Reviewer's report

Title: Prevalence of naturally occurring NS5A resistance-associated substitutions in patients infected with hepatitis C virus subtype 1a, 1b, and 3a, co-infected or not with HIV in Brazil

Version: 0 Date: 02 Jun 2017

Reviewer: Joerg Timm

Reviewer's report:

The authors analyzed the prevalence of RAVs in NS5A in 257 HCV infected patients from Brazil. The study included HCV mono-infected as well as HIV co-infected patients. Although the prevalence of naturally occurring RAVs is well characterized for most genotypes, regional data are of clinical interest to address if local differences exist. The study is overall well performed and adds important knowledge to the scientific community. I have the following comments:

1. I would omit the historical perspective on interferon-treatments from the introduction, I believe that combinations of DAAs with interferon are not in use anymore. It would be more interesting to present the currently available DAA treatment regimens and information on the particular DAAs that are currently used in Brazil? Moreover, what are the important RAVs in NS5A in the different genotypes? 2. Please highlight also somewhere in the manuscript (introduction or discussion) the evidence for the clinical benefit of resistance testing and also the controversy, as most guidelines do not recommend testing or recommend testing only in certain cases. 3. The manuscript should be edited by a native English speaker. Some sentences seem incomplete or are quite confusing. Here are a few examples:

- Line 174: RAV were observed in subtype 1a sequences. 
- Line 274: "Special attention must be driven to NS5A resistance" 
- Line 288: ..., once NS5A profile resistance is dependent to HCV subtype and geographic origin.

4. The first part of the discussion is a repetition of the results section. Overall, the discussion is overly long and could be shortened. It is sufficient to highlight substantial differences between RAV frequencies in Brazil and reported frequencies in the literature. 5. To my knowledge R30Q in GT1b is no RAV (see your Citation 7+20) 6. Did the authors look for minor populations at RAV positions? 7. Were the HIV co-infected patients under treatment? 8. What was the viral load of the patients in the cohort (median; range)? Please include a phylogenic analysis of the mono vs the co-infected to rule out that your higher prevalence of RAVs in mono infected is due to a founder effect. 10. The absence of RAVS in HIV co-infected 1b is most likely a consequence of the small number. Please discuss. 11. In the chapter" Resistance-associated variants in HCV/HIV co-infected patients" the Y93H mutation is not listed for GT1b. 12. Page 10 line 230-234: The authors state that the discrepancies between the reported RAV frequencies and the frequencies in this study may be the result of different classifications of RAVs. Can the authors give an example? 13. Page 7 Line 178-180 multiple variants were only detected in 4/77 GT1a sequences: Page 11 Line 260-263: 8/77 GT1a? The discrepancy should be clarified. 14. "Also, the presence of NS5A associated resistance variants at baseline has been associated with virologic failure, mainly when low genetic barrier resistance drugs are used." Please give a reference for this statement.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.
Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

Yes

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If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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