*Dear reviewers,*

*We appreciate all your time and efforts in reviewing our clinic manuscript. We have addressed all issues indicated in your reports. Please see our responses below:*

***Response to Comments from Reviewer 1:***

I read with great interest the paper by Lytvyak et al, regarding the use of anti-retroviral therapy for PBC patients. It is a very well written paper providing a detailed review and update information on the use of anti-retroviral drugs for UDCA non-responders PBC patients. It will be interesting for the reader to see results, regarding the detection of human betaretrovirus in normal subjects or subjects with other liver diseases apart from PBC. For example Xu et al (Hepatology 2014) failed to detect betaretrovirus nucleic acid sequences in six liver disease control patients at the time of liver transplantation in contrast to 2 out of 4 PBC patients. Moreover, Johal et al (J Hepatol 2009) could not identify MMTLV-LV envelope sequences in 20 patients with histologically normal liver tissue, but they did in 50/184 (27%) patients with other liver diseases such as viral hepatitis, alcoholic and non-alcoholic liver disease etc. Finally, Wang et al (Aliment Pharmacol Ther 2015) detect HBRV integrations in 7% and HBRV RNA in BEC lysates in 15% of "liver controls" (including AIH and cryptogenic liver disease). A discussion is needed about these discrepancies and what may be the potential impact of retroviruses in this set of patients without PBC.

*We have rewritten this section as detailed below:*
*The role that HBRV plays in the pathogenesis of PBC is still debated [2, 19]. In early studies, the virus was predominantly detected in lymph nodes rather than in the liver, similar to observations of MMTV infection in mice [20]. Approximately 75% of peri-hepatic lymph node samples derived from PBC patients at the time of liver transplantation were positive for HBRV protein and RNA, whereas only 1 in 3 PBC patients had detectable HBRV RNA in the liver [14]. Other groups experienced difficulty with detection virus in the liver. For example, one lab was unable to detect viral DNA in PBC liver using a single round of PCR and a separate group found HBRV in 5% of patients with PBC during a survey of liver disease patients for infection [21]. In agreement, our lab rarely found hepatic HBRV DNA (~5%) using nested-PCR. Taken together, these studies are concordant and suggest that more sensitive techniques have a higher detection rate in different tissue compartments [4, 14]. Nevertheless, the perceived lack of detection of*

*HBRV at the site of disease has caused considerable controversy and confusion [22],[21]. Indeed, Selmi and colleagues suggested [22], "In our opinion, the only possible final evidence for a role of a betaretrovirus in PBC could be provided by the direct demonstration, possibly through chromatograms, of the insertion of viral sequences in the genome of a large number of patients with PBC."*

*It is generally agreed that the detection of proviral integrations is considered the gold standard to confirm retroviral infection. To address this issue, ligation mediated-PCR was used to identify the junction regions where the betaretroviral long terminal repeat joins up with the human genome. Next generation sequencing was employed to characterize the proviral integrations and increase the sensitivity of the reactions. In these studies, HBRV proviral integrations and HBRV RNA were detected in two thirds of PBC patients' biliary epithelium samples [23]. Viral integrations studies also established the presence of HBRV in PBC patients' lymph nodes, whereas integrations were rarely observed in the liver, in keeping with clinical observations from most laboratories. In vitro studies confirmed that PBC patients' lymph nodes harbored infectious virus following the isolation of the HBRV in cell culture [24]. Taken together, these data suggest that HBRV can be found at the site of disease and isolated from patients with PBC.*

*The prevalence studies also revealed the presence of HBRV in patients without PBC, bringing up the concern with lack of specificity. In our viral integration studies, infection was commonly found in patients with cryptogenic liver disease and autoimmune hepatitis (AIH) as well as a small a proportion of control samples [23]. We had previously observed HBRV in patients with AIH [25], which is consistent with the knowledge that up to 20% of patients with PBC have overlap features with AIH [26, 27]. These data suggest a hypothesis that HBRV may be associated with different phenotypic manifestations of liver disease modulated by genetic and other factors. However, another lab using nested PCR found HBRV in patients with various hepatic diagnoses - but not healthy controls [21]. If HBRV infection is associated with the development of liver disease per se, these data could be compared with early observations following the discovery of hepatitis C virus. Viral infection was not just confined to those with blood transfusions and high risk behavior but also found in patients with various diagnostic categories, such as alcoholic liver disease, hepatitis B virus co-infection, autoimmune hepatitis and cryptogenic cirrhosis to name a few. Another consideration is that better diagnostic methods will be required to determine the true prevalence of HBRV infection in patients and healthy subjects as PCR studies can be prone to artifact.*

A minor comment: as Combivir, Kaletra and Truvada are the commercial names of the drugs, the symbol of trademark must be added.

*As advised, we have added $^{TM}$ symbol when commercial drug names have been mentioned.*

### *Response to Comments from Reviewer 2*

The paper is extremely well written and is an excellent summary of the data implicating retroviruses in the development of and progression of PBC. . There are a few minor errors in syntax- in the core tip last sentence "The use of digital droplet PCR has markedly improved the sensitivity of viral detection in peripheral ? and should enable' Also HBRV interchanged with human betaretrovirus. It should be defined once and the the abbreviation used. Page 4 second sentence- poor construction:

*We have checked and corrected all syntax errors and abbreviations.*