Supplementary Online Content


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This supplementary material has been provided by the authors to give readers additional information about their work.
eMethods and Other Information

Paritaprevir was identified as a lead compound by AbbVie and Enanta Pharmaceuticals.

Investigators

Abbreviations
3D, ombitasvir/paritaprevir/ritonavir and dasabuvir
ART, antiretroviral therapy
ATV, atazanavir
DAA, direct-acting antiviral
PTW, post-treatment week
HCV, hepatitis C virus
HIV, human immunodeficiency virus
ITT, intent-to-treat
LLOQ, lower limit of quantitation
pegIFN, pegylated interferon
RAL, Raltegravir
RBV, ribavirin
SVR, sustained virologic response
SVR12, sustained virologic response 12 weeks post-treatment
Eligibility Criteria

Main Inclusion:

1. Patients must be HCV treatment-naïve or pegIFN/RBV-experienced. If pegIFN/RBV-experienced, patient must have documentation that they were adherent to prior pegIFN/RBV combination therapy and meet one of the following categories:

   - Null responder:
     - received at least 12 weeks of pegIFN/RBV for the treatment of HCV and failed to achieve a 2 log10 IU/mL reduction in HCV RNA at Week 12 (Patients will be considered to meet this definition if the lack of treatment response was documented between Weeks 10 – 16 of treatment); or
     - received at least 4 weeks of pegIFN/RBV for the treatment of HCV and achieved a < 1 log10 IU/mL reduction in HCV RNA at Week 4 (Patients will be considered to meet this definition if the lack of treatment response was documented after ≥ 25 days of treatment); or

   - Partial responder: received at least 20 weeks of pegIFN/RBV for the treatment of HCV and achieved ≥ 2 log10 IU/mL reduction in HCV RNA at Week 12 (Patients will be considered to meet this definition if the treatment response was documented between Weeks 10 – 16 of treatment), but failed to achieve HCV RNA undetectable at or after Week 20 of treatment; or

   - Relapser: received at least 36 weeks of pegIFN/RBV for the treatment of HCV and was undetectable at or after Week 36 of treatment, but HCV RNA was detectable within 52 weeks of treatment follow-up.

2. Body Mass Index (BMI) is from ≥ 18 to < 38 kg/m2 at the time of screening. BMI is calculated as weight measured in kilograms (kg) divided by the square of height measured in meters (m).

3. Chronic HCV infection prior to study enrollment. Chronic HCV infection is defined as one of the following:

   - Positive for anti-HCV antibody (Ab) or HCV RNA at least 6 months before Screening, and positive for HCV RNA and anti-HCV Ab at the time of Screening; or
   - Positive for anti-HCV Ab and HCV RNA at the time of Screening with a liver biopsy consistent with chronic HCV infection.

4. Screening laboratory result indicating HCV genotype 1-infection.

5. Patient has HCV RNA level > 10,000 IU/mL at Screening.

6. Positive test result for anti-Human Immunodeficiency Virus antibody (HIV Ab) at Screening.

7. Plasma HIV-1 RNA < 40 copies/mL during screening using Abbott RealTime HIV-1 assay, and plasma HIV-1 RNA below LLOQ by an approved plasma HIV-1 RNA quantitative assay (including but not limited to: COBAS® Ampliprep/COBAS® Taqman® HIV-1 Test, v 2.0 or Abbott RealTime HIV-1 assay) at least twice during the 24 weeks prior to screening including one qualifying result at least 8 weeks prior to screening. Patients with a solitary (unconfirmed) plasma HIV-1 RNA above LLOQ and < 200 copies/mL within 24 weeks of screening may be eligible for enrollment with approval of the AbbVie Study Designated Physician.

8. CD4+ count ≥ 200 cells/mm3 or CD4+% ≥ 14% during screening.
9. On a stable, qualifying HIV-1 ART regimen for at least 8 weeks prior to screening. The HIV-1 ART regimen must include two nucleoside/nucleotide reverse transcriptase inhibitors plus the ritonavir-boosted protease inhibitor atazanavir, or the integrase inhibitor, raltegravir.

The nucleoside/nucleotide reverse transcriptase inhibitor combinations in the stable qualifying HIV-1 ART regimen must be either:

- Tenofovir disoproxil fumarate (TDF) PO QD plus emtricitabine (FTC) PO QD (Individual components or as the fixed dose combination TDF/FTC, Truvada®), or
- Tenofovir disoproxil fumarate (TDF) PO QD plus lamivudine (3TC) PO QD or 3TC PO BID (Individual components or as the fixed dose combination TDF/3TC).

The ritonavir boosted protease inhibitors in the stable qualifying HIV-1 ART regimen must be either:

- Atazanavir (ATV) PO QD coadministered with ritonavir PO QD, or

The integrase inhibitor in the stable qualifying HIV-1 ART regimen must be:

- Raltegravir (RAL) PO BID.

Patients receiving any other HIV-1 ART in addition to those noted above would not be eligible for enrollment in the study.

10. Patients will be considered to be without cirrhosis and included if the following criteria are met:

Per local standard practice, documented results of one of the following:

- A liver biopsy within 24 months prior to or during screening demonstrating the absence of cirrhosis, e.g., a METAVIR Score of 3 or less, Ishak score of 4 or less; or
- A screening FibroScan result of < 12.5 kPa (FibroScan must be approved by the local regulatory agency to qualify for entrance criteria); or a screening FibroTest score of ≤ 0.72 and Aspartate Aminotransferase to Platelet Ratio Index (APRI) ≤ 2 (Patients with a screening FibroTest result that is ≤ 0.72 and an APRI > 2, or a FibroTest result that is ≥ 0.73 and an APRI ≤ 2 must have a FibroScan or liver biopsy to determine the presence or absence of cirrhosis).

Main Exclusion:

1. Positive test result at screening for Hepatitis B surface antigen (HBsAg).
2. Prior therapy with DAAs for the treatment of HCV, including telaprevir and boceprevir.
3. HCV genotype performed during screening indicating unable to genotype or co-infection with any HCV genotype other than GT 1.
4. Evidence of past virologic failure to more than one HIV-1 ART regimen.
5. Chronic human immunodeficiency virus, type 2 (HIV-2) infection.
HCV Virologic Failure Criteria for Patient Management
The following criteria will be considered evidence of HCV virologic failure. Patients demonstrating any of the following will be discontinued from study drug:

- Confirmed increase from nadir in HCV RNA (defined as 2 consecutive HCV RNA measurements of $> 1 \log_{10}$ IU/mL above nadir) at any time point;

- Confirmed HCV RNA $\geq$ LLOQ (defined as 2 consecutive HCV RNA measurements $\geq$ LLOQ) at any point after HCV RNA < LLOQ during treatment.

HIV Virologic Failure Criteria for Patient Management
HIV RNA was assessed at each scheduled study visit. If a patient's HIV RNA was $\geq 40$ copies/mL, the HIV RNA was to be repeated approximately 2 weeks later, and every 2 weeks thereafter until the patient met criteria for study treatment discontinuation as noted below or until plasma HIV RNA was $< 40$ copies/mL. Failure to maintain HIV-1 virologic suppression was defined as three consecutive visits with HIV-1 RNA $\geq 40$ copies/mL, or one visit $\geq 40$ copies/mL followed by repeat plasma HIV-1 RNA $\geq 200$ copies/mL, at which time patients were to be discontinued from study drugs.

Virologic Resistance Testing
HCV RNA was extracted from samples obtained at baseline and at the time of virologic failure. Only samples with an HCV RNA level of $\geq 1000$ IU/mL underwent sequence or phenotype analysis in order to allow accurate assessment of amplification products. Therefore if the HCV RNA level at the time of virologic failure (VF) was $<1000$ IU/mL, the sample closest in time after the failure with an HCV RNA level $\geq 1000$ IU/mL was used.

The target genes were amplified by RT-PCR and then nested PCR using primers appropriate for subtype 1a or 1b sequences encoding NS3/4A protease, NS5A, and NS5B polymerase. The nested PCR amplification product was used as the template for population DNA sequencing. The DNA sequence from each baseline sample was translated into amino acid sequence and compared to the appropriate reference sequence (1a-H77 [NC004102] or 1b-Con1 [AJ238799]) in order to identify pre-existing resistance-associated variants. The DNA sequence from each post-baseline sample was translated into amino acid sequence and compared to the sequence from the corresponding baseline sample to identify resistance-associated amino acid variants that emerged as a result of treatment.

Phylogenetic Analysis of HCV Sequences from Patients Experiencing Virologic Failure
Methods. For patients experiencing virologic failure, the HCV NS3/4A, NS5A and NS5B gene sequences from baseline and virologic failure samples were phylogenetically analyzed to determine genetic relatedness between samples. Nucleotide sequences from each full-length genomic target were aligned using the MAFFT sequence alignment method, and phylogenetic trees were constructed using the neighbor-joining tree-building method with the HKY85 nucleotide substitution model. Reliability of the tree topology was examined using bootstrap analysis, and 1000 bootstrapping replicates were utilized to generate a consensus tree with a 50% threshold cutoff for each phylogenetic analysis. Nucleotide alignments and phylogenetic trees were generated using the Geneious and MEGA6 software packages.

Results. Phylogenetic analyses of HCV NS3/4A, NS5A, and NS5B sequences revealed that HCV re-infection likely occurred in 2 patients with post-treatment HCV recurrence in the TURQUOISE-I study; both patients were HCV treatment-naïve prior to TURQUOISE-I enrollment, had F0-1 fibrosis, had received 24 weeks of 3D + RBV.

Based on sequence and phylogenetic analyses, the post-treatment recurrence of HCV viremia observed in Patients 4 and 5 (main text Table 2) appears to have resulted from a new HCV infection with a different genotype 1a isolate than was present at baseline prior to treatment. The phylogenetic analyses of NS3/4A,
NS5A, and NS5B (eFigure 2) revealed a low level of genetic relatedness among sequences from Patient 4 or 5, indicated by a lack of phylogenetic clustering between the patient’s baseline and post-treatment samples. HCV sequences from Patients 4 and 5 sorted as distinct isolates, similar to other genotype 1a sequences used as references in the analysis. In contrast, for virologic failure Patients 2 and 3 the analysis revealed phylogenetic clustering with strong bootstrap support between HCV baseline and post-treatment sequences indicating a high degree of genetic relatedness.

In the sequence analysis of NS3/4A, NS5A, and NS5B, the nucleotide sequence identity between the baseline sample and the sample taken after HCV recurrence ranged between 89.3-93.8% for Patient 4 and 90.3-92.4% for Patient 5, similar to that usually observed between distinct HCV isolates within a genotype. In addition, the NS5A sequence from Patient 4 had a 7 amino acid insertion at position 260 at baseline (relative to the 1a-H77 reference strain) that was not present in the post-treatment sample. Treatment-emergent resistance-associated variants were also not observed in any of the targets for either Patient 4 or 5. In contrast, the nucleotide sequence identity between the baseline and failure samples for the three targets was 96.3-98.1% for Patient 2, and 99.2-99.5% for Patient 3, confirming high genetic relatedness between HCV sequences from each patient. Collectively, these data strongly suggest that while Patients 2 and 3 experienced virologic failure, both Patients 4 and 5 were re-infected with a new isolate of HCV genotype 1a that was distinct from the one present prior to treatment.
### eTable. Baseline Fibrosis Stage Scoring

<table>
<thead>
<tr>
<th>Baseline Fibrosis Stage, Metavir Equivalents</th>
<th>Liver Biopsy Metavir, Batts-Ludwig, Knodell, IASL, Scheuer, or Laennec Score</th>
<th>Liver Biopsy Ishak Score</th>
<th>FibroScan® (kPa)</th>
<th>FibroTest®</th>
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<tbody>
<tr>
<td>F0-F1</td>
<td>0 or 1</td>
<td>0, 1, or 2</td>
<td>&lt;8.8</td>
<td>≤0.48 with APRI ≤2</td>
</tr>
<tr>
<td>F2</td>
<td>2</td>
<td>3</td>
<td>≥8.8 to &lt;9.6</td>
<td>0.49 to 0.58 with APRI ≤2</td>
</tr>
<tr>
<td>F3</td>
<td>3</td>
<td>4</td>
<td>≥9.6 to &lt;12.5</td>
<td>0.59 to 0.72 with APRI ≤2</td>
</tr>
<tr>
<td>F4</td>
<td>4</td>
<td>5 or 6</td>
<td>≥12.5</td>
<td>≥0.73 with APRI &gt;2</td>
</tr>
</tbody>
</table>

Baseline fibrosis stage is defined for patients with liver biopsy scores, FibroScan scores, or FibroTest scores available. Fibrosis score were determined by a single score in patients with multiple scores available. If a biopsy score was present, it was used to categorize the patient, regardless of the FibroScan/FibroTest score. Similarly, if a FibroScan score was present along with a FibroTest score, then the FibroScan score was used to categorize the patient. If biopsy and FibroScan scores were not present and more than one FibroTest result was available, then the baseline FibroTest result (i.e., last non-missing FibroTest result on or before baseline) and concordant APRI result (i.e., last non-missing APRI result on or before baseline) was used to categorize the patient. APRI, aspartate aminotransferase to platelet ratio index.
**eFigure 1. TURQUOISE-I Part 1a Study Design.** TURQUOISE-I Part 1a is a phase 2, open-label trial of 12- or 24-week treatment with ombitasvir/paritaprevir/r and dasabuvir with ribavirin in HCV genotype 1/HIV-1 co-infected patients. Sustained virologic response was assessed 12 weeks after the end of treatment. 3D, ombitasvir/paritaprevir/ritonavir and dasabuvir; RBV, ribavirin; SVR12, sustained virologic response at post-treatment week 12.
eFigure 2. Phylogenetic Analysis of HCV Sequences at Baseline and at the Time of Virologic Failure for Patients in the TURQUOISE-I Study. Neighbor-joining phylogenetic trees are displayed for (A) NS3/4A, (B) NS5A and (C) NS5B. HCV patient isolates are represented by a colored square identifying the patient number, and labeled with the time point of collection for each sequence (PTW, post treatment week). Reference sequences are labeled by the subtype followed by the GenBank accession number. Bootstrap values ≥50 are listed at appropriate nodes, and the genetic distance scale bar indicates the number of nucleotide substitutions per site between sequences. The phylogenetic analyses provide evidence of HCV re-infection in Patients 4 and 5 based on genetic relatedness of samples taken at baseline and at the time of virologic failure.
eFigure 3. Mean Change from Baseline in CD4+ T-cell Count and Lymphocyte Count. Mean CD4+ T-cell count and lymphocyte count changes from baseline are plotted for patients in the 12-week 3D + ribavirin treatment group (A) or the 24-week 3D + ribavirin treatment group (B). Colored lines with square symbols denote CD4+ T-cell count (cells/mm$^3$) and are plotted on the left y-axis. Black dashed lines with circles denote lymphocyte count (x10$^9$/L) and are plotted on the right y-axis. I bars indicate ± standard deviation. 3D, ombitasvir/paritaprevir/ritonavir and dasabuvir; RBV, ribavirin.
eFigure 4. Mean Change from Baseline in CD4+ T-cell Percentage and White Blood Cell Count. Mean CD4+ T-cell percentage and white blood cell count changes from baseline are plotted for patients in the 12-week 3D + ribavirin treatment group (A) or the 24-week 3D + ribavirin treatment group (B). Colored lines with square symbols denote CD4+ T-cell percentage and are plotted on the left y-axis. Black dashed lines with circles denote white blood cell count (x10^9/L) and are plotted on the right y-axis. I bars indicate ± standard deviation. 3D, ombitasvir/paritaprevir/ritonavir and dasabuvir; RBV, ribavirin; WBC, white blood cell.