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IL28B polymorphism and hepatitis C: A genetic marker of peginterferon-α sensitivity devoid from classical interferon-α side effects?

To the Editor:

Single nucleotide polymorphism (SNP) in the region of the interleukin-28B (*IL28B*) gene has recently been associated with spontaneous and treatment-induced clearance of hepatitis C virus infection [1–3]. The SNPs are located near the *IL28B* gene of chromosome 19, implicating a role for its gene product, interferon-λ3, in the immune response to hepatitis C virus. The mechanisms by which *IL28B* polymorphism effects the sensitivity to exogenous peginterferon-α therapy is unclear at present.

We have read with interest the recent genome-wide association study by Thompson *et al.* on peginterferon-α-induced cytopenia in chronic hepatitis C patients [4]. The authors state that none of the *IL28B* polymorphisms known to be associated with treatment induced viral clearance, correlated with the typical peginterferon-α side effects thrombocytopenia and neutropenia after 4 weeks of treatment. The authors suggest a possible liver specificity of interferon-λ by which the favorable *IL28B* polymorphism might result in increased viral clearance from the liver with a reduced propensity for interferon-α-induced bone marrow suppression.

We have three concerns regarding the study that might influence interpretation of the observations made by the authors.

In the Individualized Dosing Efficacy versus Flat Dosing to Asses Optimal Pegylated Interferon Therapy (IDEAL) study, 3070 patients were treated, of whom 1654 completed the treatment [5]. The patient group of the current GWAS study consisted of 1284 out of 1604 patients who consented to genetic testing and fulfilled the 80/80/80 adherence criteria. The majority of patients studied in this analysis, therefore, probably derive from the 1654 who completed treatment. Limiting the analysis to adherent patients when studying dose-related side effects such as peginterferon-induced cytopenia may appear justified at first glance. This approach, however, excludes the data from patients who had dose reductions or were not able to complete treatment for other peginterferon side effects, but who may have had concomitant peginterferon-induced cytopenias.

In the IDEAL study, patients were treated with either peginterferon-α-2b 1 μg/kg, 1.5 μg/kg or peginterferon-α-2a 180 μg/kg weekly on a 1/1/1 randomized basis in combination with ribavirin oral therapy [5]. The proportions of patients with neutropenia who met the criterion for per protocol peginterferon-dose reduction in this study were 12.5%, 19.4% and 21.1% of the patients treated with the low-dose peginterferon-α-2b (1 μg/kg) versus the standard-dose peginterferon-α-2b (1.5 μg/kg) and peginterferon-α-2a (180 μg/kg), respectively. The inclusion of the low-dose peginterferon-α-2b arm in the current analysis therefore obscures the severity of the observed cytopenias in the two arms that were treated with the standard dose of either peginterferon-α that is relevant for current clinical practice.

In two recent publications by our group on peginterferon-α-induced cytopenia, thrombocytopenia and neutropenia progressed to a nadir after 12 and 14 weeks of peginterferon-α treatment, respectively [6,7]. The analysis performed by the authors after only 4 weeks of treatment might therefore underscore the severity of true peginterferon-α-induced cytopenia, obscuring a possible link with baseline *IL28B* polymorphism as a marker of interferon sensitivity.

To rule out an inclusion bias, it is necessary to demonstrate that the cytopenias observed in the reported 1284 patients do not differ from those observed in the 1466 patients who did not consent to genetic testing or in the 320 patients who did consent but are not included in the analysis. We are also interested to learn from the authors whether the absence of correlation with *IL28B* polymorphism and peginterferon-induced cytopenia would hold if the analysis were to be performed in both adherent and non-adherent patients under treatment at week 12 and 18 from the two standard peginterferon-α-2b 1.5 μg/kg and peginterferon-α-2a 180 μg dosing groups, omitting the data from the low-dose peginterferon-α-2b (1.0 μg/kg) group. An analysis of the *IL28B* genotype distribution in the so-called "non-adherent" population that consented to genetic testing in comparison to the reported "adherent" group would further help to clarify whether a favorable *IL28B* polymorphism does in fact enhance viral clearance without the occurrence of classical interferon-α side effects.

Conflict of interest

H.O. has no conflict of interest to disclose. R.J.dK. Received research funding and has been invited speaker from Merck and Roche. H.L.A.J. received grants from and is a consultant for: Bristol Myers Squibb, Gilead Sciences, Novartis, Roche and Merck.

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Reply to "*IL28B* polymorphism and hepatitis C: A genetic marker of peginterferon-α sensitivity devoid from classical interferon-α side effects?"

To the Editor:

We appreciate the interest of Orlent *et al.* in our recent study [1] and would like to comment on the issues raised in their letter.

(i) The primary endpoint we selected for all genome-wide analyses of peginterferon (pegIFN)-induced cytopenia was quantitative reduction in cell counts, as a continuous variable, at week 4. We chose the continuous variable as the primary endpoint for two reasons. Firstly, this method maximizes the statistical power of the dataset. Secondly, it avoids the pitfalls of defining pre-specified thresholds for cytopenia, where timing and the chosen level of the threshold are arbitrary. We chose the week 4 timepoint to minimize confounding by dose adjustment/adherence. It is true that week 4 preceded the nadir for pegIFN-related thrombocytopenia and neutropenia in this study, which occurred between week 8 and 12. However, the rate of decline was most profound in the first 4 weeks, with significant reductions in both platelet counts and neutrophil counts by week 4 [1]. We have subsequently tested for association between the *IL28B* polymorphism rs12979860 and quantitative reduction of platelet/neutrophil counts at week 12. No significant association was observed.

(ii) Orlent *et al.* suggested that inclusion of patients treated with low-dose pegIFN-α-2b in our analysis may have confounded the results. We included these patients to maximize the sample size. We did adjust for pegIFN dose however, as stated in the methodology [1], by coding a binary variable for full *vs.* low dose pegIFN. This variable was included in all statistical models. PegIFN dose was significantly associated with both thrombocytopenia and neutropenia as expected. *IL28B* polymorphism was not significantly associated with pegIFN-cytopenia when analysis was limited to patients receiving full-dose pegIFN.

(iii) Orlent *et al.* also raised the possibility of inclusion bias. It is true that we limited the analysis of week 4 cytopenia to patients who were at least 80% adherent to study treatment to this timepoint. This decision was taken to refine the clinical phenotype. However, it should be noted that only 26 patients were excluded from the analyses of platelet/neutrophil counts on the basis of adherence at the week 4 timepoint [1]. Inclusion of these 26 patients into the analysis does not change the results of the analyses. The majority of the patients who consented to genetic testing, but were not included in this analysis, were excluded on the basis of genotyping quality control or missing data points (only patients with complete datasets for all relevant variables could be included in the analyses). There was no significant difference in the median reductions of platelet counts or neutrophil counts comparing patients who were included in the final analysis *vs.* patients who consented to genetic testing but were not included in the final analyses (patients of European American, African American and Hispanic ethnicity considered separately). We therefore feel that inclusion bias within this study was unlikely. We have recently analyzed the overall IDEAL cohort for patient factors that might influence consent to the performance of genetic testing and the data will be presented at AASLD 2011 [2]. There were no differences in treatment outcomes or overall adverse events (including cytopenia) between patients who did, or did not consent, to participation in the pharmacogenomics substudy.