Importance of Minimal Residual Viremia for Relapse Prediction in Patients With Chronic Hepatitis C Genotype 1 Infection

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This study demonstrates that a more precise prediction of the individual relapse risk in chronic hepatitis C virus genotype 1 infection can be obtained by kinetics of minimal residual viremia at weeks 4, 8, and 12 in combination with levels of baseline viremia. These data may also help to further individualize new protease inhibitor–based triple therapy regimens.

The success of individually tailored treatment regimens depends on the prevention of viral relapse in patients with chronic hepatitis C virus (HCV) infection. Persistence of minimal residual viremia must be considered as one important factor that accounts for relapse occurrence. In comparison of polymerase chain reaction (PCR; detection limit, 100 IU/L) with the more sensitive transcription-mediated amplification (TMA) technology, persisting HCV RNA levels were observed in 37%, 27%, and 20% of PCR-negative samples at week 12, 20, and 24 of therapy, respectively [1]. Furthermore, stratification according to baseline viremia [2] and inclusion of week 8 response in combination with HCV RNA assessments at weeks 4 and 12 [3] are useful tools for improving relapse prediction.

We conducted a multicenter randomized trial to determine whether treatment duration of 18–48 weeks is as efficient as a fixed treatment period of 48 weeks [4]. To further define the risk of relapse we analyzed 4 criteria: (1) baseline viral load; (2) virologic response at week 4, 8, and 12; (3) branched DNA (bDNA) and TMA assay; and (4) influence of the major interleukin 28B (interferon, lambda 3) gene (IL28B) single-nucleotide polymorphisms (SNPs).

These observations will also be relevant for the further development of the new treatment standard with triple regimens containing directly antiviral agents in treatment-naïve patients with chronic hepatitis C genotype 1 infection. A rapid and profound decrease of viral load within the first 4–8 weeks of triple therapy will be necessary to allow response-guided adaptation of treatment duration and minimize the risk for viral resistance against protease inhibitors [5–8].

Methods

The present analysis included 225 patients with chronic hepatitis C genotype 1 infection who were treated with peginterferon alfa-2b (1.5 μg/kg/week subcutaneously [s.c.]) plus weight-based ribavirin (800–1400 mg/day) for 48 weeks within the prospective individualized treatment strategy (INDIV-1 study) [4]. HCV RNA levels were quantified at baseline, weekly up to week 8, at weeks 12, 24, and 48, and at the end of follow-up by bDNA assay (Versant 3.0; formerly Bayer Diagnostics, Leverkusen, Germany; currently provided by Siemens, Eschborn, Germany; detection limit, 615 IU/mL). In addition, a more sensitive TMA assay (Versant qualitative HCV RNA; formerly Bayer Diagnostics, Leverkusen, Germany; currently provided by Siemens, Eschborn, Germany; detection limit, 5.3 IU/mL) was performed only for those patients who had HCV RNA levels of <1000 IU/mL by the bDNA test. Virologic relapse was defined as reappearance of HCV RNA during follow-up after stopping therapy in patients with an end-of-treatment virologic response.

Calculation of relapse rates was based on a per protocol analysis of individuals with end-of-treatment response and available HCV RNA results at weeks 4, 8, and 12 of treatment and after 24 weeks of follow-up, respectively. In addition, results were stratified according to baseline viral load (HCV RNA cutoff, 800 000 IU/mL).
Four different virologic response patterns were defined at weeks 4, 8, and 12: (1) complete virologic response (negative bDNA and TMA assays), (2) partial virologic response with minimal residual viremia (negative bDNA assay, positive TMA assay), (3) patients with a HCV RNA level decrease of ≥2 log measured by bDNA assay, and (4) patients with a HCV RNA level decrease of ≤2 log measured by bDNA assay.

The SNP rs12979860, located ~3 kilobases upstream of the IL28B gene, was selected for genotyping [9] and tested with a PCR-based endpoint genotyping assay using custom designed rs12979860 primers and hydrolysis probes (Applied Biosystems; forward primer, 5’-GCTGTCGTAATGACCA-3’; reverse primer, 5’-GCCTGGCGAACACACTTG-3’; and 5’-FAM-CTGGTTCGCGCCTTC-BHQ-3’). Results were defined as CC, CT, or TT (IL28B genotype), respectively.

Relapse rates were compared by χ² tests and the Fisher exact test where appropriate. The level of significance was set at α = .05. All statistical analyses were performed using PASW Statistics (version 18.0.0; SPSS).

All patients provided written informed consent. The study was approved by the local ethics committees according to the Declaration of Helsinki and the International Conference on Harmonization and Committee for Proprietary Medicinal Products Good Clinical Practice.

**Results**

At week 4, a quantitative HCV RNA response (negative bDNA assay) was observed in only 34% of patients, whereas by week 12 already 75.8% of patients were bDNA negative. The number of nonresponders (<2 log HCV RNA level decrease from baseline), decreased from 42.8% at week 4 to 14.2% at week 12.

A minimal residual viremia (negative bDNA assay, positive TMA assay) could be detected in 45 (20.3%) of 222 patients at week 4, in 57 (26.6%) of 214 patients at week 8, and in 42 (19.9%) of 211 patients at week 12. The relative percentage of bDNA responder patients defined by persistence of minimal residual viremia decreased progressively during therapy (59% at week 4, 42% at week 8, and 26% at week 12, respectively).

Relapse rates depended on viral kinetics and differed according to different virologic response patterns. Minimal residual viremia was associated with a relapse risk of 20.6% (6 of 29 patients) at week 4, 31.8% (14 of 44 patients) at week 8, and 55.2% (16 of 29 patients) at week 12, whereas in patients with negative TMA assays, the relapse rates were only 0% (0 of 29 patients), 4.2% (3 of 71 patients), and 8.9% (9 of 101 patients) at the same 3 time points.

We also evaluated relapse rates in complete responders (bDNA and TMA negative) as well as the period necessary to become TMA negative (Figure 1). Virologic relapse was not observed in any of the patients with low baseline viremia (≤800 000 IU/mL) and TMA response within the first 12 weeks of therapy. In contrast, in cases with high baseline viremia, the percentage of individuals with virologic relapse was 0% (0 of 3 patients), 15.8% (3 of 19 patients), and 37.5% (6 of 16 patients) if HCV RNA first became undetectable at week 4, 8, or 12, respectively (P < .0005).

Relapse rates in TMA-negative patients were independent of IL28B genotype. In addition, relapse rates did not significantly differ between cases with low or high baseline viral load. In multiple regression analyses, only high baseline viremia, but not IL28B genotype, was predictive of viral relapse after therapy (P = .016; data not shown).

**Discussion**

We showed that (1) a significant percentage of patients who achieve undetectable HCV RNA levels by quantitative assays (bDNA) still have detectable viremia by use of highly sensitive assays (TMA) and that (2) these patients have an increased risk for relapse. The risk for viral relapse was associated with the duration of minimal residual viremia and increased from 20.6% at week 4 to 55.2% at week 12. During the same period, the likelihood of residual HCV RNA decreased from 59% to 26%. Thus, our study links the presence of minimal residual viremia to the risk of suffering a relapse.

Prediction of relapse rates was further improved by including baseline HCV RNA level and viral kinetics at week 8. The subgroup of patients with baseline viremia of ≥800 000 IU/mL and an initial late TMA response at week 12 displayed the highest relapse risk. In contrast, none of the patients with HCV RNA level of ≤800 000 IU/mL at baseline and a negative TMA assay at week 8 relapsed.

In our proof-of-concept INDIV-1 trial, individually tailored treatment durations were not superior to a standard therapeutic regimen because therapeutic decisions were based on the bDNA assay and irrespective of baseline viral load [4]. It is tempting to speculate that sustained virologic response rates of the individualized regimens would have been equivalent to the fixed treatment period of 48 weeks if therapy had been adjusted to TMA results. This hypothesis was proven successfully in the subsequent INDIV-2 study [10].

Prior to the introduction of real-time PCR-based techniques such as the TMA-assay used by us or the Roche Cobas Ampliprep/Cobas-Taqman (CAP-CTM) HCV test with HCV RNA detection limits of <15 IU/mL, the qualitative Cobas Amplicor assay with a lower detection limit of 50 IU/mL was the one most widely used in clinical practice. A comparison between CAP-CTM and Cobas Amplicor revealed that treatment individualization can be reliably performed on the basis of both methods [11]. Because HCV RNA was not quantified with the Cobas Amplicor assay in our study, the effect of a minimal residual viremia between 5.3 and 50 IU/mL on relapse rates has yet to be determined.
What is the significance of our results for new standard triple therapy regimens including HCV protease inhibitors? So far, response-guided therapies rely on HCV RNA kinetics at weeks 4 and 8 to define landmarks for shortened treatment duration or futility rules \[5, 8\]. In the future, all available parameters such as baseline viral load, early viral kinetics, \textit{IL28B} genotype, and histological staging have to be included in treatment algorithms to further individualize treatment strategies and minimize the risk of viral resistance \[5–8\]. Only highly sensitive HCV RNA assays should be used to adequately monitor viral kinetics and predict the risk of relapse. If even interferon-free treatment regimens do eventually become available, an extension of the current follow-up period of 24 weeks after therapy to rule out viral relapse should be considered in order to ensure that late relapers are not missed.

**Notes**

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**References**