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-α (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 cytopenia, 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 minimize confounding by dose adjustment/adherence. 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 at 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.

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

References


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The data suggest that IL28B polymorphism does not influence the occurrence or severity of IFN-related cytopenia. However, we acknowledge that the methodology of genome-wide association studies has some limitations, including the statistical requirement to correct for multiple testing. As a consequence, small effect sizes may not be detected. Therefore, we cannot exclude the possibility that there may be a small contribution (odds ratio <1.5) of common genetic variants to IFN-related cytopenias. The clinical importance of such a contribution would be expected to be modest.

Conflict of interest

Drs. Thompson, Muir and Sulkowski report having received research and grant support and receiving consulting fees or acted in an advisory capacity for Schering-Plough (now Merck, Inc). Dr. Thompson is co-inventor of a patent application based on the ITPA finding. Dr. Clark has no conflicts to report.

References


Transient elevation of serum bile salts after partial hepatectomy is due to metabolic overload and not to cholestasis

To the Editor:

In a recent publication Miura et al. reported on the mechanism of cholestasis after partial hepatectomy (PH) in rats [1]. The first day after PH, they find a transient doubling of serum bile salts, a down-regulation of the bile acid uptake protein Ntcp and an up-regulation of Mrp4 and Cyp7A1. With these findings they confirm a number of earlier studies [2–4]. Miura et al. consider this as evidence of cholestasis. We like to take issue with this view.

Vos et al. (in 1999) showed that 24 h after PH in rats, bile flow, expressed per gram of liver, was increased from 1.5 ± 0.1 to 2.6 ± 0.4 µl/g liver/min. Bile flow per 100 g body weight did not change. The same is true for bile salt secretion; it increased from 80 ± 4 to 152 ± 22 nmol/g liver/min and did not change when calculated per 100 g body weight [2]. Other authors have reported similar findings both in rats and mice [5,6]. Thus, if cholestasis is defined as an impairment of bile flow, there is no cholestasis after PH. To the contrary, for the remnant liver there is increased bile flow and bile salt flux. The remnant liver maintains a normal bile flow despite a decrease of organ mass. The transiently elevated serum bile salt levels may be the consequence of de novo bile salt synthesis. Thus, elevated serum bile salt and bilirubin levels not always mean cholestasis but they can be a sign of hepatic overload. This is not a trivial statement because this overload has profound physiological consequences in that it may trigger hepatic regeneration. This is in line with the metabolic overload theory as a trigger of liver regeneration [7]. There is ample new evidence that bile salts are instrumental for this response [8,9].

Bile salts are ligands for the nuclear hormone receptor FXR. Activation of FXR in the liver simulates regeneration. In agreement with this, FXR^-/- mice show delayed liver regeneration after PH [10]. Although the precise mechanism has not been clarified yet, this activation depends on the intracellular free concentration of bile salts and this depends in part on a balance between hepatic uptake, secretion and de novo synthesis. The findings of Miura et al. and others suggest that uptake is impaired and basolateral secretion is increased. This, together with data showing that canalicular secretion is increased, makes it difficult to predict what happens to the intracellular bile salt concentration but a fair guess would be that there would have been no change if de novo synthesis would not have been activated. Indeed, Csanyk et al. reported that after PH in mice, the hepatic concentrations of glycine-conjugated and unconjugated bile salts were increased [6]. Up-regulation of Cyp7A1 is most likely responsible for the increased serum and liver bile salt levels and we suggest that this is a key event in the regenerative response of the liver. Cyp7A1 expression is mainly controlled by a protein called Fgf15 that is produced in the ileum and acts on the FGFR4 receptor in the liver. In the ileum, Fgf15 synthesis is activated by binding of bile salts to FXR. Since bile salt flux through the ileum after partial hepatectomy is not decreased, we propose that FGFR4 in the liver may be down-regulated in order for Cyp7A1 to be up-regulated. In conclusion: in order for bile salts to play a controlling role, bile salt flux through the liver has to be maintained within very strict limits. This is achieved by a detailed regulation of uptake, secretion and synthesis. These regulations are probably more

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