ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 2010, p. 2681–2683 Vol. 54, No. 6
0066-4804/10/$12.00 doi:10.1128/AAC.01796-09
Copyright © 2010, American Society for Microbiology. All Rights Reserved.

Characterization of V36C, a Novel Amino Acid Substitution Conferring Hepatitis C Virus (HCV) Resistance to Telaprevir, a Potent Peptidomimetic Inhibitor of HCV Protease▽†

Laetitia Barbotte,[1]‡ Abdelhakim Ahmed-Belkacem,[1]‡ Stéphane Chevaliez,[1,2] Alexandre Soulier,[1,2] Christophe Hézode,[1,3] Henri Wajcman,[1] Doug J. Bartels,[4] Yi Zhou,[4] Andrzej Ardzinski,[4] Nagraj Mani,[4] B. Govinda Rao,[4] Shelley George,[4] Ann Kwong,[4] and Jean-Michel Pawlotsky[1,2]*

INSERM U955, Créteil, France[1]*; National Reference Center for Viral Hepatitis B, C and Delta, Department of Virology, Henri Mondor Hospital, University of Paris 12, Créteil, France*[2]*; Department of Hepatology and Gastroenterology, Henri Mondor Hospital, University of Paris 12, Créteil, France*[3]*; and Vertex Pharmaceuticals Incorporated, Cambridge, Massachusetts*[4]

Received 18 December 2009/Returned for modification 18 February 2010/Accepted 30 March 2010

We characterized a novel substitution conferring moderate resistance to telaprevir, a peptidomimetic inhibitor of hepatitis C virus protease. V36C conferred a 4.0-fold increase in the telaprevir 50% inhibitory concentration in an enzyme assay and a 9.5-fold increase in the replicon model. The replication capacity of a replicon harboring V36C was close to that of the wild-type protease. This case emphasizes the complexity of hepatitis C virus resistance to protease inhibitors.

Advances in virology have led to the development of novel therapeutics specifically targeting hepatitis C virus (HCV) (4). Telaprevir (VX-950; Vertex Pharmaceuticals Incorporated, Cambridge, MA) is a novel, highly selective, potent peptidomimetic inhibitor of the HCV nonstructural protein 3/4A (NS3/4A) protease (1, 3) which has reached phase III clinical development in combination with pegylated alpha interferon (IFN-α) and ribavirin. Amino acid substitutions conferring telaprevir resistance have been reported at positions Val 36, Thr 54, Arg 155, and Ala 156 of the NS3 protease (2, 5). In patients treated with telaprevir and pegylated IFN-α with and without ribavirin, breakthroughs during treatment and relapses after treatment are characterized by the recurrence of telaprevir-resistant HCV variant replication (1).

Here, we characterized a novel, so far unknown, telaprevir resistance substitution at position Val 36 in a 38-year-old treatment-naïve woman with chronic hepatitis C due to HCV genotype 1b infection. The patient was enrolled in PROVE2, a phase II randomized clinical trial assessing the efficacy and safety of telaprevir in combination with pegylated IFN-α2a with or without ribavirin (1). The patient was treated with telaprevir at 750 mg/8 h, pegylated IFN-α2a at 180 μg/week, and ribavirin at 1.0 g/day. HCV RNA became undetectable (<10 IU/ml) on therapy, but after 43 days of treatment, the patient withdrew consent and stopped therapy. She continued to be followed up after treatment withdrawal.

Figure 1 shows the kinetics of HCV RNA levels in the patient during the 43 days of therapy. HCV RNA was detected 8 weeks after treatment withdrawal, and HCV RNA levels returned to nearly baseline levels. Twenty to 24 full-length NS3 protease clones were sequenced at each HCV RNA-positive time point. The patient was infected with a wild-type, telaprevir sensitive viral population at the baseline (Fig. 1). At the time of posttreatment relapse, the HCV variants all bore a Val-to-Cys substitution at position 36 (V36C). The V36C substitution remained dominant throughout posttreatment follow-up, up to day 512 after the start of therapy (Fig. 1). This substitution was associated with a Leu-to-Phe substitution at position 14 of the protease (L14F). L14F is present in approximately 6% of all HCV sequences and 8.5% of HCV subtype 1b sequences available in the European HCV database (http://euhcvdb.ibcp.fr/euHCVdb/). Based on molecular modeling, it is predicted to be located at more than 20 Å of the telaprevir binding site and is therefore unlikely to alter telaprevir-NS3 protease interaction.

The V36C substitution was further characterized and compared to other substitutions at NS3 protease position 36 observed in dominant populations in the PROVE2 trial (Table 1). The methods are described in the supplemental material. As shown in Table 1, the V36C substitution conferred a 4.0-fold increase in the telaprevir 50% inhibitory concentration (IC_{50}) in our NS3/4A protease enzyme assay. This moderate increase in the IC_{50} was on the same order as that conferred by V36M and slightly greater than that conferred by V36L. The K_m and V_{max} values of the NS3 protease-catalyzed enzymatic reaction were on the same order for the V36C variant and the other V36 variants and close to that of the wild-type protease (Table 1).

In the subgenomic Con1-mADE replicon model, the V36C substitution conferred a 9.5-fold increase in the telaprevir 50% effective concentration (EC_{50}), a level of resistance on the same order as those reported for other substitutions at position

* Corresponding author. Mailing address: Department of Virology, Hôpital Henri Mondor, 51 avenue du Maréchal de Lattre de Tassigny, 94010 Créteil, France. Phone: 33-1-4981-2827. Fax: 33-1-4981-4831. E-mail: jean-michel.pawlotsky@hmn.aphp.fr.
‡ Contributed equally to this article.
† Supplemental material for this article may be found at http://aac.asm.org/.
▽ Published ahead of print on 5 April 2010.

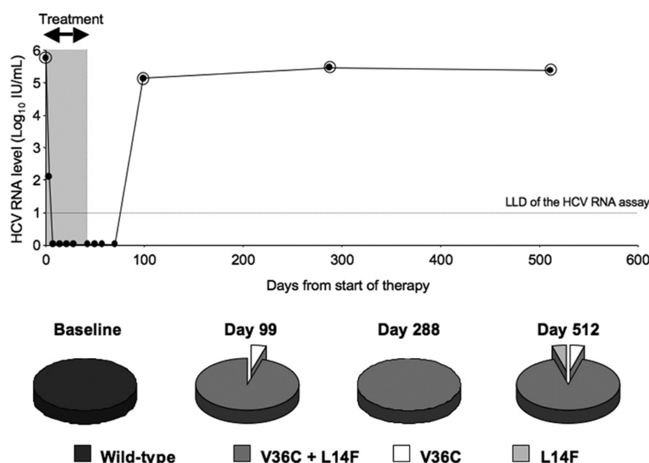

FIG. 1. (Top) HCV RNA kinetics in a patient who selected a V36C amino acid substitution on triple therapy with pegylated IFN-α2a, ribavirin, and telaprevir. The HCV RNA levels are expressed in $\log_{10}$ IU/ml. LLD, lower limit of detection. The circled dots represent the four time points at which quasispecies sequence analysis was performed. (Bottom) Dynamics of NS3 quasispecies populations after treatment withdrawal relative to the baseline.

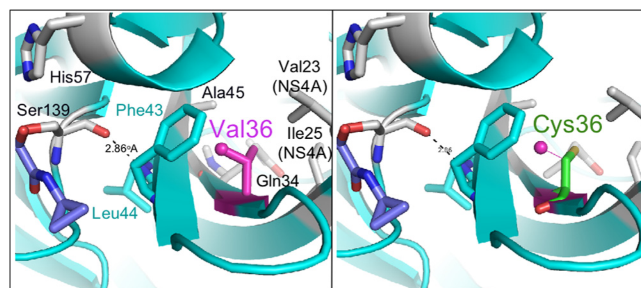

FIG. 2. Computationally predicted three-dimensional models of the V36 (wild-type, left) and V36C (resistant, right) NS3 protease variants complexed with telaprevir. The wild-type Val side chain is purple, whereas the Cys substitution is green. The four side chains located around Val at position 36 that make contact with Phe at position 43 are white, as the active-site Ser at position 139 and His at position 57. Telaprevir carbon atoms are light blue.

36 (6). The replication capacity of a transiently transfected cell system expressing a luciferase replicon harboring V36C was on the same order as that of the wild-type virus (Table 1). As already reported (6), V36A and V36M did not substantially alter the replication capacity of HCV replicons.

The previously described modeled complex of the NS3 protease with telaprevir (6) was used to generate a model of the V36C NS3 protease variant (Fig. 2). Like other substitutions at position V36, V36C was predicted to affect inhibitor binding for the following two reasons. (i) The loss of contact with Phe at NS3 position 43 may result in increased flexibility of the latter, subsequently leading to weaker contact with the prime-side part of telaprevir. (ii) The increased flexibility of Phe at position 43 may affect the hydrogen bond between active Ser 139 main-chain carbonyl and the main-chain NH of Leu 44, thus affecting the covalent interaction of Ser 139 with the ketoamide moiety of telaprevir. The V36C amino acid substitution leaves a void near the Phe 43 side chain and lowers binding via the above-mentioned mechanisms. The Cys 36 variants were predicted to fit comfortably in the mainly hydrophobic enzymatic pocket, and the telaprevir binding loss was predicted to be of a magnitude similar to that observed for the V36A and V36M substitutions, a result in keeping with the results of resistance testing in the enzyme assay and the replicon system.

We describe here a novel amino acid substitution at position Val 36 of the NS3 protease, V36C, which confers reduced *in vitro* susceptibility to telaprevir. Its characteristics were close to those of the other substitutions at the same position associated with HCV resistance to telaprevir. Computational modeling suggested that V36C affects telaprevir binding to the protease catalytic pocket through weaker contact with the prime-side part of telaprevir and alteration of the covalent interaction of Ser 139 with the ketoamide moiety of telaprevir.

The rarity of V36C compared to other substitutions at po-

TABLE 1. V36 substitutions observed in patients included in the PROVE2 clinical trial

V36 variant	No. (%) of V36 variants in PROVE 2 trial		NS3/4A protease enzyme assay[a]				HCV replicon assay[a]		
	Variant found as dominant (100%) viral population at baseline (n = 241)	Variant found as dominant (100%) viral population at the time of breakthrough or relapse (n = 64)	Mean IC_{50} (μM) ± SD	Mean fold change in IC_{50} relative to wild type ± SD	Mean K_m (μM) ± SD	Mean V_{max} (μM/μg/s) ± SD	Mean EC_{50} (μM) ± SD[c]	Mean fold change in EC_{50} relative to wild type ± SD	Mean relative replication capacity ± SD[d]
V36 (wild type)	227 (94)	40 (62.5)	0.6 ± 0.2	1.0 ± 0.5	10.1 ± 6.1	0.0026 ± 0.0007	0.4 ± 0.1	1.0	100
V36M	2 (0.8)	18 (28.1)	3.0 ± 0.8	5.0 ± 1.8	5.1 ± 1.3	0.0018 ± 0.0002	3.4 ± 0.8[b]	7.0 ± 1.6[b]	77 ± 12[b]
V36A	0 (0)	4 (6.3)	NA[e]	NA	NA	NA	3.6 ± 1.1[b]	7.4 ± 2.2[b]	104 ± 26[b]
V36L	10 (4.1)	6 (9.4)	1.3 ± 0.1	2.2 ± 0.6	4.6 ± 1.0	0.0022 ± 0.0002	1.1 ± 0.2[b]	2.2 ± 0.4[b]	NT[f]
V36C	0 (0)	1 (1.6)	2.4 ± 1.3	4.0 ± 1.3	6.4 ± 2.6	0.0023 ± 0.0004	3.8 ± 0.5	9.5 ± 0.9	98 ± 9

[a] Results were calculated based on three independent experiments.
[b] Data from Zhou et al. (6); fold change calculated with respect to their wild-type sequence.
[c] Data generated with a stable replicon.
[d] Data generated with a transient replicon.
[e] NA, no soluble protein obtained.
[f] NT, not tested.

sition Val 36 could be explained by the genetic barrier to the emergence of this variant. Indeed, Val is the most frequent amino acid at position 36 of the NS3 protease in both HCV genotype 1a (GUG)- and 1b (GU[U/C])-infected patients. While substitutions such as V36M, V36A, or V36L generally require a single nucleotide substitution to occur, making the preexistence of such variants as minor less fit populations at the baseline likely in most infected patients, V36C requires 3 and 2 nucleotide substitutions in HCV genotypes 1a and 1b, respectively. Therefore, the V36C variant is less likely to be generated during replication, as it needs a two- or three-step process, and is thus less likely to preexist at the baseline in a majority of patients.

Interestingly, the virological relapse occurred 8 weeks after treatment withdrawal and the full population bore the V36C substitution at this time. This finding is in keeping with the low-level resistance to telaprevir conferred by this amino acid substitution. During the short treatment period, the fully sensitive wild-type viral population was fully controlled. It may have been eradicated rapidly, as this population never reappeared over several months of posttreatment follow-up. Given the EC_{50}s, the V36C viral population is likely to have been only partially inhibited by telaprevir. It is possible that the low-replication V36C viral population could have been eradicated, although less rapidly than the wild-type variant, if therapy with pegylated IFN-α and ribavirin had been continued. The fact that this patient withdrew consent and stopped therapy after only 43 days may have been a unique opportunity for this rare, most likely preexisting, relatively poor fitness V36 variant to emerge and grow as a dominant viral variant *in vivo*.

In conclusion, we reported the characteristics of a previously unreported variant with a V36C substitution conferring moderate resistance to telaprevir. This case emphasizes the complexity of HCV resistance to NS3/4A protease inhibitors in the context of pegylated IFN-α-based therapy.

Laetitia Barbotte is a recipient of a predoctoral fellowship from the Ministère de l'Enseignement Supérieur et de la Recherche. Abdelhakim Ahmed-Belkacem is a recipient of a postdoctoral fellowship from the Agence Nationale de Recherche sur le SIDA et les Hépatites Virales (ANRS).

REFERENCES

1. **Hézode, C., N. Forestier, G. Dusheiko, P. Ferenci, S. Pol, T. Goeser, J. P. Bronowicki, M. Bourlière, S. Gharakhanian, L. Bengtsson, L. McNair, S. George, T. Kieffer, A. Kwong, R. S. Kauffman, J. Alam, J. M. Pawlotsky, and S. Zeuzem.** 2009. Telaprevir and peginterferon-alfa-2a with or without ribavirin for chronic HCV infection. N. Engl. J. Med. **360:**1839–1850.
2. **Kieffer, T. L., C. Sarrazin, J. S. Miller, M. W. Welker, N. Forestier, H. W. Reesink, A. D. Kwong, and S. Zeuzem.** 2007. Telaprevir and pegylated interferon-alpha-2a inhibit wild-type and resistant genotype 1 hepatitis C virus replication in patients. Hepatology **46:**631–639.
3. **McHutchison, J. G., G. T. Everson, S. C. Gordon, I. M. Jacobson, M. Sulkowski, R. Kauffman, L. McNair, J. Alam, and A. J. Muir.** 2009. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. N. Engl. J. Med. **360:**1827–1838. [Erratum, **361:**1516.]
4. **Pawlotsky, J. M., S. Chevaliez, and J. G. McHutchison.** 2007. The hepatitis C virus life cycle as a target for new antiviral therapies. Gastroenterology **132:** 1979–1998.
5. **Sarrazin, C., T. L. Kieffer, D. Bartels, B. Hanzelka, U. Muh, M. Welker, D. Wincheringer, Y. Zhou, H. M. Chu, C. Lin, C. Weegink, H. Reesink, S. Zeuzem, and A. D. Kwong.** 2007. Dynamic hepatitis C virus genotypic and phenotypic changes in patients treated with the protease inhibitor telaprevir. Gastroenterology **132:**1767–1777.
6. **Zhou, Y., D. J. Bartels, B. L. Hanzelka, U. Muh, Y. Wei, H. M. Chu, A. M. Tigges, D. L. Brennan, B. G. Rao, L. Swenson, A. D. Kwong, and C. Lin.** 2008. Phenotypic characterization of resistant Val36 variants of hepatitis C virus NS3-4A serine protease. Antimicrob. Agents Chemother. **52:**110–120.