Reviewer's report

Title: Mechanistic biomarkers of liver safety and benign elevations in serum aminotransferases - A study in healthy volunteer treated with cholestyramine

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Reviewer: shashi K ramaiah

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- Minor Essential Revisions

  The author can be trusted to make these. For example, missing labels on figures, the wrong use of a term, spelling mistakes.

  o The authors use “mechanistic Biomarkers” in the manuscript to refer to new biomarkers. It is not clear if miR-122, CK, HMGB1, SDH are biomarkers that help explain the mechanism or liver injury; this wording should be deleted. May be the authors interpret “necrosis versus apoptosis” as mechanistic biomarkers.

  o Study design: it is somewhat difficult to understand the clinical study design of which should be modified (similar comment is made on the figure 1). There is a washout phase after investigational drug with cholestyramine. The rationale for the inclusion of a positive control should be included. Also Figure 1 bidirectional arrows may not be easy for the reader to interpret and should be rewritten.

  o Table 1: Peak ALT, AST, ALP and total bili are post dosing fold changes as shown in the table which should be clarified in the title of the table. It can be confusing to readers when they read both the absolute value as well as fold-change.

  o The rationale for including only the proposed biomarkers is briefly included in the introduction section. Since there are several novel biomarkers (including PON1, MDH, purine nucleoside phosphorylase) that represent several aspects of liver injury, it is not clear why the authors hypothesized only a select biomarkers. It is understandable to not measure a long list of biomarkers but the authors may have some previous knowledge why their proposed biomarkers were short listed which should be stated.

  o The authors measured investigational biomarker samples only from samples containing the highest level. Is it possible that these biomarkers were also increased in samples at other timepoints when ALT increase was not high. This is less likely but measuring such samples would further add value to the hypothesis.

  o The authors claim to test the liver injury mechanism (apoptosis versus necrosis) by measuring total and cleaved CK 18. Based on the lack of differences in the ratio of cleaved to total CK18, the authors interpret that there is no shift from apoptosis to necrosis. Did the authors measure additional parameters to test apoptosis versus necrosis mechanisms? Without such data
the interpretation is speculative.

It does not appear that the authors measured these new biomarkers when same subjects were assessed after the treatment was stopped after the patient follow up. Did these parameters decline or normalize? Would measuring these parameters during the drug free period provide some idea on the ongoing liver necrosis/apoptosis?

• Discussion: The ‘new’ biomarker data generated is quite interesting to support additional hepatic changes beyond benign transaminase elevations. The authors discuss the mechanistic aspects (apoptosis versus necrosis) as well as additional mechanisms to understand whether the increases are due to true hepatic injury or a benign change. There is no new data generated to explain or confirm the authors speculations other than citing literature changes that these new biomarkers change due to necrosis and/or apoptosis. It would have been compelling to test some of the mechanistic parameters of liver injury.

• Discretionary Revisions

Figure 2: X-axis: EOS should be defined.

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 declare that i have no completing interests’.