Manuscript N° 25877: Point-by-point answers to the reviewers

We thank the reviewers for the helpful critique, suggestions and comments. We believe, and hope the editors / referees agree, that the manuscript is now improved.

The reviewer n° 00502973 commented: *In the current submission, Lagaye et al reported the anti-HCV effect of a new autophagy inhibitor GNS-396. This would be helpful to develop new medicine for anti-HCV with new mechanism. However, concerns existed. And the manuscript could not be accepted before the concerns being addressed.*

Major concern: *As the development of new DAA, more powerful anti-HCV drugs are available at present. The author compared the EC50 of GNS-396 with some anti-HCV drugs. However, the comparison with new DAAs, e.g. Sofosbuvir were not present. This should be addressed.*

We thank the reviewer for this helpful comment. We made new experiments with the new DAA Sofosbuvir and added the obtained results in the Table 1.

Minor concerns:

1. *In the 2nd paragraph of the Introduction: "Over the past few years, other direct acting antivirals (DAAs) were developed[10-14] as second generation of PI with higher antiviral potency, HCV NS5A replication complex inhibitors and nucleotide analogue HCV NS5B polymerase inhibitors[13] as well host-targeted indirect antivirals like cyclophilin inhibitors[15] and lambda interferon[15]." change to "Over the past few years, other direct acting antivirals (DAAs) were developed[10-14] as second generation of PI with higher antiviral potency, HCV NS5A replication complex inhibitors and nucleotide analogue HCV NS5B polymerase inhibitors[13] as well as host-targeted indirect antivirals like cyclophilin inhibitors[15] and lambda interferon[15]."*

We apologised for this mistake and changed in the text to "Over the past few years, other direct acting antivirals (DAAs) were developed[10-14] as second generation of PI with higher antiviral potency, HCV NS5A replication complex inhibitors and nucleotide analogue HCV NS5B polymerase inhibitors[13] as well as host-targeted indirect antivirals like cyclophilin inhibitors[15] and lambda interferon[15]."

2. *Under the title "Liver slices preparation, culture and infection": "Cell number for tissue slices was estimated at ~ 2.7 106 cells per slice based on a 14-cell thick slice (cell diameter ~ 25µm)[41]." change to "Cell number for tissue slices was estimated at ~ 2.7 ×106 cells per slice based on a 14-cell thick slice (cell diameter ~ 25µm)[41]."*

We apologised for this mistake and changed in the text to "Cell number for tissue slices was estimated at ~ 2.7 × 10^6 cells per slice based on a 14-cell thick slice (cell diameter ~ 25µm)[41]."

Also in this paragraph, "One day post-culture in twelve-transwell plates, human primary liver slices were inoculated with HCV Con1/C3 at a multiplicity of infection (MOI) equal to 0.1 at 37°C in the same culture conditions than described above, for overnight." change to "One day post-culture in twelve-transwell plates, human primary liver slices were inoculated with HCV Con1/C3 at a multiplicity of infection (MOI) equal to 0.1 at 37°C in the same culture conditions as described above, for overnight."

We apologised for this mistake and changed in the text to "One day post-culture in twelve-transwell plates, human primary liver slices were inoculated with HCV Con1/C3 at a multiplicity of infection (MOI) equal to 0.1 at 37°C in the same culture conditions **as** described above, for overnight."

3. *The 2nd paragraph under the title "Quantification of HCV strands RNA by real-time quantitative Reverse Transcription- Polymerase Chain Reaction (qRT-PCR)": "Reverse transcription was performed using primers described previously located in the 5' NCR region of HCV genome, tag-RC1 (5'-GGC CGT CAT GGT GGC GAA TAA GTC TAG CCA TGG CGT TAG TA-3')[47]" I suppose this sequence comprises of 2 primers as it was used as 2 primers later (5'-GGC CGT CAT GGTGGC GAA TAA-3', . 5'-GTC TAG CCA TGG CGT TAG TA-3').*

The reviewer n° 00502973 are right, it is a long primer named tag-RC1 (5'-GGC CGT CAT GGT GGC GAA TAA GTC TAG CCA TGG CGT TAG TA-3') containing the tag primer sequence (5′-GGC CGT CAT GGTGGC GAA TAA-3′) + RC1 primer sequence (5'-GTC

TAG CCA TGG CGT TAG TA-3'). This tag-RC1 primer is used with RC21 for the first amplification.
Then, RC1 (5′-GTC TAG CCA TGG CGT TAG TA-3′) and RC21 primers were used for positive-strand amplification, and tag (5′-GGC CGT CAT GGTGGC GAA TAA-3′) with RC21 primers were used for negative strand amplification.

4. *Under the title "Western blotting": "Horseradish peroxidase-conjugated anti-mouse IgG (GeHealthCare Life Sciences) taken 1:50000 were used as secondary antibodies." change to " Horseradish peroxidase-conjugated anti-mouse IgG (GeHealthCare Life Sciences) at the dilution of 1:50000 were used as secondary antibodies."*

We apologised for this mistake and changed in the text to" Horseradish peroxidase-conjugated anti-mouse IgG (GeHealthCare Life Sciences) at the dilution of 1:50000 were used as secondary antibodies."

5. *The 1st paragraph of the Discussion: "2. GNS-396 was a potent autophagy inhibitor, acting as "lysosomotropic agent" which was able to inhibit HCV replication in primary human adult HCVcc infected liver slices culture." This is the 2nd point. The 1st point was missing or not labeled.*

We apologised for this mistake and changed in the text to "Our study evidenced that 1. the *ex vivo* model of human liver slices HCVcc Con1 infection may be efficiently used for the assay of the antiviral potency of a new inhibitor (GNS-396 compared to HCQ) which interfered with autophagy; 2. GNS-396 was a potent autophagy inhibitor, acting as "lysosomotropic agent" which was able to inhibit HCV replication in primary human adult HCVcc infected liver slices culture."

6*. The 2nd paragraph of the Discussion: "Since the permissive cell lines are transformed and poorly differentiated." This sentence was incomplete.*

We apologised for this mistake and changed in the text to "Moreover, the study of virus/host cell interactions is limited since the permissive cell lines are transformed and poorly differentiated."

7. *The last paragraph of the Discussion: "Moreover, the infection of human liver slices culture with primary viral isolates from patients that we succeed to establish[41], should allow highlighting the potential early emergence of drug resistant viral variants during the anti-viral treatments." change to "Moreover, the infection of human liver slices culture with primary viral isolates from patients that we succeed to establish[41], should allow highlighting the potential of early emergence of drug resistant viral variants during the anti-viral treatments.*

We apologised for this mistake and changed in the text to "Moreover, the infection of human liver slices culture with primary viral isolates from patients that we succeed to establish[41], should allow highlighting the potential of early emergence of drug resistant viral variants during the anti-viral treatments."

The reviewer n° 00053644 commented: "The manuscript is clear and comprehensive. I believe there are no changes or modifications to do" and accepted the manuscript.