Format for ANSWERING REVIEWERS

Dear Editor,

Please find enclosed the edited manuscript in word format (file name: 15294-Review).

Title: Virological response and resistance mutations to NS3/4A inhibitors in hepatitis C virus-human immunodeficiency virus coinfection

Author: Alissa Naqvi, Valérie Giordanengo, Brigitte Dunais, Francine de Salvador-Guillouet, Isabelle Perbost, Jacques Durant, Pascal Pugliese, Aline Joulié, Pierre Marie Roger, Eric Rosenthal

Name of Journal: *World Journal of Hepatology*

ESPS Manuscript NO: 15294

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated.

2 Revision has been made according to the suggestions of the reviewer:

Reviewer 1: 00504019

1. In Methods section, In NS3 genotyping part, there is no sufficiently detailed information provided for the primers used in RT-PCR, and nested PCR, and their sites in HCV genome.

   To correct these points, we included:

   (1) Table 1 with primer sequences and their sites in the HCV-H77 reference strain used in the study to compare our sequences
   (2) Extra details in the Methods section.

2. The meaning of the sentence "Then, in the case of negative PCR amplification, a nested PCR was performed" is not so easy to understand.

   We changed to: When needed, a nested PCR was performed involving the inner genotype-specific primers in 40 μl of reagent mixture…..

3. In Results section, there is no sufficiently detailed description for genotyping the cloned HCV sequences. Is it performed according the NS3 protease region or not?

   This was completed with the description of the sequence analysis: "Their NS3 sequences were compared to the HCV-H77 reference strain (reference sequence AF009606) using Sequence Navigator softwareTM (Applied Biosystems) and were analysed with Geno2pheno HCV. Both

these methods yielded the same resistance mutations."

4. There is no detailed information about the nucleotide or amino acid sequences about the analyzed cloned gene provided.

   Table 3 details the NS3 amino acid mutations responsible for resistance.

5. It is known that in the plasma of the chronic HCV infected patient harbors many of mutations or quasispecies, so only a single sequence within the NS3 protease region could not provide significant information about the real conditions of the infection. In the manuscript, the information about the mutations of nucleotide or amino acid sequence does not support the validity of the therapeutic efficiency and antiviral resistance.

   Due to the high rate of viral turnover and the error-prone activity of the HCV polymerase, HCV replication results in the production of numerous variants known as quasispecies, as in HIV infection. While several HIV studies have indicated that the presence of minority resistant variants (using deep-sequencing) in HIV patients should be detected and monitored because their presence can increase the risk of virological failure, this fact is not clear for HCV infection. So currently drug-resistance mutations are routinely detected using standard Sanger sequencing, which does not detect minor variants with a frequency below 20%. The impact of detecting minor variants generated by ultra-deep sequencing (UDS) on HCV drug-resistance interpretations has not yet been studied.
   This argument is developed in the discussion.

6. There is no information about the criterion for HIV infection provided. Is it only based HIV-RNA detection or not? Why all the HIV-RNA in the patient plasma was undetectable after they received antiretroviral treatment? Is there any clinical practice references could be listed?

   In France HIV infection is always confirmed by ELISA and Western Blot tests. Combined antiretroviral therapies (cART) currently available in France are generally very effective and achieve undetectable HIV-RNA levels in the vast majority of patients. The French Hospital Database on HIV includes data on 120 000 HIV-infected patients. In 2009, cohort uptake of cART was 85.2% and 93% of patients on cART for at least 6 months had viral load ≤500 copies/ml. (Mary-Krause *et al*. Cohort profile: French hospital database on HIV (FHDH-ANRS CO4). Int J Epidemiol 2014; 43:1425-36. The patient population in this study was recruited among regularly followed outpatients. These patients therefore received optimal care and follow-up.

7. The tables and figure are not so formal and easy to understand.

   As mentioned above, an extra table has been added describing HCV primer sequences. As for the remaining tables and graphs, we consider they are clear and cannot be further improved.

Reviewer 2 : 02860761

This manuscript needed for English editing. But however it of very good idea and provide important clinical answers.

We consider the English to be reasonably good.

Reviewer 3 : 00069855

1. No approved research protocol from institutional ethic committee and a signed informed consent from patients or their representative.

This study was not a clinical trial:

a) The anti-HCV regimens administered to the patients were approved by the French national drug safety agency (Agence nationale de sécurité du medicament) and had been licensed at the time of the study, so that no Ethics Committee approval was required.

b) Patients followed in our University Hospital departments provide initial informed consent as soon as they present at the hospital and this consent covers both medical management and approval for data entries in the Nadis® database. Briefly, Nadis® (Fedialis Medica, Marly le Roi, France) is an electronic medical record (EMR) for HIV, hepatitis B virus (HBV)- or hepatitis C virus (HCV)-infected adults seeking care in French public hospitals. The collected data and details of the networking organization have been submitted to the French National Commission on Informatics and Rights (CNIL). All patients provide written informed consent prior to the inclusion of their data in the EMR. Puglièse P *et al*. A large French prospective cohort of HIV-infected patients: the Nadis Cohort. HIV Med 2009; 10: 504-511 (Reference 6 of our manuscript).

2. No placebo-controls or other kind of controls for your observation.

The explanations provided above justify the absence of controls in this study. Moreover, two twin trials (ANRS HC26 TelapreVIH and HC27 BocepreVIH) referenced in our manuscript (Poizot-Martin I *et al* and Cotte L *et al*) allowed the approval of telaprevir and boceprevir in HIV-infected-patients by the European Medicines Agency. These were open-label, single-arm, phase 2 clinical trials. Our study aimed to provide additional data from "real-life" setting, more particularly in very difficult to treat-patients not included in the ANRS HC26 and HC27 clinical trials.

3. As if your manuscript is still in writing and unfinished.

The manuscript in its present form is complete.

3 References and typesetting were corrected.

Thank you again for publishing our manuscript in the *World Journal of Hepatology*.