

# Pretreatment resistance to hepatitis C virus protease inhibitors boceprevir/telaprevir in hepatitis C subgenotype 1a-infected patients from Manitoba

Anton Andonov PhD<sup>1</sup>, Kamran Kadkhoda PhD<sup>2</sup>, Carla Osiowy PhD<sup>1</sup>, Kelly Kaita MD<sup>3</sup>

A Andonov, K Kadkhoda, C Osiowy, K Kaita. Pretreatment resistance to hepatitis C virus protease inhibitors boceprevir/telaprevir in hepatitis C subgenotype 1a-infected patients from Manitoba. *Can J Gastroenterol* 2013;27(7):414-416.

**BACKGROUND:** Traditional therapy with pegylated interferon and ribavirin combined with the new protease inhibitors boceprevir or telaprevir has demonstrated improved outcomes in hepatitis C virus (HCV)-infected patients. Prevalence data regarding pre-existing drug-resistant variants to these two new virus inhibitors in the Canadian population are not available.

**OBJECTIVE:** To detect pre-existing mutations conferring resistance to boceprevir and/or telaprevir in Canadian patients infected with HCV genotype 1a.

**METHODS:** Resistance-associated mutations (RAMs) were evaluated in 85 patients infected with HCV genotype 1a who had not yet received antiviral therapy. The NS3 protease gene was sequenced and common RAMs were identified based on a recently published list.

**RESULTS:** The overall prevalence of pre-existing RAMs to boceprevir and telaprevir was higher compared with other similar studies. All of the observed RAMs were associated with a low level of resistance. A surprisingly high proportion of patients had the V55A RAM (10.6%). None of the mutations associated with a high level of resistance were observed. The simultaneous presence of two low-level resistance mutations (V36L and V55A) was observed in only one patient. Three other patients had both T54S RAM and V55I mutations, which may require a higher concentration of the protease drugs. The prevalence of various mutations in Aboriginal Canadian patients was higher (37.5%) compared with Caucasians (16.39%) ( $P=0.038$ ).

**CONCLUSIONS:** The present study was the first to investigate pre-existing drug resistance to boceprevir/telaprevir in Canadian HCV-infected patients. A relatively high proportion of untreated HCV genotype 1a patients in Manitoba harbour low-level RAMs, especially patients of Aboriginal descent, which may contribute to an increased risk of treatment failure.

**Key Words:** Boceprevir; HCV infection; Resistance-associated mutations; Telaprevir

More than one dozen antiviral compounds directly targeting hepatitis C virus (HCV) replication have recently been developed. The most promising include inhibitors of the NS3/NS4A protease and the NS5B polymerase. In 2011, boceprevir and telaprevir were the first antivirals to be approved in combination with pegylated interferon-alpha and ribavirin for the treatment of chronic HCV (genotype 1) infection in North America and Europe. The use of these drugs has led to the emergence of HCV resistance-associated mutations (RAMs), which are generally characterized by different levels of reduced susceptibility of the dominant virus population. Moreover, there is evidence that some of these RAMs already exist to different extents, even

**La résistance préthérapeutique au bocéprévir et au télaprévir, des inhibiteurs de la protéase du virus de l'hépatite C, chez des patients du Manitoba infectés par le sous-génotype 1a de l'hépatite C**

**HISTORIQUE :** La thérapie habituelle à l'interféron pégylé et à la ribavirine associée au bocéprévir ou au télaprévir, de nouveaux inhibiteurs de la protéase, donne une meilleure issue démontrée chez les patients infectés par le virus de l'hépatite C (VHC). On ne possède pas de données de prévalence au sujet des variantes de résistance préthérapeutique à ces deux inhibiteurs de virus au sein de la population canadienne.

**OBJECTIF :** Déceler les mutations préexistantes conférant une résistance au bocéprévir, au télaprévir ou à ces deux médicaments chez les patients canadiens infectés par le génotype 1a du VHC.

**MÉTHODOLOGIE :** Les chercheurs ont évalué les mutations associées à la résistance (MAR) chez 85 patients infectés par le génotype 1a du VHC qui n'avaient pas encore reçu d'antivirothérapie. Ils ont séquencé le gène de protéase NS3 et déterminé les MAR courantes d'après une liste récemment publiée.

**RÉSULTATS :** La prévalence globale des MAR préexistantes au bocéprévir et au télaprévir était plus élevée que dans des études similaires. Toutes les MAR observées s'associaient à un faible taux de résistance. Une proportion étonnamment élevée de patients présentaient la MAR V55A (10,6 %). Les chercheurs n'ont observé aucune mutation associée à un fort taux de résistance, mais ont observé un seul cas de présence simultanée de deux mutations de faible niveau de résistance (V36L et V55A). Trois autres patients présentaient une MAR T54S et une mutation V55I, qui peut exiger une plus forte concentration d'inhibiteurs de la protéase. La prévalence de diverses mutations chez les patients autochtones du Canada était plus élevée (37,5 %) que chez les blancs (16,39 %) ( $P=0,038$ ).

**CONCLUSIONS :** La présente étude était la première à porter sur la résistance préexistante au bocéprévir et au télaprévir chez des patients canadiens infectés par le VHC. Une proportion relativement élevée de patients du Manitoba atteints du génotype 1a du VHC non traité présentent de faibles niveaux de MAR, notamment ceux d'origine autochtone, ce qui peut contribuer à un risque plus élevé d'échec du traitement.

before treatment, in some, if not most, patients (1-3). To complicate matters further, a significant cross-resistance is observed between these two new drugs – namely, a single resistance mutation profile for boceprevir is common for telaprevir or vice versa (1). Furthermore, the resistance profile associated with HCV antivirals against the same target – the HCV protease or polymerase – is also distinct depending on the HCV genotype or subgenotype (1,4).

Approximately one-half of all Canadian patients with chronic hepatitis C, including those in the province of Manitoba, are infected with HCV subgenotype 1a, which is more difficult to treat (5-7). Most of the currently published investigations on NS3/NS4A drug

<sup>1</sup>Public Health Agency of Canada, National Microbiology Laboratory; <sup>2</sup>Cadham Provincial Laboratory; <sup>3</sup>University of Manitoba, Department of Internal Medicine, Winnipeg, Manitoba

Correspondence: Dr Anton Andonov, Public Health Agency of Canada, National Microbiology Laboratory, 1015 Arlington Street, Winnipeg, Manitoba R3E 3R2. Telephone 204-789-6062, fax 204-789-2082, e-mail anton.andonov@phac-aspc.gc.ca

Received for publication January 7, 2013. Accepted February 21, 2013

resistance mutations, although focusing on HCV genotype 1, do not reliably differentiate their findings between subgenotypes 1a and 1b, the latter being significantly more common in Europe, especially among the adult population (8,9). The objective of the present study was to investigate the level of pre-existing boceprevir/telaprevir-relevant HCV RAMs among HCV subgenotype 1a treatment-naive patients in Manitoba.

## METHODS

EDTA-plasma samples from 85 randomly selected HCV-infected patients with known genotype 1a were identified in 2011 through routine HCV diagnostic testing. The main criterion for selection was sufficient residual volume because the samples were previously tested for anti-HCV antibodies and HCV RNA. All patients were treatment naive for protease inhibitors. None of the patients were HIV or hepatitis B virus coinfecting. HCV subgenotype was determined using a commercially available assay (Versant HCV Genotype 2.0 Assay [LIPA], Siemens, Belgium). In cases for which reliable differentiation was not possible, reverse transcription polymerase chain reaction (RT-PCR) followed by complementary DNA sequencing was used.

### Amplification and sequencing of the HCV NS3 gene

HCV RNA was extracted from 250 µL of serum using an automated nucleic acid extraction system (NucliSENS easyMag, bioMérieux Inc, USA) and amplified by RT-PCR using primers specific for the HCV subgenotype 1a NS3 protease catalytic domain as described previously (10). Amplicon products were gel purified before cycle sequencing on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, USA) using BigDye version 3.1 terminator chemistry. Sequence data obtained were used to confirm HCV subgenotype 1a of each viral sample; further sequence alignment and analysis was performed to determine common protease inhibitor resistance mutations based on a recently published list by the HCV Phenotype Working Group of the HCV Drug Development Advisory Group (11).

## RESULTS

The median age of the patients was 48 years (range 20 to 73 years), male to female ratio was 2:1, 61 were Caucasian and 24 were First Nations (various Aboriginal peoples in Canada who are neither Inuit nor Métis).

Phylogenetic analysis using HCV subgenotype 3a as an outlier confirmed that all isolates belonged to subgenotype 1a (data not shown).

The frequency of RAMs to linear protease inhibitors (PIs) observed in the present study are shown on Table 1 and compared with several other studies from North America and Europe. The overall prevalence of pre-existing RAMs to boceprevir and telaprevir was much higher than reported in other studies. Although all of the observed RAMs are characterized by low-level resistance (<10 fold change), all were usually found in at least 10% of patients who failed treatment based on previous studies (11). A surprisingly high proportion of the patients harboured the V55A RAM (10.6%). Additionally, 4.7% had a V55I mutation, which may also confer a low level of resistance. It is important to note that none of the mutations associated with a high level of resistance were observed. The simultaneous presence of two low-level RAMs (V36L and V55A) was observed in only one patient. Three others had both the T54S RAM and V55I mutation; however, although suspected, the latter may not be resistance associated. Two additional mutations were observed (T54W and A156G); while there are no data for the former in terms of its relevance to linear PI resistance, the latter has a minor fold change of 2.1 resistance effect on boceprevir (12). RAMs observed in other studies (Q41, F43) were not detected among patients in the present study. Although the focus of the present study was on linear PI resistance, it is of interest to note that Q80K, known to be associated with resistance to macrocyclic PIs such as TMC43535, was present in almost one-half of the study population (47%).

The prevalence of various mutations in First Nations patients was higher (37.5%) compared with Caucasians (16.39%) (P=0.038).

**TABLE 1**  
Prevalence of naturally occurring hepatitis C virus (HCV) protease inhibitor (PI) resistance-associated mutations at baseline in treatment-naive HCV subgenotype 1a patients from Winnipeg, Manitoba

PI resistance-associated mutations	Study (reference)			
	Present (n=85)	Kuntzen et al (14)* (n=362)	Vicenti et al (15)* (n=62)	Gaudieri et al (16)* (n=112-159)†
V36L	2.3%	1.7%	6%	1.8%
V36M	1.2%	0.5%	0%	0%
T54S	5.9%	1.9%	3%	4.4%
V55A	10.6%	ND	1.5%	ND
V55I‡	4.7%	ND	ND	ND
R155K	0%	0.6%	0%	0.5%
A156/S,T,V,I	0% (A156G: 1.2%)	0%	0%	0%
D168/A,V,E	0%	0.3%	0	1.3%
Total§	20%	5%	10.5%	

Note: Low level of resistance fold change <10; intermediate to high fold change = 11 to 100; high level of resistance fold change >100. \*Only HCV subgenotype 1a samples included for analysis from these references; †Variable number of samples tested for different mutations; ‡V55I may result in low-level resistance such as V55A; §Does not include V55I mutation. ND Not done

## DISCUSSION

We analyzed the prevalence of pre-existing drug resistance mutations associated with boceprevir and telaprevir among 85 patients infected with HCV subgenotype 1a, which is the most common subgenotype among Canadians with chronic HCV infection. Well-known RAMs conferring a low level of resistance to linear PIs (V36L, V36M and T54S) were detected in a small proportion of cases, similar to previous studies (13-16). A surprisingly high prevalence of the V55A mutation was observed in our study population. This RAM is associated with a low level of resistance to boceprevir, both in vitro (6.9-fold increase in half maximal inhibitory concentration [ie, IC50]) and in vivo (10,13). An additional 4.7% of patients harboured a similar mutation (V55I), which may also be associated with resistance to boceprevir. These findings are somewhat unusual because the naturally occurring V55A amino acid change was observed in only 0.3% and V55I in 1.1%, respectively, among 621 HCV subgenotype 1a strains from GenBank (4) and require additional studies.

None of the patients exhibited either single mutations associated with a high level of drug resistance or a previously known combination of two low-level mutations, such as V36M and R155K, leading to high PI resistance.

The high proportion of cases harbouring the Q80K mutation (47%) conferring resistance to one of the macrocyclic inhibitors TMC435 is consistent with some previous findings (15,16) and indicates that this amino acid site is polymorphic in nature rather than a naturally pre-existing mutation.

Due to the direct sequencing approach, only the dominant bases were called after analyzing the sequences; therefore, the observed RAMs represent the principal virus population. Although clinical in vivo data suggest that sustained virological response could be achieved, albeit not always, in the presence of RAMs during triple combination therapy, it is possible that in some instances at least pre-existing low-level RAMs can result in reduction of the early treatment response and perhaps contribute to poor treatment outcomes in patients with sub-standard adherence to the PI component. In addition, the probability of subsequent RAMS developing in the presence of a pre-existing dominant mutation, thus leading to virus breakthrough, is higher compared with de novo formation of two complementing RAMS.

Presently, screening for PI RAMs in treatment-naive patients is not warranted; however, as more information regarding the cost

efficiency of this process becomes available and, in view of the increased cost and hematological side effects associated with triple therapy, the need for drug resistance prescreening, at least in previous non- or partial responders to peginterferon and ribavirin therapy, may be better justified.

Although approved in 2011, triple therapy with peginterferon, ribavirin and boceprevir or telaprevir has only been recently implemented in some Canadian provinces. To the best of our knowledge, our data regarding detection of pre-existing drug resistance to boceprevir/telaprevir are the first to be reported in Canada. Albeit preliminary, our observations regarding an increased rate of low-level

primary drug resistance warrant further studies with larger HCV-infected populations from different provinces including First Nations HCV-infected patients.

---

**INSTITUTION FROM WHICH WORK ORIGINATED:** Public Health Agency of Canada, National Microbiology Laboratory.

---

**ACKNOWLEDGEMENTS:** The authors are indebted to Paige Isabey and Jamie Borlang for excellent technical assistance.

---

## REFERENCES

1. Halfon P, Locarnini S. Hepatitis C virus resistance to protease inhibitors. *J Hepatol* 2011;55:192-206.
2. Sarrazin C, Zeuzem S. Resistance to direct antiviral agents in patients with hepatitis C virus infection. *Gastroenterology* 2010;138:447-62.
3. Lauck M, Alvarado-Mora MV, Becker EA, et al. Analysis of hepatitis C virus intrahost diversity across the coding region by ultradeep pyrosequencing. *J Virol* 2012;86:3952-60.
4. Cento V, Mirabelli C, Salpini R, et al. HCV genotypes are differently prone to the development of resistance to linear and macrocyclic protease inhibitors. *PLoS One* 2012;7:1-19.
5. Andonov A, Chaudhary RK. Genotyping of Canadian hepatitis C virus isolates by PCR. *J Clin Microbiol* 1994;32:2031-4.
6. Chaudhary RK, Tepper M, Elsaadany S, Gully P. Distribution of hepatitis C virus genotypes in Canada: Results from the LCDC sentinel health unit surveillance system. *Can J Infect Dis* 1999;10:53-6.
7. Dawood M, Smart, G, Wood M, Wu HX, Paton S, Wu J. Hepatitis C virus infection among First nation and non-First Nation people in Manitoba, Canada: A public health laboratory study. *Can J Microbiol* 2006;52:999-1005.
8. Sy T, Jamal MM. Epidemiology of hepatitis C virus (HCV) infection. *Int J Med Sci* 2006;3:41-6.
9. Esteban JI, Sauleda S, Quer J. The changing epidemiology of hepatitis C virus infection in Europe. *J Hepatol* 2008;48:148-62.
10. Susser S, Welsch C, Wang Y, et al. Characterization of resistance to the protease inhibitor boceprevir in hepatitis C virus-infected patients. *Hepatology* 2009;50:1709-18.
11. 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.
12. 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;54:1878-87.
13. Vermehren J, Susser S, Lange CM, et al. 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;19:120-7.
14. Kuntzen T, Timm J, Berical A, et al. Naturally occurring dominant resistance mutations to hepatitis C virus protease and polymerase inhibitors in treatment-naïve patients. *Hepatology* 2008;48:1769-78.
15. 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;67:984-7.
16. Gaudieri S, Rauch A, Pfafferoth K, et al. Hepatitis C virus drug resistance and immune-driven adaptations: Relevance to new antiviral therapy. *Hepatology* 2009;49:1069-82.