Reviewer’s report

**Title:** Peretinoin, an acyclic retinoid, improves hepatic gene signature of chronic hepatitis C following curative therapy of hepatocellular carcinoma

**Version:** 2  **Date:** 13 December 2012

**Reviewer:** Ingeborg Tinhofer

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The study of Honda et al was designed for determining to molecular basis of the beneficial effects observed in a previously performed phase II/III clinical trial for peretinoin maintenance treatment after curative treatment of hepatocellular cancer patients. Liver biopsies from 12 HCC patients who had been treated with curative resection or ablation were taken at baseline and week 8 of peretinoin treatment. Biopsies were subjected to gene expression analysis of mRNA levels. The predictive value of gene expression pattern for recurrence was assessed. The rational of the study is clearly defined and the experiments were well performed. The authors should be congratulated for their molecular translational study with clinical samples collected before and under treatment. Nonetheless, there are some critical issues which should be addressed before the manuscript would be acceptable for publication.

Major compulsory revisions:

1) As mentioned in the Introduction, the authors have performed a previous multicenter, double-blinded Phase II/III study in which 401 HCC patients have been randomized to receive peretinoin at a dose of 300mg or 600mg or a placebo. The results of this previous study have not been reported in a full paper, but preliminary efficacy data were presented at ASCO 2010. As mentioned in the ASCO abstract, recurrence-free survival after 2 years was significantly improved in the patient group receiving 600 mg per day compared to placebo (HR 0.27; 0.07-0.96). However, a daily dose of 300 mg peretinoin did not improve but rather slightly increased the recurrence risk when compared to placebo (HR 1.19, 0.55-2.60).

In their current study, the authors evaluated the molecular response of liver cells to peretinoin treatment again in a 300-mg and 600-mg dose group. However, the authors did not separately report on the expression profile analysis from these two dose groups and it remains unclear why they have chosen two different concentrations. Considering the different outcome reported before, it would be interesting to know whether or not differences were also observed on the molecular level. Since only a very small number of patients was included in the molecular pharmacokinetic study the rationale for choosing two different concentrations should be given.

2) It remained also unclear to the reviewer whether or not the results from expression profiling of the 300-mg group was also included in the model for
identification of a predictive gene signature. Given that the subsequent maintenance treatment after the experimental 8-week start phase consisted of 600 mg peretinoin for all patients the inclusion of the expression profiles from the 300-mg group in the class prediction might represent a significant bias in their model. This is important since the authors conclude from their study that response assessment during the early period of administration might predict recurrence-free survival. The authors should explain their procedure for the hierarchical clustering, especially in terms of which samples were included, and should also discuss more in detail potential biases in this analysis.

3) As stated on page 6 all patients were clinically tumor-free which means that the gene expression profiles were generated from normal liver tissue. Even if the presence of putative tumor stem cells or residual tumor cells cannot be excluded the molecular pattern mainly represents the non-tumor cell fraction. The authors should discuss in more detail how the expression profile in normal liver cells might determine the recurrence risk? Did the authors also evaluate the expression profiles at the time point when the tumor recurred in order to see whether the same signaling pathways were still activated?

4) The rationale for selecting week 8 for the second biopsy should be given. How can the authors rule out that an earlier assessment of gene expression would not be superior for predicting the beneficial peretinoin effect?

5) Liver peretinoin concentrations in 10 of 12 patients were below the detection limit and, as discussed by the authors on page 17 the analysis of a possible relationship between peretinoin concentrations and the molecular expression profiles was not possible. In contrast, plasma concentrations were detectable and dose-dependent. Was there a dose-relationship between plasma levels and expression profiles? If not, why did the authors include these data here?

6) As seen in Table 1, 5 of 6 patients with recurrences at 4.5 years were female and only 1 of 5 males experienced a tumor recurrence at that time. Did the authors observe a difference in the expression profiles in females and males? Is there evidence that the action of peretinoin might differ depending on the gender?

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I have no conflict of interest to declare.