Towards optimal treatment for chronic hepatitis C infection
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Neopterin and ALT as markers of inflammation in chronic hepatitis C patients during administration of the HCV NS3•4A protease inhibitor telaprevir (VX-950) and/or peginterferon alfa 2a

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Submitted
Abstract

Background Neopterin is a marker of monocyte/macrophage activity. ALT is a marker of hepatocyte injury. We determined changes in neopterin and ALT levels, as markers of inflammation, in 2 ancillary studies during two phase 1b trials of Hepatitis C virus (HCV) NS3•4A protease inhibitor telaprevir (VX-950), with or without peginterferon alfa 2a (Peg-IFN).

Methods 54 chronic hepatitis C patients (genotype 1) received placebo or telaprevir, with or without Peg-IFN, for 14 days in 2 multiple-dose studies.

Results During administration of telaprevir, every patient demonstrated a >2-log decrease in HCV RNA. Mean neopterin and ALT levels decreased in all 4 telaprevir alone groups. In contrast, mean neopterin levels increased and ALT levels decreased in the Peg-IFN plus telaprevir and Peg-IFN plus placebo groups.

Conclusion These data suggest that treatment of chronic hepatitis C patients with an HCV NS3•4A protease inhibitor ameliorates inflammation. The increase in neopterin levels and decrease in ALT levels during administration of Peg-IFN with or without telaprevir is in accordance with earlier observations that IFN reduces hepatocyte injury but increases monocyte/macrophage activity. The IFN-mediated immunomodulatory effects appear to remain intact when IFN is combined with telaprevir.
**Introduction**

Neopterin (6-d-erythro-trihydroxypropylpteridine) is a pteridine derivative that is produced during the metabolism of guanosine triphosphate (GTP), and is a marker of inflammation. Neopterin is produced primarily by monocytes and macrophages upon activation by interferon (IFN) gamma or IFN alfa [1]. Neopterin levels are elevated in hepatitis C virus (HCV) infection [2-5], hepatitis A infection [6], hepatitis B infection [6], HIV-1 infection [7, 8], severe acute respiratory distress syndrome (SARS) [9], and a variety of other infectious and inflammatory diseases [7].

In advanced HIV-1 infection, neopterin levels are elevated and independently predict disease progression [8]. Neopterin levels decline during highly active antiretroviral therapy (HAART), but levels remain elevated compared with healthy controls [10-13]. Neopterin levels decline during treatment of SARS [9], or as disease activity naturally subsides in hepatitis A and B [6]. Administration of IFN alfa, IFN gamma, or TNF alfa induces an increase in neopterin in healthy volunteers [14-16].

The current treatment of choice for patients with chronic hepatitis C genotype 1 infection consists of administration of pegylated IFN alfa (PegIFN alfa) in combination with the nucleoside analog ribavirin for 48 weeks. In chronic hepatitis C patients, treatment with IFN alfa based regimens induces an increase in neopterin levels, irrespective of treatment outcome [5, 17-19].

Alanine aminotransferase (ALT) is an enzyme that is present in hepatocytes. ALT is released from damaged hepatocytes into the blood after hepatocellular injury or death. The HCV NS3•4A serine protease mediates proteolysis of the HCV polyprotein at the NS3/NS4A, NS4A/NS4B, NS4B/NS5A, and NS5A/NS5B junctions and is essential for HCV replication [20]. Telaprevir (VX-950) is a highly selective peptidomimetic inhibitor of the HCV NS3•4A serine protease that is currently under development for the treatment of chronic HCV infection.

We determined changes in neopterin as a marker of monocyte/macrophage activity, and ALT as a marker of hepatocyte injury, in 2 ancillary studies during two phase 1b trials of telaprevir with or without Peg-IFN alfa 2a, in chronic hepatitis C patients.

**Methods**

These were 2 ancillary studies to 2 placebo controlled phase 1b trials investigating the safety, pharmacokinetics and HCV RNA kinetics during administration of telaprevir (VX-950, Vertex Pharmaceuticals Incorporated, Cambridge, MA, USA), a specific inhibitor of HCV genotype 1 NS3•4A protease with or without PegIFN alfa 2a, in chronic hepatitis C patients [21, 22].

Clinical and demographic characteristics of the patients are summarized in table 1. Briefly, patients ranged in age from 21 to 64 years, 34 were men and 20 were women. All patients...
were infected with HCV genotype 1, with HCV RNA levels ≥ 100,000 IU/mL. All patients had compensated liver disease, and were HBsAg and anti-HIV negative.

Telaprevir or placebo was administered during 14 days in both studies. In study VX04-950-101 (hereafter referred to as the 101 study), patients were allocated to: 450 mg telaprevir q8h (n=10); 750 mg telaprevir q8h (n=8); 1250 mg q12h (n=10); or telaprevir matched placebo (n=6). In study VX05-950-103 (hereafter referred to as the 103 study), patients were allocated to: 750 mg telaprevir q8h (n=8); 750 mg telaprevir q8h plus 180 μg Peg-IFN alfa 2a (PEGASYS®, Hoffman-La Roche, Basel, Switzerland) on days 1 and 8 (n=8); telaprevir matched placebo plus 180 μg Peg-IFN alfa 2a on days 1 and 8 (n=4). Telaprevir dosing started at day 2 in both studies. The first telaprevir dose in the 103 study was a 1250 mg loading dose.

Serum neopterin levels were measured by a quantitative competitive ELISA (ELItest® Neopterin, Brahms, Hennigsdorf, Germany), according to the manufacturer's instructions, at pretreatment, day 8 and day 15. The expected plasma level of neopterin in healthy individuals is between 3.1 and 7.7 nmol/L. The minimum level of detection is 2 nmol/L.

ALT levels were assessed by a routine technique at frequent intervals during the study. HCV RNA was assessed at frequent intervals during the study by real-time PCR (COBAS® TaqMan HCV Test with HPS extraction; linear dynamic range 3.0 x 10^1 to 2.0 x 10^8 HCV RNA IU/mL; lower limit of detection 10 HCV RNA IU/mL; Roche Diagnostics, Branchburg, NJ, USA), according to the manufacturer's instructions.

Statistical analysis was performed using SPSS version 12.0.2 for Windows (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 4.0b for Macintosh (GraphPad Software, San Diego, CA, USA). Values are given as means ± SD. We used the Wilcoxon signed rank test to compare neopterin, log HCV RNA and ALT levels before and during treatment. A p-value <0.05 was considered to represent a significant difference. The scores of a treatment group at any point in time were discarded if the number of observations was 50% or less.

Results

Fifty-seven patients were enrolled in the 2 studies. Three patients dropped out after randomization but before the first dose of study drug. Fifty-four patients completed the studies as planned. Clinical data and safety data for both studies have been reported previously [21, 22].

Neopterin

Baseline neopterin levels were elevated in 38/54 patients (mean 9.89 nmol/L; ULN 7.7 nmol/L). Mean neopterin levels decreased in all dose groups during administration of telaprevir alone, but increased during administration of Peg-IFN alfa 2a plus telaprevir or placebo (Figure 1, Table 2). Compared to baseline, mean neopterin levels were significantly lower during administration of telaprevir alone in the 750 mg q8h [101 study] group at day 15, and in the 750 mg q8h [103 study] group at day 8 (Wilcoxon signed rank test, p<0.05). Compared to baseline, mean neopterin levels were significantly higher during administration of Peg-
HCV protease inhibitor ameliorates inflammation

IFN alfa 2a plus telaprevir at day 8 and day 15 (Wilcoxon signed rank test, p<0.05). After day 8, mean log HCV RNA increased in the 450 mg q8h telaprevir alone dose group, but mean neopterin remained low (Figure 1, Table 2). In the 1250 mg q12h telaprevir alone dose group only 5 samples were available at day 15, this time point was excluded from the analysis.

ALT
Mean ALT levels were elevated at baseline and decreased during dosing in all groups (Figure 1, Table 2). Compared to baseline, mean ALT levels were significantly lower during treatment
### Table 1. Patient Baseline Characteristics

<table>
<thead>
<tr>
<th>Study</th>
<th>Telaprevir (mg)</th>
<th>PegIFN alfa (μg qw)</th>
<th>HCV RNA, log10 IU/mL</th>
<th>Sex, n (%)</th>
<th>Age, years</th>
<th>Sex, n (%)</th>
<th>Age, years</th>
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<td></td>
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<tr>
<td></td>
<td>750 mg q8h</td>
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<td>47–64</td>
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<td>PegIFN 2a 180 μg qw</td>
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<td>PegIFN 2a 180 μg qw</td>
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<td>n = 8</td>
<td>n = 10</td>
<td>n = 8</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>103</td>
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### Table 2. Mean neopterin, ALT and HCV RNA at baseline and during dosing

<table>
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<tr>
<th>Study</th>
<th>Telaprevir (mg)</th>
<th>PegIFN alfa (μg qw)</th>
<th>HCV RNA, log10 IU/mL</th>
<th>Neopterin</th>
<th>ALT</th>
<th>Log HCV RNA</th>
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<td>day 15</td>
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<td>73 ± 39</td>
<td>73 ± 39</td>
<td>73 ± 39</td>
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<tr>
<td></td>
<td>1250 mg q12h</td>
<td>PegIFN 2a 180 μg qw</td>
<td></td>
<td>41 ± 32**</td>
<td>38 ± 32**</td>
<td>41 ± 32**</td>
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<td>n = 10</td>
<td>n = 8</td>
<td>n = 10</td>
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<tr>
<td></td>
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<td>PegIFN 2a 180 μg qw + Placebo</td>
<td>n = 6</td>
<td>n = 8</td>
<td>n = 4</td>
</tr>
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</table>

**NOTE.** Data are presented as means ± SD. Neopterin in nmol/L (upper limit of normal, ULN 7.7nmol/L), ALT in U/L (ULN 34 U/L for females, ULN 43 U/L for males), log HCV RNA in IU/mL. Values in bold typeface indicate a significantly different value compared to baseline (*p<0.05, **p<0.005, Wilcoxon signed rank test). HCV, hepatitis C virus; q8h, every 8 hours; q12h, every 12 hours; qw, every week; SD, standard deviation.
in all groups, except the placebo group and the Peg-IFN alfa 2a plus placebo group (Wilcoxon signed rank test, p<0.05). The decline in mean ALT levels was sustained during the second week of dosing in the telaprevir alone groups and the Peg-IFN alfa 2a plus telaprevir group. In the 4 telaprevir alone groups, 26 out of 36 patients had an elevated baseline ALT level; ALT levels normalized in 21 out of these 26 patients, 18 out of these 26 patients had normal ALT levels at day 9. After day 8, mean log HCV RNA increased in the 450 mg q8h dose and 1250 mg q12h dose groups, but mean ALT continued to decrease (Figure 1, Table 2). Six out of 12 patients in the Peg-IFN alfa 2a plus telaprevir or placebo groups had an elevated baseline ALT level; ALT levels normalized in 2 out of these 6 patients in the Peg-IFN alfa 2a plus telaprevir or placebo groups (Figure 1, Table 2).

HCV RNA
During administration of telaprevir, every patient demonstrated a >2-log\textsubscript{10} drop in HCV RNA in all dose groups. In the 750 mg q8h telaprevir plus PegIFN alfa 2a group, mean HCV RNA dropped 3.8 log\textsubscript{10} at day 4, and 5.5 log\textsubscript{10} at day 15 (Figure 1, Table 2). In the 750 mg q8h telaprevir alone group in the 101 study, mean HCV RNA dropped 3.6 log\textsubscript{10} at day 4, and 4.2 log\textsubscript{10} at day 15 (Figure 1, Table 2). In the 450 mg q8h, 1250 mg q12h and the 750 mg q8h (103 study) telaprevir alone groups, maximal effect was seen at day 4 to day 8 followed by an increase in mean viral load between day 8 and day 15 (Figure 1, Table 2). In the PegIFN alfa 2a plus placebo group, mean HCV RNA dropped 1.2 log\textsubscript{10} at day 15 (Figure 1, Table 2). Compared to baseline, mean log HCV RNA levels were significantly lower in all treatment groups during administration of telaprevir with or without Peg-IFN alfa 2a (Wilcoxon signed rank test, p<0.05).

Discussion
In this study we have shown that serum neopterin levels and ALT levels decrease concurrently with HCV RNA during administration of an inhibitor of HCV NS3•4A protease in chronic hepatitis C patients, indicating a reduction in both monocyte/macrophage activity and hepatocyte injury. In contrast, neopterin levels increase while ALT and HCV RNA levels decrease during administration of an inhibitor of HCV NS3•4A protease combined with Peg-IFN alfa 2a.
Mean neopterin levels were elevated at baseline in all groups. Mean neopterin levels declined to normal and near normal levels in the telaprevir alone groups. However, mean neopterin levels increased in the PegIFN alfa 2a plus telaprevir or placebo groups. Mean neopterin levels did not change in patients receiving placebo alone. The pattern of ALT decline was comparable to the pattern of HCV RNA decline. Mean ALT levels were elevated at baseline in all groups and had decreased to normal levels in the 750 mg q8h dose group [101 study] at day 7, in the 1250 mg q12h and the Peg-IFN alfa 2a plus telaprevir dose groups at day 9, in the 450 mg q8h dose group at day 11, and in the 750 mg q8h dose group [103 study] at day 12.
Taken together, these results demonstrate that inhibition of HCV replication by telaprevir with or without Peg-IFN alfa 2a ameliorates HCV-induced liver inflammation. After day 8, an increase in mean HCV RNA levels was seen in the 450 mg q8h and 1250 mg q12h telaprevir alone dose groups but mean neopterin levels continued to decrease in the 450 mg q8h group, and mean ALT levels continued to decrease in the 450 mg q8h and 1250 mg q12h dose groups. This suggests that inflammation and HCV levels are to some extent independent, and this is an argument in favour of the theory that HCV itself is not a direct cytopathic virus [23-25]. Moreover, although HCV RNA levels were increasing after day 8, they were lower than before administration of telaprevir. This suggests that minor reductions in HCV RNA levels may result in a decrease in inflammation. The increase in HCV RNA levels in the 450 mg q8h and 1250 mg q12h dose groups after day 8 appears to be due to selection of HCV variants with decreased sensitivity to telaprevir [26-28]; the results of sequence analysis are reported in detail in separate manuscripts [29, 30]. Neopterin is produced by monocytes/macrophages. The decrease in neopterin levels upon administration of telaprevir alone indicates a decrease of monocyte/macrophage activation. This is in contrast to the monocyte/macrophage activation and the increase in neopterin levels during treatment with IFN alfa with or without telaprevir. IFN alfa is a pleiotropic cytokine with direct antiviral, immunomodulatory, and both pro- and anti-inflammatory effects [15, 31]. The increase in neopterin during IFN alfa based therapy is probably due to the pro-inflammatory effects of IFN alfa. During ribavirin monotherapy ALT decreases but not HCV RNA or neopterin [18]. These differences in ALT, neopterin and HCV RNA patterns suggest that HCV induces a multifaceted inflammatory response. Similar decreases in neopterin levels and reduction of monocyte activation are seen during treatment with HIV-1 specific protease inhibitors (HAART) [10-13]. These results confirm that these protease inhibitors are direct inhibitors of viral replication that act without help of the immune response. Determination of neopterin levels next to other markers of inflammation (e.g., ALT, cytokines, chemokines) can be applied to assess the degree of monocyte/macrophage activation and activation of other cell types involved in inflammation (as has been demonstrated by Biezeveld et al. [32] in Kawasaki disease). The decline in neopterin and ALT during administration of telaprevir alone indicates a rapid decrease in both monocyte/macrophage activity and hepatocyte injury during administration of an HCV specific protease inhibitor. Recent studies suggest that inhibition of HCV NS3•4A serine protease may lead to a restoration of intracellular host defenses [33]. This may be a contributing factor in the decline in HCV RNA in patients during dosing with telaprevir. In summary, these results suggest a significant reduction in HCV-induced inflammation, as demonstrated by a decrease in both neopterin and ALT levels, and HCV RNA in chronic hepatitis C patients during dosing with an HCV NS3•4A serine protease inhibitor.
HCV protease inhibitor ameliorates inflammation

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