PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	A protocol for a phase IV, open-label, feasibility study investigating non-invasive markers of hepatic fibrosis in people living with HIV-1 and non-alcoholic fatty liver disease randomised to receiving optimised background therapy (OBT) plus maraviroc or OBT
AUTHORS	Bradshaw, Daniel; Gilleece, Yvonne; Verma, Sumita; Abramowicz, Iga; Bremner, Stephen; Perry, Nicky

VERSION 1 – REVIEW

REVIEWER	Emmanuel Tsochatzis UCL Institute for Liver and Digestive Health, Royal Free Hospital, UK
REVIEW RETURNED	03-Dec-2019

GENERAL COMMENTS	No specific comments. Well written protocol and interesting study.

REVIEWER	Chiara Dentone IRCCS Policlinico San Martino Hospital Genoa, Italy
REVIEW RETURNED	29-Dec-2019

GENERAL COMMENTS	This protocol is well described. The objectives are ambitious in the era of antiretroviral therapy as 'single tablet regimen'. In literature there are few data concerning the 'anti-fibrotic' effect of Maraviroc and we need a randomised trial to confirm these data. I suggest, if possible, to add in the schedule of trial procedures, blood samples at week 24, 48 and 96 to search inflammatory biomarkers. If not possible to do the analysis at the same time of the protocol, I suggest to authors to forecast frozen samples for future analyses to strengthen the data of the study.

REVIEWER	H. Nina Kim University of Washington, USA
REVIEW RETURNED	18-Feb-2020

GENERAL COMMENTS	The authors make a reasonably compelling argument for this pilot study of maraviroc to assess feasibility and acceptability of this regimen for the amelioration of NALFD in people living with HIV. However their primary aims do not include any mention of a measure for acceptability (qualitative or otherwise). Self-reported adherence is noted but this in itself is not acceptability. A carefully outlined approach with objective criteria to define NAFLD is indicated. Simply relying on clinician documentation or referral and imaging may lead to misclassification. If imaging is to be used, then

	criteria for imaging needs to be specified clearly. If ultrasound is used, what kind of grading would qualify for steatosis? Why for instance is CAP (which is routinely available in many settings) not used to identify eligible patients? Along these same lines, cirrhosis needs to be clearly defined in the exclusion criteria. Incidentally, one might argue that these patients are particularly in need of maraviroc if it is found to work in this pilot, and excluding these individuals for the main study would limit the scope and generalisability of these findings. The recruitment procedure is somewhat vague; more detail around how many sites and how patients will be actively identified needs to be provided. Alcohol use needs to be clearly documented throughout this trial as this can certainly change and result in potential confounding of results. If this is an exclusion criterion, it would be important to specify the timeframe during which the drinking thresholds apply to exclude moderate/heavy drinkers up front. I am concerned that the PI will be permitted at their discretion to withdraw a patient if their adherence is <80%. Every effort should be made to retain participants in this pilot trial and to minimize withdrawals. Participants could certainly choose at any time to withdraw from the study but poor adherence should not be a reason for the PI to withdraw a participant. Particularly if adherence is to be one of the primary outcomes. This kind of selective withdrawal by the PI can introduce bias to these results. It is worth noting that pilot studies are usually underpowered (and often not formally powered) to assess treatment effect. But they can provide, as suggested here, a preliminary assessment of benefit. If this is the case, then a careful discussion re the choice of significance levels and perhaps avoidance of p-value presentation (over confidence intervals) should be considered as a responsible approach to conducting this research. NOTE: the abstract notes that NAFLD is the leading cause of liver disease in PLWH but this is debatable depending on the setting. HCV or HBV may still dominate in some parts of the world. Our lack of adequate surveillance systems make this a difficult-to-verify statement. The reference (24) by Irvine that is cited for the unit change justification for ELF is actually based on a convenience sample of patients who underwent liver biopsy at a single hospital center and includes a heterogeneous mix of patients that may not be entirely consistent with this population of interest. It would be helpful if a reference could be cited that draws on a NAFLD population (or at least PLWH).

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Emmanuel Tsochatzis
Institution and Country: UCL Institute for Liver and Digestive Health, Royal Free Hospital, UK

Please state any competing interests or state ‘None declared’: None declared

No specific comments. Well written protocol and interesting study.

Reviewer: 2
Reviewer Name: Chiara Dentone
Institution and Country: IRCCS Policlinico San Martino Hospital, Genoa, Italy
Please state any competing interests or state ‘None declared’: None declared

This protocol is well described. The objectives are ambitious in the era of antiretroviral therapy as 'single tablet regimen'.
In literature there are few data concerning the 'anti-fibrotic' effect of Maraviroc and we need a randomised trial to confirm these data.
I suggest, if possible, to add in the schedule of trial procedures, blood samples at week 24, 48 and 96 to search inflammatory biomarkers.
If not possible to do the analysis at the same time of the protocol, I suggest to authors to forecast frozen samples for future analyses to strengthen the data of the study.

We agree that this would be worth exploring. The primary endpoint of the current study is acceptability and feasibility, and unfortunately there will be insufficient available resources for the current study to assess for additional secondary endpoints. However, assessment of changes in serum inflammatory biomarkers will be included in the future efficacy study.

Reviewer: 3
Reviewer Name: H. Nina Kim
Institution and Country: University of Washington, USA
Plase state any competing interests or state ‘None declared’: None

The authors make a reasonably compelling argument for this pilot study of maraviroc to assess feasibility and acceptability of this regimen for the amelioration of NALFD in people living with HIV. However their primary aims do not include any mention of a measure for acceptability (qualitative or otherwise). Self-reported adherence is noted but this in itself is not acceptability.

We will deduce acceptability of using maraviroc as a therapy for NAFLD in PLWH by considering multiple factors including the recruitment rate, retention rate and adherence rates. Although none of these variables individually describes acceptability, high rates of participant recruitment, retention and adherence will serve as a surrogate for this. This has been added into the manuscript.

A carefully outlined approach with objective criteria to define NAFLD is indicated. Simply relying on clinician documentation or referral and imaging may lead to misclassification. If imaging is to be used, then criteria for imaging needs to be specified clearly. If ultrasound is used, what kind of grading would qualify for steatosis? Why for instance is CAP (which is routinely available in many settings) not used to identify eligible patients?

Whilst histological diagnosis is the gold standard for diagnosis of NAFLD, we are undertaking a non-invasive study in order to avoid the requirement for participants to submit to liver biopsy with the potential impact on recruitment, acknowledging that this is a limitation of the study. In our criteria for the diagnosis of NAFLD, we are following the guidelines of the European Association for the Study of the Liver which state that ultrasound is the preferred first-line diagnostic procedure for imaging of NAFLD. We acknowledge that ultrasound may miss some cases, particularly in the context of minimal steatosis; however, the use of ultrasound reflects the modality of choice for NAFLD diagnosis in the UK. CAP score is determined at baseline and the change in this variable is a secondary outcome

measure. In addition, the primary end points are related to feasibilityrather than efficacy, of use of maraviroc in PLWH and NAFLD, and therefore the impact of missing mild cases of NAFLD through use of ultrasound is unlikely to alter these primary outcomes.

Reference to the recommendation by EASL for the use of ultrasound for diagnosis of NAFLD has been incorporated into the manuscript.

Along these same lines, cirrhosis needs to be clearly defined in the exclusion criteria.

Cirrhosis is defined as an exclusion criterion. This may be defined through one or more different modalities including biopsy, appearance on imaging (nodular liver with features of portal hypertension such as splenomegaly > 13cm and/or varices), or liver stiffness measurement on Fibroscan > 13 kPa. The additional detail has been added into the manuscript.

Incidentally, one might argue that these patients are particularly in need of maraviroc if it is found to work in this pilot, and excluding these individuals for the main study would limit the scope and generalisability of these findings.

We have focused on a group of patients without cirrhosis as the aim of NAFLD treatments in the first instance is to prevent progression of hepatic fibrosis, including development of cirrhosis. In this small feasibility study, inclusion of patients with and without cirrhosis would result in too heterogeneous a population to be able to make conclusions about acceptability and feasibility. However, we do agree that maraviroc could be of benefit in patients with cirrhosis and therefore, if maraviroc is found to be acceptable in the current study, a larger trial will be undertaken including patients with cirrhosis.

This additional detail has been added into the manuscript.

The recruitment procedure is somewhat vague; more detail around how many sites and how patients will bactively identified needs to be provided.

The following additional information has been added into the manuscript: “Sixty participants will be recruited in seven UK National Health Service (NHS) HIV clinics”. The following sentence provides detail on how patients will be actively identified by the direct care team or clinical research team: “(i) review of a database of patients in the HIV department with NAFLD, (ii) pre- identification of patients due to attend a routine pre-arranged follow up appointment in the HIV or hepatology service and (iii) review of medical notes during routine clinical follow up. Individuals will be contacted either in person, in clinic or by telephone.”

Alcohol use needs to be clearly documented throughout this trial as this can certainly change and result in potential confounding of results. If this is an exclusion criterion, it would be important to specify the timeframe during which the drinking thresholds apply to exclude moderate/heavy drinkers up front.
A detailed alcohol history is taken at screen and further questions on alcohol consumption are also included in the diet history sequentially through the trial. As in other similar trials [Anstee et al, Contemp Clin Trials 2019], a specific timeframe for drinking thresholds is not given and this is consistent with EASL guidance on NAFLD, which state that the relationship between alcohol and liver injury depends on multiple cofactors including type of alcoholic beverage, drinking patterns and duration of exposure. Therefore, some clinician discretion is used in determining whether or not alcohol may be a factor in liver disease and hence any need for exclusion.

I am concerned that the PI will be permitted at their discretion to withdraw a patient if their adherence is <80%. Every effort should be made to retain participants in this pilot trial and to minimize withdrawals. Participants could certainly choose at any time to withdraw from the study but poor adherence should not be a reason for the PI to withdraw a participant. Particularly if adherence is to be one of the primary outcomes. This kind of selective withdrawal by the PI can introduce bias to these results.

The manuscript has been revised to emphasise that where a participant discontinues IMP for any reason, every effort will be made to continue to follow them up on the study.

“The percentage of IMP compliance for each participant will be calculated. Where this figure is <80%, this will lead to a likely recommendation to discontinue IMP, although this will be at the discretion of the PI . In all circumstances, every effort will be made to continue to follow up the participant on the study. Of note, where a discrepancy exists between self- reported compliance and compliance identified via pill counting, any decision to discontinue IMP will rest with the study PI.”

It is worth noting that pilot studies are usually underpowered (and often not formally powered) to assess treatment effect. But they can provide, as suggested here, a preliminary assessment of benefit. If this is the case, then a careful discussion re the choice of significance levels and perhaps avoidance of p-value presentation (over confidence intervals) should be considered as a responsible approach to conducting this research.

Pilot studies should not be used to test hypotheses and, in line with this, our primary objective is to assess the feasibility of conducting a definitive trial. We have not proposed any significance testing in the protocol and, as stated, will not be presenting any p-values. Instead, we will be presenting 95% confidence intervals to convey the precision of between-groupifferences in the outcome measures.

NOTE: the abstract notes that NAFLD is the leading cause of liver disease in PLWH but this is debatable depending on the setting. HCV or HBV may still dominate in some parts of the world. Our lack of adequate surveillance systems make this a difficult-to-verify statement.
This has been changed to ‘a leading cause of liver disease in PLWH’.

The reference (24) by Irvine that is cited for the unit change justification for ELF is actually based on a convenience sample of patients who underwent liver biopsy at a single hospital center and includes a heterogeneous mix of patients that may not be entirely consistent with this population of interest. It would be helpful if a reference could be cited that draws on a NAFLD population (or at least PLWH).

Many thanks for highlighting this. Additional references have been cited which refer to the use of ELF for assessing hepatic fibrosis in PLWH and people with NAFLD. These are also listed below.

Sherman KE, Abdel-Hameed EA, Ehman RL, Rouster SD, Campa A, Martinez SS, et al. Validation and Refinement of Noninvasive Methods to Assess Hepatic Fibrosis: Magnetic Resonance Elastography Versus Enhanced Liver Fibrosis Index. Dig Dis Sci. 2020;65(4):1252-7.

Inadomi C, Takahashi H, Ogawa Y, Oeda S, Imajo K, Kubotsu Y, et al. Accuracy of the Enhanced Liver Fibrosis test, and combination of the Enhanced Liver Fibrosis and non-invasive tests for the diagnosis of advanced liver fibrosis in patients with non-alcoholic fatty liver disease. Hepatol Res. 2020.

Staufer K, Halilbasic E, Spindelboeck W, Eilenberg M, Prager G, Stadlbauer V, et al. Evaluation and comparison of six noninvasive tests for prediction of significant or advanced fibrosis in nonalcoholic fatty liver disease. United European Gastroenterol J. 2019;7(8):1113-23.

Guillaume M, Moal V, Delabaudiere C, Zuberbuhler F, Robic MA, Lannes A, et al. Direct comparison of the specialised blood fibrosis tests FibroMeter(V2G) and Enhanced Liver Fibrosis score in patients with non-alcoholic fatty liver disease from tertiary care centres. Aliment Pharmacol Ther. 2019;50(11-12):1214-22.