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Reference


DOI: 10.1371/journal.pone.0017232
PMID: 21390311
Response Prediction in Chronic Hepatitis C by Assessment of IP-10 and IL28B-Related Single Nucleotide Polymorphisms

Martin Lagging¹*, Galia Askarieh¹, Francesco Negro², Stephanie Bibert³, Jonas Söderholm¹, Johan Westin¹, Magnus Lindh¹, Ana Romero¹, Gabriele Missale⁴, Carlo Ferrari⁴, Avidan U. Neumann⁵, Jean-Michel Pawlotsky⁶, Bart L. Haagmans⁷, Stefan Zeuzem⁸, Pierre-Yves Bochud³*, Kristoffer Hellstrand¹*, for the DITTO-HCV Study Group

1 Department of Infectious Diseases/Virology, University of Gothenburg, Gothenburg, Sweden, 2 University Hospital of Geneva, Geneva, Switzerland, 3 Infectious Diseases Service, University Hospital and University of Lausanne, Lausanne, Switzerland, 4 Azienda Ospedaliera di Parma, Parma, Italy, 5 Bar-Ilan University, Ramat-Gan, Israel, 6 Hôpital Henri Mondor - Université Paris XII, Créteil, France, 7 Department of Virology, Erasmus MC, Rotterdam, The Netherlands, 8 Department of Medicine I, J. W. Goethe University Hospital, Frankfurt, Germany

Abstract

Background: High baseline levels of IP-10 predict a slower first phase decline in HCV RNA and a poor outcome following interferon/ribavirin therapy in patients with chronic hepatitis C. Several recent studies report that single nucleotide polymorphisms (SNPs) adjacent to IL28B predict spontaneous resolution of HCV infection and outcome of treatment among HCV genotype 1 infected patients.

Methods and Findings: In the present study, we correlated the occurrence of variants at three such SNPs (rs12979860, rs12980275, and rs8099917) with pretreatment plasma IP-10 and HCV RNA throughout therapy within a phase III treatment trial (HCV-DITTO) involving 253 Caucasian patients. The favorable SNP variants (CC, AA, and TT, respectively) were associated with lower baseline IP-10 (P = 0.02, P = 0.01, P = 0.04) and were less common among HCV genotype 1 infected patients than genotype 2/3 (P < 0.0001, P < 0.0001, and P = 0.01). Patients carrying favorable SNP genotypes had higher baseline viral load than those carrying unfavorable variants (P = 0.0013, P = 0.029, P = 0.0004 respectively). Among HCV genotype 1 infected carriers of the favorable C, A, or T alleles, IP-10 below 150 pg/mL significantly predicted a more pronounced reduction of HCV RNA from day 0 to 4 (first phase decline), which translated into increased rates of RVR (62%, 53%, and 39%) and SVR (85%, 76%, and 75% respectively) among homozygous carriers with baseline IP-10 below 150 pg/mL. In multivariate analyses of genotype 1-infected patients, baseline IP-10 and C genotype at rs12979860 independently predicted the first phase viral decline and RVR, which in turn independently predicted SVR.

Conclusions: Concomitant assessment of pretreatment IP-10 and IL28B-related SNPs augments the prediction of the first phase decline in HCV RNA, RVR, and final therapeutic outcome.

Introduction

Hepatitis C virus (HCV) infects 170 million people worldwide [1] and is a leading cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma [2]. Treatment with pegylated interferon-α (peg-IFN) and ribavirin results in a sustained viral response (SVR) in approximately 50% of patients infected with HCV of genotype 1 and 80% of those with HCV genotypes 2 or 3 [3,4,5]. Recently, several genome-wide association studies have revealed that single nucleotide polymorphisms (SNPs) in the 19q13 region, in close proximity to three genes (IL28A, IL28B, and IL29) encoding cytokines of the IFN-λ family, predict spontaneous clearance of HCV infection [6,7] as well as SVR following peg-IFN/ribavirin therapy among patients infected with HCV genotype 1 [6,8,9,10]. Three of these SNPs are reportedly highly predictive of a favorable treatment response among HCV genotype 1 infected patients: n12979860 [7,8], rs12980275 [10], and rs8099917 [6,9,10], with a strong linkage disequilibrium noted between n12979860 and rs8099917 [6]. Aside from differences
in the assays utilized, SVR was the defined endpoint in the genome-wide association study (GWAS) identifying rs12979860 [8], whereas null responsiveness to interferon was used in the analysis that endorsed rs12980275 and rs8099917 [10].

The C allele at rs12979860 also is associated with higher baseline viral load [8,11], which otherwise is an established negative predictor of response to peg-IFN/ribavirin therapy [3,4,5]. Similarly counterintuitive is the report that a C allele at rs12979860 is more common in Caucasians with HCV genotype 2 and 3 infection than in genotype 1 infected or in HCV uninfected individuals [11,12]. In addition, two studies reported that homozygous carriage of GG at rs8099917 was associated with slightly lower PBMC mRNA expression of IL28 in 49 and 20 individuals, respectively [9,10], although another study reported no difference in IL28B mRNA expression when stratified regarding rs12979860 genotype in PBMC from 80 individuals [8]. In line with this latter finding, G allele carriage at rs8099917 has been reported to be associated with elevated intrahepatic mRNA expression of a panel of 37 interferon-stimulated genes (ISGs) but not IL28B in 91 HCV infected patients [13].

Interferon-gamma inducible protein 10 kDa (IP-10 or CXCL10) is a chemotactic CXC chemokine of 77 amino acids in its mature form [14,15]. IP-10 targets the CXCR3 receptor but, unlike other CXC chemokines, lacks chemotactic activity for neutrophils and instead attracts T lymphocytes, NK cells, and monocytes to sites of infection [15,16,17]. IP-10 is produced by a variety of cells, including hepatocytes, and levels of IP-10 at onset of therapy are reportedly elevated in patients infected with HCV of genotypes 1 or 4 who do not achieve SVR [18]. In difficult-to-treat genotype 1 infected HCV patients, cut-off levels of IP-10 in plasma of 150 pg/mL have yielded positive and negative predictive values for SVR of 71% and 100%, respectively [19]. IP-10 in plasma is mirrored by intrahepatic IP-10 mRNA and strongly predicts the HCV RNA decline during the first 48 weeks ("first phase decline") during interferon/ribavirin therapy for all HCV genotypes [20].

The impact of IP-10 on the elimination of HCV RNA during therapy in the setting of IL28B genetic variants is not known. We therefore assessed plasma IP-10 in relation to genetic variants at three major IL28B SNPs (rs12979860, rs12980275, and rs8099917) in patients chronically infected with HCV genotypes 1-4. Our results demonstrate a significant association between IL28B genetic variants and IP-10 in plasma and imply that combined assessment of these predictive factors may improve prognostication of the rate of first phase elimination of HCV RNA, as well as achieving a rapid virological response (RVR) and SVR.

### Methods

#### Ethical Aspects

The treatment study conformed to the guidelines of the 1975 Declaration of Helsinki and was approved by ethical committees at each center (Medicinska fakultetens forskningsetikkomité, Goteborgs Universitet, Gothenburg, Sweden, CPE-ILE-De-France IX, CHU Henri Mondor, Creteil, France, Comitato Etno Indipendente (IRB/IEC) dell’Azienda Ospedaliera di Parma, Parma, Italy, Comite d’Ethique du department de Medicine, Hopitaux Universitaires de Genève, Geneva, Switzerland, the Helsinki committee of the Kaplan Medical Center, Rehovot, Israel, the Ethics Committee of Hospital General Vall d’Hebron, Barcelona, Spain, the Ethics Committee of Aristotle University of Thessaloniki, Thessaloniki, Greece, the Ethics Committee of Klinikum der Johann Wolfgang Goethe-Universitat, Frankfurt, Germany, the Ethics Committee of University Hospital Rotterdam Dijkzigt, Rotterdam, Netherlands). Written informed consent was obtained from each patient included in this study.

#### Patients

Between February 2001 and November 2003, 270 patients (180 men and 90 women) were recruited in a phase III, open-label, randomized, multicenter trial conducted by the DITTO-HCV study group at 9 centers in France, Germany, Greece, Israel, Italy, Netherlands, Spain, Sweden, and Switzerland, as previously described [8].

### Table 1. Baseline Characteristics with Patients Grouped According to IL28B Genetic Variations.

<table>
<thead>
<tr>
<th></th>
<th>rs12979860 (n = 93)</th>
<th>rs12980275 (n = 123)</th>
<th>rs8099917 (n = 153)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>41.6 (10.1)</td>
<td>41.9 (9.5)</td>
<td>41.9 (11.4)</td>
</tr>
<tr>
<td>Gender</td>
<td>64/29</td>
<td>77/46</td>
<td>78/37</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.1 (3.6)</td>
<td>25.0 (3.5)</td>
<td>25.0 (3.5)</td>
</tr>
<tr>
<td>HCV-RNA level (log10 IU/mL)</td>
<td>6.3 (0.8)</td>
<td>6.1 (0.7)</td>
<td>6.0 (0.6)</td>
</tr>
<tr>
<td>HCV Genotype</td>
<td>44/13/33/3</td>
<td>33/6/15/5</td>
<td>33/15/5/3</td>
</tr>
<tr>
<td>Fibrosis Stage ( Ishak 0/1/2/3/4/5/6)</td>
<td>3/18/27/5</td>
<td>7/35/27/5</td>
<td>4/22/28/5</td>
</tr>
<tr>
<td>Steatosis Grade (0/1/2/3)</td>
<td>3/13/12/5</td>
<td>14/1</td>
<td>13/12/1</td>
</tr>
</tbody>
</table>

*Data presented as mean (SD) or n.

Chi-squared test.
reported [21]. All patients were adults, had compensated liver disease, were treatment naïve for hepatitis C, and fulfilled the following inclusion criteria: a positive test for anti-HCV antibody, an HCV RNA level greater than 1000 IU/mL, and two serum alanine aminotransferase values above the upper limit of normal within 6 months of treatment initiation. Two hundred and fifty three Caucasian patients had samples available for IL28B analysis (baseline characteristics shown in Table 1), and 252 of these patients had pretreatment plasma available for evaluation of IP-10.

**Treatment**

All patients in the DITTO-HCV trial were treated for 6 weeks with 180 μg pegylated interferon-α2a sc once weekly (Pegasys, F. Hoffmann-LaRoche, Basel, Switzerland) and ribavirin orally twice daily (Copegus, F. Hoffmann-LaRoche) at a total daily dose of 1,000 mg for patients weighing less than 75 kg and 1,200 mg daily for above 75 kg. Thereafter, patients were randomized 1:1 based on their viral kinetic classification to receive individualized therapy or to continue on standard combination therapy for a total of 48 weeks. There were no major differences in treatment outcome for patients receiving individualized or standard therapy [21].

**HCV Genotyping**

Genotyping of HCV was performed using INNO-LiPA HCV II (Innogenetics N.V., Ghent, Belgium).

![Figure 1. Frequency distribution of IL28B variants in relation to HCV genotypes 1-3.](doi:10.1371/journal.pone.0017232.g001)
HCV RNA Quantification
HCV RNA was determined by RT-PCR using Cobas Amplicor HCV Monitor version 2.0 (Roche Diagnostics, Branchburg, NJ), and quantified on days 0, 1, 4, 7, 8, 15, 22, 29, at end of treatment, and 24 months after the completion of treatment.

Classification of Treatment Outcome
Patients were classified as having achieved RVR if HCV RNA was undetectable (≤50 IU/mL) in plasma on treatment day 29, and were classified as having SVR if HCV RNA was undetectable in plasma 24 weeks after the completion of therapy.

Liver Biopsies
Liver biopsies were obtained from patients in the DITTO-HCV trial within 12 months prior to inclusion in the study, and liver biopsy samples were processed for both histological evaluation (≥1.5 cm) and for RNA analysis (≥1 cm). Only biopsies with a length exceeding 1.5 cm and containing more than 6 portal tracts were evaluated. In total, liver biopsies from 228 infected patients in the DITTO-HCV trial were retrieved and evaluated. For each biopsy, a hematoxylin-eosin stain and a Sirius Red stain were centrally staged and graded by two independent observers experienced in pseudo-numerical scoring of liver biopsies in a blinded fashion according to the Ishak protocol [22]. Equivocal issues were debated after the independent scores were noted, and a consensus score was obtained. In addition, steatosis was graded as follows: absent = 0, less than 30% of hepatocytes involved = 1, 30–70% of hepatocytes involved = 2, and more than 70% of hepatocytes involved = 3 [23].

IP-10 Quantification in Plasma
Quantification of IP-10 was performed using Quantikine (R&D SYSTEMS Minneapolis, MN), a solid-phase ELISA, on plasma samples obtained during the week prior to the start of therapy. All samples were stored at −70°C until assayed.

DNA extraction
DNA from peripheral blood mononuclear cells was isolated using the QIAamp DNA mini kit (Qiagen) and quantitated on the NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific).

IL28-B genotyping
DNA samples from patients and controls were genotyped for the IL28B rs8099917, rs12979860 and rs12980275 polymorphism with TaqMan SNP genotyping assays (Applied Biosystems Inc, Foster City, CA), using the ABI 7500 Fast real time thermocycler, according to manufacturers recommended protocols. TaqMan probes and primers were designed and synthesized by Applied Biosystems Inc. Automated allele calling was performed using SDS software from Applied Biosystems Inc. Positive and negative controls were used in each genotyping assay. The primers and probes utilized were:

1. NCBI dbSNP ID rs8099917 Applied Biosystems (AB) reference: C_11710096_10
2. NCBI dbSNP ID rs12979860 Forward primer: TGTACTG-AACCAGGGAGCTTC, Reverse primer: GCGGGGAGT-GCAATTCACC, Vic probe: TGGTTCGGCGCTTTC, Fam probe: TGTTTCAGCTTTC
3. NCBI dbSNP ID rs12980275 Forward primer: GTGCTG-AGAGAAGTCAAATTCC, Reverse primer: CCCTCACCAGGAAATATT, Vic probe: AGACAGCTCTGTGGTTTC, Fam probe: AGACGTCCGTTTCTA

Statistical Methods
Individual characteristics between groups were evaluated using the Wilcoxon-Mann-Whitney U-test, Kruskal-Wallis test and Chi squared ($\chi^2$) test, and Spearman's rank correlation coefficient $r$, test was utilized to evaluate relationships between variables. All abovementioned statistical analyses were performed using StatView for Macintosh (Version 5.0, SAS Institute Inc., Cary, NC, USA). After univariate analyses, multivariate analyses were performed among the HCV genotype 1 infected patients using...
Stata (version 9.1, StataCorp, College Station, TX, USA). For multivariate models, all variables associated with the endpoint (P<0.2) were entered, with age and gender forced into the model. Because of strong linkage disequilibrium noted between rs12979860 and rs12980275 ($R^2 = 0.86$ assuming Hardy-Weinberg equilibrium, as compared to $R^2 = 0.42$ for rs12979860 and rs8099917 as well as $R^2 = 0.41$ for rs12980275 and rs8099917) and because rs8099917 was not significantly associated with SVR, only SNP variants for rs12979860 were included in the multivariate analyses. SNPs were tested using three models assuming one of the following modes of inheritance: dominant (comparing presence of one or two copies of the minor allele versus none), recessive (comparing presence of two copies of the minor allele versus none or one copy), and additive (none, one or two copies of the minor allele were coded 0, 1 and 2, respectively, assuming greater effect with increased copy number of the minor allele).
Allele). Linkage disequilibrium was calculated using the pwld software implemented in Stata. All reported p-values are two-sided, and p-values <0.05 were considered significant.

**Results**

A strong association was noted between the distribution of HCV genotypes and IL28B SNP variants (P<0.0001 for rs12979860 and rs12980275, and P=0.01 for rs8099917, Chi squared test; Table 1) with the favorable CC at rs12979860, AA at rs12980275, and TT at rs8099917 being significantly more common in patients with HCV genotype 2 or 3 infection than genotype 1 (Figure 1). The 11 patients infected with HCV genotype 4 had a similar distribution of IL28B variants as patients infected with genotype 1. Patients who were homozygous for the favorable SNP genotypes had higher baseline viral load (mean 6.3 log10 IU/mL; Table 1); heterozygous patients had intermediate (mean 6.1 log10 IU/mL for rs12979860 and rs12980275, and 5.9 log10 IU/mL for TG at rs8099917); and patients who were homozygous for the risk alleles had lower (mean 5.9 log10 IU/mL for TT at rs12979860, 6.0 log10 IU/mL for GG at rs12980275, and 5.8 log10 IU/mL for GG at rs8099917, P=0.0013, P=0.029, and P=0.0004 respectively; Table 1).

Significantly lower baseline plasma IP-10 levels were observed in homozygous carriers of the favorable CC at rs12979860 (median 189 vs. 258 pg/mL, P=0.02, Mann-Whitney U-test; Figure 2), AA at rs12980275 (median 189 vs. 258 pg/mL, P=0.01), and TT at rs8099917 (median 224 vs. 288 pg/mL, P=0.04), as compared with patients carrying one or two copies of the risk alleles.

Among genotype 1 infected patients, homozygous carriers of the three favorable IL28B alleles had significantly more pronounced first phase viral decline, as reflected by the reduction of HCV RNA from treatment day 0 to 4, when compared with patients

**Table 2.** The impact of IL28B genetic variations and baseline plasma IP-10 on the likelihood of achieving RVR among patients infected with HCV genotype 1.

<table>
<thead>
<tr>
<th>NCBI dbSNP ID</th>
<th>&lt;150 pg/mL</th>
<th>150–600 pg/mL</th>
<th>&gt;600 pg/mL</th>
<th>P</th>
<th>Total</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs12979860</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>8/13 (62%)</td>
<td>8/25 (32%)</td>
<td>0/4 (0%)</td>
<td>0.05</td>
<td>16/42 (38%)</td>
<td>0.006</td>
</tr>
<tr>
<td>CT</td>
<td>4/22 (18%)</td>
<td>11/55 (20%)</td>
<td>0/16 (0%)</td>
<td>0.15</td>
<td>15/93 (16%)</td>
<td>0.007</td>
</tr>
<tr>
<td>TT</td>
<td>0/6 (0%)</td>
<td>3/17 (18%)</td>
<td>0/3 (0%)</td>
<td>0.41</td>
<td>3/27 (11%)</td>
<td>0.007</td>
</tr>
<tr>
<td>rs12980275</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>9/17 (53%)</td>
<td>9/29 (51%)</td>
<td>0/4 (0%)</td>
<td>0.10</td>
<td>18/50 (51%)</td>
<td>0.18</td>
</tr>
<tr>
<td>AG</td>
<td>3/19 (16%)</td>
<td>10/51 (20%)</td>
<td>0/15 (0%)</td>
<td>0.18</td>
<td>13/85 (15%)</td>
<td>0.18</td>
</tr>
<tr>
<td>GG</td>
<td>0/5 (0%)</td>
<td>3/17 (18%)</td>
<td>0/4 (0%)</td>
<td>0.41</td>
<td>3/27 (11%)</td>
<td>0.18</td>
</tr>
<tr>
<td>rs8099917</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>11/28 (39%)</td>
<td>13/53 (25%)</td>
<td>0/14 (0%)</td>
<td>0.02</td>
<td>24/95 (25%)</td>
<td>0.18</td>
</tr>
<tr>
<td>TG</td>
<td>1/12 (8%)</td>
<td>9/40 (22%)</td>
<td>0/8 (0%)</td>
<td>0.20</td>
<td>10/61 (16%)</td>
<td>0.18</td>
</tr>
<tr>
<td>GG</td>
<td>0/1 (0%)</td>
<td>0/4 (0%)</td>
<td>0/1 (0%)</td>
<td>0.01</td>
<td>0/6 (0%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Total</td>
<td>12/41 (29%)</td>
<td>22/97 (23%)</td>
<td>0/23 (0%)</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All P-values using Chi squared test.

*P-values for SNP genotypes and frequency of RVR irrespective of baseline IP-10.

**Figure 4.** Mean HCV RNA reduction according to IP-10 in HCV genotype 1 with favorable IL28B genotype.

doi:10.1371/journal.pone.0017232.g004
carrying the risk alleles (mean 2.0, 0.9 and 0.6 log_{10} IU/mL for rs12979860 CC, CT and TT, 1.8, 0.9 and 0.7 log_{10} IU/mL for rs12980275 AA, AG and TT, and 1.4, 0.8 and 0.6 for rs8099917 TT, TG and GG respectively, P < 0.001 for all 3 SNPs, Kruskal-Wallis test). Among homozygous or heterozygous carriers of the favorable alleles, IP-10 was highly significantly associated with the first phase reduction of HCV RNA (rs = 2, 0.50, P = 0.001 and rs = 2, 0.40, P = 0.04 for rs12979860 CC and CT, rs = 2, 0.39, P = 0.003 for rs12980275 AA and AG, and rs = 2, 0.40, P < 0.0001 and rs = 2, 0.25, P = 0.046 for rs8099917 TT and TG respectively, Figure 3). This association was also significant for the maximum decline in HCV RNA from day 0 to 4 as well as the decline from day 0 to 1, thus emphasizing an association with the first phase decline in HCV RNA, and translated into a more rapid reduction of HCV RNA during the first 4 weeks of therapy among patients with lower baseline IP-10, and a slower decline among those with higher (Figure 4). Similarly, among genotype 2/3 infected patients, CC carriers of rs12979860 had significantly more pronounced first phase viral decline, as reflected by the reduction of HCV RNA from treatment day 0 to
or and

(92% vs. 83% for RVR and patients who had no dose reductions, i.e. per-protocol analysis even when the analysis was restricted to the genotype 1 infected RVR (P = 0.048 and P = 0.016 respectively), and RVR was in turn IL28B below 150 pg/mL respectively; n = 126). In contrast to genotype IL28B AA, and rs8099917, in HCV RNA (P = 0.009 and P = 0.007), respectively. Notably, achieving RVR was associated with a significantly greater first phase decline of HCV RNA during therapy irrespective of the first phase decline in HCV RNA even in genotype 2/3 infected patients, which translated into improved prediction of RVR and SVR. Considering the high SVR rates among HCV genotype 1 infected homozygous carriers of CC at rs12979860, AA at rs12980275, or TT at rs8099917 with baseline IP-10 levels below 150 pg/mL (85%, 76%, and 75% respectively), these patients, although few in number, should be encouraged to initiate therapy and may be candidates for shortened duration of therapy in line with current treatment guidelines considering the high likelihood of achieving RVR [24,25]. Additionally, these patients may be suitable for initial inclusion in pending trials with interferon-free DAA regimes, because of the favorable odds of successful salvage therapy with interferon in the event of development of resistance towards these new therapeutic agents.

The finding that IL28B variants primarily impact on the first phase decline of HCV RNA [26], as previously reported for IP-10 [20], suggests that both IL28B variants and IP-10 are linked to the antiviral effectiveness of peg-IFN and ribavirin in the blocking of the production or release of infectious virions rather than on the clearance of HCV infected cells. The risk alleles, T at rs12979860, or G at rs12980275 and rs8099917, were found to be significantly associated with modest elevations of baseline IP-10 levels. This finding is in line with the recent report by Honda et al. that G at rs8099917 entails higher intrahepatic expression of a panel of 37 representative ISGs not including IP-10 [13], which is typically observed in patients who respond less favorably to treatment [27]. The elevated baseline induction of ISGs among risk allele carriers may explain why these alleles are associated with a lower viral load observed in our study among patients with chronic HCV infection, corroborating previous reports [8,11].

Our finding that homozygous CC at rs12979860, AA at rs12980275, and TT at rs8099917 were significantly more common in the setting of HCV genotype 2 or 3 infection than 1 in a population consisting only of Caucasian patients confirms and extends the findings reported by McCarthy et al. [11]. Indeed, the proportion of CC at rs12979860 among HCV genotype 2 and 3 infected patients (56% and 67%, respectively) in our study is higher than the reported prevalence in HCV uninfected Caucasians (~40%), suggesting that this SNP genotype entails a higher likelihood of development of chronic infection following exposure to HCV genotype 2 or 3. This observation, however, may be considered counter-intuitive since homozygous carriage of CC at rs12979860 was associated with a significantly greater first phase decline of HCV RNA even in genotype 2/3 infected patients. A possible hypothesis for these seemingly contradictory findings is that carriage of the favorable allele C at rs12979860, A at rs12980275 and T at rs8099917, being associated with a slightly diminished baseline activation of ISGs, is beneficial for clearance of all HCV genotypes as reflected by the association with a greater first phase decline in HCV RNA during therapy irrespective of

| Table 3. Sensitivity, specificity, positive and negative predictive values of the likelihood achieving SVR among patients infected with HCV genotype 1 (n = 170). |
|-----------------|-------|-------|-------|-------|
|                  | PPV   | NPV   | Sensitivity | Specificity |
| rs12979860 CC   | 66%   | 52%   | 32%   | 81%   |
| Baseline plasma IP-10 | 66% | 52% | 33% | 81% |
| <150 pg/mL (n = 44) | 63% | 56% | 53% | 65% |
| CC at rs12979860 or | 85% | 50% | 12% | 98% |
| IP-10<150 pg/mL (n = 75) | 91% | 59% | 37% | 96% |

Discussion

In spite of the pending introduction of direct antiviral agents (DAA) in routine clinical practice, interferon-α and ribavirin are likely to retain pivotal roles in the management of chronic HCV infection, and thus predicting responsiveness to interferon/ribavirin-based therapies will remain important. In this setting, the main finding in the present study was that pretreatment plasma levels of IP-10 increased the level of prediction of the first phase decline in HCV RNA among patients carrying IL28B SNP variants, which translated into improved prediction of RVR and SVR. Considering the high SVR rates among HCV genotype 1 infected homozygous carriers of CC at rs12979860, AA at rs12980275, or TT at rs8099917 with baseline IP-10 levels below 150 pg/mL (85%, 76%, and 75% respectively), these patients, although few in number, should be encouraged to initiate therapy and may be candidates for shortened duration of therapy in line with current treatment guidelines considering the high likelihood of achieving RVR [24,25]. Additionally, these patients may be suitable for initial inclusion in pending trials with interferon-free DAA regimes, because of the favorable odds of successful salvage therapy with interferon in the event of development of resistance towards these new therapeutic agents.
HCV genotype, but more advantageous for genotype 1 in comparison to 2/3. In the event of continuous re-exposure to a variety of HCV genotypes following a possible initial spontaneous clearance of HCV, as is often the case among intravenous drug users in addition to the lack of a lasting protective immune response [29], this skewness will exert selective pressures and over time lead to an under-representation of these favorable alleles among genotype 1 patients and an over-representation among 2/3 patients as compared to the non-infected population. This would be the case even if carriage of this SNP genotype were slightly beneficial in the event of a single point exposure to HCV genotype 2 or 3. Supportive of this concept is the non-significant trend observed among 27 HCV genotype 2/3 patients infected through exposure to contaminated blood products as compared to 322 genotype 2/3 patients with other routes of infection in another study [NORDynamIC trial] [29]. Similarly, in an elderly population in southern Italy predominantly infected with HCV genotype 2 likely secondary to past iatrogenic exposure, a non-significant trend towards a lower proportion of CC at rs12979860 (30%) was noted among genotype 2/3 infected patients than among non-infected controls (37% vs. 42%) [30].

In conclusion, baseline plasma IP-10 is significantly associated with IL28B-related SNPs, and augments the level of predictiveness of the first phase decline in HCV RNA, RVR, and final treatment outcome. Therefore, pre-treatment screening of IL28B genetic variants, together with measurement of IP-10 in plasma, may provide useful prognostic information prior to initiating antiviral therapy for HCV.

Acknowledgments
We thank Elke Verhey-Hart, Marie-Louse Landleius and Ulla Gingsjo for expert technical assistance.

Author Contributions
Conceived and designed the experiments: ML PYB KH. Performed the experiments: GA SB JS AR PYB JMP. Analyzed the data: ML PYB KH FN AUN. Contributed reagents/materials/analysis tools: KH BLH PYB FN SB AR GM CF AUN JMP SZ. Wrote the paper: ML KH PYB FN.

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