Virology 443 (2013) 278–284

Contents lists available at SciVerse ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Emergence of resistance-associated variants after failed triple therapy with vaniprevir in treatment-experienced non-cirrhotic patients with hepatitis C-genotype 1 infection: A population and clonal analysis ☆

Richard J.O. Barnard [a,*], Carolyn M. McHale [a], William Newhard [a], Carol A. Cheney [a], Donald J. Graham [a], Amy L. Himmelberger [a], Julie Strizki [a], Peggy M.T. Hwang [a], Amber A. Rivera [b], Jacqueline D. Reeves [b], David Nickle [a], Mark J. DiNubile [a,**], Daria J. Hazuda [a], Niloufar Mobashery [a]

[a] Merck Sharp & Dohme, Whitehouse Station, NJ, USA
[b] Monogram Biosciences Inc., South San Francisco, CA, USA

ARTICLE INFO

Article history:
Received 26 March 2013
Returned to author for revisions
18 April 2013
Accepted 10 May 2013
Available online 10 June 2013

Keywords:
Vaniprevir
Hepatitis C-genotype 1
Resistance-associated variants

ABSTRACT

Background: Vaniprevir with P/R improved SVR rates over P/R alone in treatment-experienced patients with chronic HCV-genotype 1 infection, but treatment failure presents therapeutic challenges. We identified RAVs from non-cirrhotic patients failing to achieve SVR on vaniprevir-containing regimens from a dose/duration-ranging trial of triple-combination therapy.
Methods: Using population analysis, resistance sequencing was performed on all baseline samples and on samples at virologic failure in the vaniprevir arms. Longitudinal clonal analyses were performed on viral isolates from six vaniprevir recipients experiencing breakthrough viremia.
Results: Baseline RAVs were detected in two patients subsequently experiencing virologic failure. At virologic failure, the majority of RAVs had substitutions at R155, A156, or D168. Clonal analyses identified novel double/triple variants emerging with continuing vaniprevir dosing.
Conclusions: RAVs were predominantly observed at R155, A156, and/or D168 during virologic failure on vaniprevir/P/R. Double/triple RAVs were identified in patients remaining viremic on triple therapy, suggesting evolution of resistance under selective pressure.

Introduction

Peginterferon-alfa and ribavirin (P/R) had been the standard of care for chronic hepatitis C virus (HCV) infection until the recent introduction of direct-acting antiviral agents (Manns et al., 2001; Fried et al., 2002; Ghany et al., 2011). The two licensed NS3/4A serine protease inhibitors [boceprevir (VICTRELIS™; Merck, Whitehouse Station, NJ, USA)] and [telaprevir (INCIVEK™; Vertex Pharmaceuticals, Cambridge, MA, USA)] when added to P/R therapy significantly improved sustained virologic response (SVR) rates in previously untreated patients as well as in patients who had partially responded to or relapsed after a standard course of P/R alone (Poordad et al., 2011; Jacobson et al., 2011; Sherman et al., 2011; Bacon et al., 2011; Zeuzem et al., 2011). Accumulating evidence indicates that even previous null responders have an appreciable chance at SVR with the newer regimens. However, virologic failure ultimately occurs in >20% of patients with genotype 1 HCV infection given triple therapy (Poordad et al., 2011; Jacobson et al., 2011; Sherman et al., 2011; Bacon et al., 2011; Zeuzem et al., 2011).

The low fidelity of the HCV RNA-dependent RNA polymerase coupled with the high rate of virion production gives rise to a population of genetically related viruses in an infected patient that harbor minor differences in their RNA genome. As a consequence, it is likely that the majority of single and double-resistant resistance-associated variants (RAVs) are present prior to treatment with direct acting antiviral (DAA) agents (Ribeiro et al., 2012). HCV protease inhibitors are routinely given as part of combination regimens with P/R in clinical practice (Ghany et al., 2011) or with other direct-acting antiviral agents in experimental trials.

Abbreviations: DAA, directly acting antiviral agent; HCV, hepatitis C virus; IC50, inhibitory concentration 50%; IU/mL, international units per milliliter; LLD, lower limit of detection; LLQ, lower limit of quantification; P/R, peginterferon alfa/ribavirin; SVR, sustained virologic response

☆The parent study and the current analysis were sponsored and funded by Merck.

* Correspondence to: Merck Research Laboratories, West Point, PA, USA.
** Corresponding author.
E-mail addresses: richard_barnard@merck.com (R.J.O. Barnard), mark_dinubile@merck.com (M.J. DiNubile).

0042-6822/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.virol.2013.05.013

Virologic failure in patients treated with DAAs is often accompanied by the emergence of viral variants with resistance to the inhibitor class, and detectable RAVs can persist up to three years after cessation of therapy (Sarrazin and Zeuzem 2010; Gambarin-Gelwan and Jacobson 2012; Welsch and Zeuzem 2012). Accordingly, the enduring clinical implications of RAVs are not yet fully understood.

Vaniprevir (MK-7009) is an investigational macrocyclic NS3/4A protease inhibitor which exhibits potent activity against HCV-genotype 1. In vitro resistance selection experiments and sequence data from Phase 1 and 2a clinical studies with vaniprevir have identified several viral NS3/4A protease variants associated with decreased susceptibility to vaniprevir. A recently completed dose/duration-ranging study (Protocol 009) of vaniprevir combined with P/R in treatment-experienced patients demonstrated higher SVR rates than those previously reported with the approved first-generation protease inhibitors combined with P/R (Lawitz et al., 2012). Using samples from the non-cirrhotic cohort of this Phase 2b trial, the current report examined the types and frequencies of vaniprevir RAVs detected in viruses isolated from patients randomized to vaniprevir/P/R at baseline and at the time of virologic failure. Since the technology for HCV population sequencing is widely available, our results can be directly applied to contemporary clinical practice. In addition, the evolution of resistance was examined by longitudinal clonal sequence analysis in six patients that experienced virologic failure with vaniprevir RAVs.

Results

Of the 169 treated non-cirrhotic patients in the four vaniprevir groups (Fig. 1), 73 (43%) had genotype 1a virus, 94 (56%) were infected with genotype 1b virus and two (1%) patients were infected with a genotype 1 virus with an indeterminate subtype. A total of 157 vaniprevir recipients (93%) were included in the primary efficacy analysis: 67 with genotype 1a virus (43%), 88 with genotype 1b virus (56%), and two (1%) with an indeterminate genotype 1 subtype. Patient histories of prior P/R failure in the primary efficacy population encompassed 62 relapses (39%), 42 null responses (26%), 30 partial responses (19%), and 23 virologic breakthroughs (15%).

Baseline population NS3/4A-sequence data were obtained for the 155 patients with typeable HCV infections included in the primary analysis. Seven (5%) patients had viruses that harbored variants with a decreased resistance to first-generation HCV protease inhibitors, including V36M, T54S, V55A/I/T/V, and R155K (Table 1). NS3/4A genes harboring variants at positions V36 and T54 confer a <4-fold decrease in sensitivity to MK-7009 in vitro with a cell-based assay. Although variants at these positions have not been identified in patients dosed with vaniprevir or in replicon resistance selection studies *in vitro*, these variants caused a synergistic decrease in sensitivity to vaniprevir when combined with the R155K variant. Notably two patients who had been null responders on their previous P/R regimen harbored R155K variants in combination with either V36M or T54S at baseline, both of whom experienced virologic failure on vaniprevir/P/R; one patient had a null response and the other had breakthrough viremia.

Population resistance analyses revealed that the majority of emergent RAVs had amino acid substitutions at positions R155, A156, or D168 (Table 2), all of which conferred decreased vaniprevir susceptibility relative to the reference strains (Table 3). Of the 28 patients who experienced virologic failure in the vaniprevir-dosing arms of this study, resistance data were available at virologic failure for 26 patients. The NS3/4a gene could not be amplified from samples obtained at virologic failure from the remaining two patients. Of the 26 patients with resistance data at virologic failure, 17 had genotype 1a virus and 9 had genotype 1b virus. RAVs were detected in 17 of 17 (100%) genotype 1a infections and 9 of 9 (100%) genotype 1b infections. The most frequently detected protease variants associated with virologic failure among genotype 1a infected patients were at positions R155, A156, and D168, whereas the most commonly detected protease variants among patients with genotype 1b infections were at positions D168 and A156. Similar to other studies with first-generation HCV proteases, R155K was only found by population sequencing in genotype 1a.

All 26 patients included in the primary efficacy analysis whose HCV isolate was sequenced at the time of virologic failure had RAVs (Table 4). Of these 26 vaniprevir recipients experiencing virologic failure with sequence data, 12 had virologic breakthrough, 1 had a null response, 2 had partial responses, and 11 had relapses. In the 12 patients who experienced virologic breakthrough, variants were detected at positions R155K, A156, and D168. Compared to patients with relapse, partial response, or null response, patients with breakthrough more often had RAVs detected at more than one locus. RAVs were detected at multiple loci (R155/D168, R155/A156, or A156/D168) in 5/12 breakthrough patients, while the remainder had variants at only R155 or D168 detected. Of the 11 relapse patients, only one had RAVs detected at more than one locus (R155K and D168V). The remaining patients

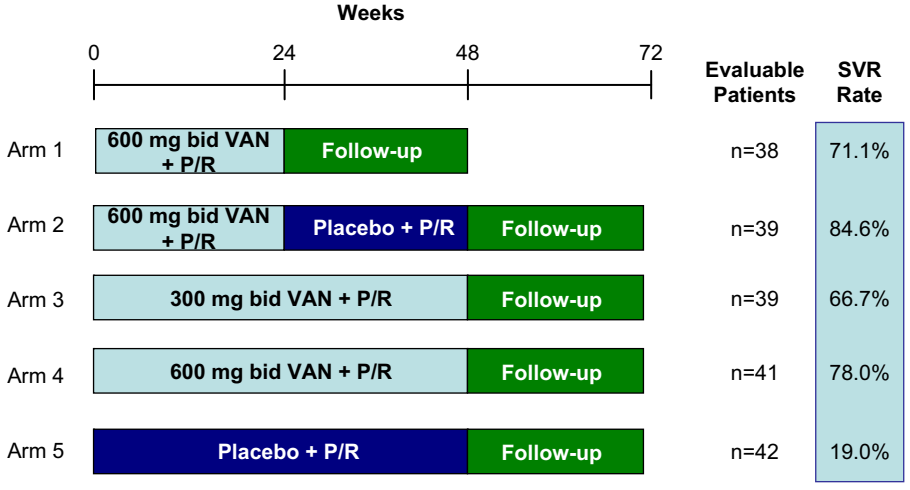

Fig. 1. Dosing arms for MK-7009 P009 Phase 2b trial in treatment-experienced non-cirrhotic patients. After stratification by type of previous failure, patients were treated with 4 regimens combining vaniprevir with P/R (arms 1–4). The control arm (arm 5) used PR alone.