarin A. Detection of hepatitis C mutants with natural resistance to NS3/4A protease inhibitors in HIV/HCV-co-infected individuals treated with antiretroviral therapy. *J Acquir Immune Defic Syndr* 2009; **51:**106–108.

©2015 International Medical Press

28. Olysio (simeprevir). *Prescribing information* 2013. Janssen Therapeutics, Titusville, NJ, USA.
29. Oliveira SB, Merchán-Hamann E, Amorim LD. HIV/AIDS coinfection with the hepatitis B and C viruses in Brazil. *Cad Saude Publica* 2014; **30:**433–438.
30. Mendes-Correa MC, Cavalheiro NP, Mello C, Barone AA, Gianini RJ. Genotypic distribution of hepatitis C among hepatitis C and HIV co-infected patients in Brazil. *Int J STD AIDS* 2008; **19:**595–599.
31. Rallón NI, Pineda JA, Soriano V, *et al*. Differences in virological response to peginterferon-α plus ribavirin in HIV-positive patients co-infected with HCV subtypes 1a or 1b. *J Acquir Immune Defic Syndr* 2012; **60:**117–123.
32. Campiotto S, Pinho JRR, Carrilho FJ, da Silva LC, Bernardini AP. Hepatitis C virus (HCV) genotypes distribution in different Brazilian regions. *XIIth International Congress of Virology (Paris)*. 27 July–1 August 2002, Paris, France. Abstract V-1014.
33. Campiotto S, Pinho JR, Carrilho FJ, *et al*. Geographic distribution of hepatitis C virus genotypes in Brazil. *Braz J Med Biol Res* 2005; **38:**41–49.
34. Nakano T, Lu L, Liu P, Pybus OG. Viral gene sequences reveal the variable history of hepatitis C virus infection among countries. *J Infect Dis* 2004; **190:**1098–1108.
35. Lenz, O, Verbinnen, T, Lin TI, *et al*. *In vitro* resistance profile of the hepatitis C virus NS3/4A protease inhibitor TMC435. *Antimicrob Agents Chemother* 2010; **54:**1878–1887.
36. Victrelis (boceprevir). *Prescribing information* 2011. Merck & Co., Whitehouse Station, NJ, USA.
37. Kim AY, Lauer GM, Ouchi K, *et al*. The magnitude and breadth of hepatitis C virus-specific CD8+ T cells depend on absolute CD4+ T-cell count in individuals coinfected with HIV-1. *Blood* 2005; **105:**1170–1178.

Accepted 24 August 2014; published online 3 October 2014