A phase IV, open-label, pilot 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

HEPMARC

Statistical Analysis Plan

Trial registration number: EudraCT number: 2017-004141-24

SAP version: 1.0, 24th March 2022

Protocol version: 5.0, 13th September 2019

Persons contributing to the analysis plan	
Name	Stephen Bremner
Position	Professor of Medical Statistics
Name	Iga Abramowicz
Position	Trial Manager

Authorisation	
Name	Chris Jones
Position	Senior Research Fellow in Medical Statistics
Signature	
Date	24/03/2022
Name	Daniel Bradshaw
Position	Consultant Virologist
Signature	
Date	04/04/2022

Contents

1. SAP revision history

Version updated	Updated version number	Summary of changes	Author of changes	Date
Pre-1.0	1.0	Authorisation by study CI and senior statistician.		

2. Abbreviations

Abbreviation	Meaning
cART	Combination antiretroviral therapy
CLDQ	Chronic liver disease questionnaire
CT	Computer tomography
CVC	cenicriviroc
ELF	Enhanced Liver Fibrosis test
HDL	High density lipo-protein
HIV	Human Immunodeficiency virus
LDL	Low density lipo-protein
MRI	Magnetic resonance imaging
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
OBT	Optimised background therapy
SAP	Statistical Analysis Plan
USS	Ultrasound scan
WPAI:SHP	Work productivity and activity impairment: specific health problem

3. Introduction

3.1 Background and rationale

Liver disease represents an important cause of morbidity and mortality in HIV-infected cohorts in industrialised countries. Data from several cohorts has shown a high prevalence of non-alcoholic fatty liver disease (NAFLD) of between 30-50% [1, 2]. Risk factors for NAFLD are widely recognised and largely associated with lifestyle factors include high BMI, type II diabetes mellitus, dyslipidaemia, smoking and high alcohol consumption as well as HIV-1 infection [3]. As NAFLD is an important risk factor for hepatic fibrosis, including cirrhosis, it represents an increasingly important comorbidity.

At least 10-20% of individuals with NAFLD are likely to progress to steatohepatitis (NASH), a risk factor for hepatic fibrosis and cirrhosis [4]. There are currently few treatments for this condition, although recent data on cenicriviroc (CVC), an R2 and R5 antagonist, suggest it may have a beneficial impact in HIV-infected subjects [5] with further data on CVC from a NASH study in HIV-uninfected participants showing antifibrotic benefit (Phase 2b) [6]. This raises the question as to whether or not the R5-antagonist MVC will demonstrate similar efficacy in NAFLD.

3.2 Study objectives

The *primary objective* is to assess the feasibility and acceptability of the addition of maraviroc to OBT, versus maintenance of OBT alone, in the treatment of NALFD in people living with HIV. This will be assessed with respect to the following:

a) Acceptability of recruitment into the study to eligible individuals
b) Monthly participant recruitment rate
c) Participant retention rate
d) Completeness of the data set
e) Proportion of individuals reporting adverse events
f) Self-reported adherence to the study drug

Secondary objectives

a) Assessment of the effect of addition of maraviroc to OBT, versus continuing OBT alone, on non-invasive serum markers of hepatic fibrosis by 48 and 96 weeks in people living with HIV and NAFLD

b) Assessment of the effect of addition of maraviroc to OBT, versus continuing OBT alone, on non-invasive markers of hepatic fibrosis in people living with HIV and NAFLD by 48 and 96 weeks as measured by transient elastography

c) Assessment of the effect of addition of maraviroc to OBT, versus continuing OBT alone, on non-invasive markers of hepatic fibrosis in people living with HIV and NAFLD by 48 and 96 weeks as measured by computerised tomography [7].

d) Assessment of the effect of addition of maraviroc to OBT, versus continuing OBT alone, on clinical signs of the metabolic syndrome in people living with HIV and NAFLD by 48 and 96 weeks.

e) Assessment of the effect of addition of maraviroc to OBT, versus continuing OBT alone on blood-derived biochemistry in people living with HIV and NAFLD by 48 and 96 weeks.

f) Assessment of the effect of addition of maraviroc to OBT, versus continuing OBT alone on HIV parameters in people living with HIV and NAFLD by 48 and 96 weeks.

g) Assessment of the effect of addition of maraviroc to OBT, versus continuing OBT alone on quality of life in people living with HIV and NAFLD by 48 and 96 weeks.

4. Study methods

4.1 Study design

Parallel group, randomised open label feasibility study

4.2 Randomisation

Randomisation, 1:1, using the web-based system, Sealed Envelope, was stratified according to:
(1) current exposure or past history of ≥ 6 months' exposure to protease inhibitor (PI)-containing antiretroviral therapy versus no current exposure and < 6 months past exposure to PI-containing therapy and
(2) BMI ≥ 25 versus < 25 and
(3) current exposure to a lipid-lowering agent*
(4) diabetes mellitus status (DM 1 or 2 versus no DM)

*HMG CoA reductase inhibitors e.g. statins; cholesterol absorption inhibitor e.g. ezetimibe; bile acid binding drugs e.g. cholestyramine; fibrates; Omega 3 fatty acids

4.3 Sample size

This is a pilot study to evaluate the feasibility and potential efficacy of addition of maraviroc to optimised background combination antiretroviral therapy and therefore no formal sample size calculation has been conducted. Results may be used to estimate the variability of the treatment effect of MVC on the ELF score which in turn may inform the sample size calculation for a larger placebo-controlled RCT.
In a previous biopsy study, a unit increase of 1 in the ELF score was associated with a 2.5-fold increased risk of a liver-related event (adjusted for age and stage of fibrosis) and therefore a unit increase of 1 is deemed to represent a clinically important entity [8]. Assuming the SD of the ELF score is 1.12, with 20 patients in each group for the analysis, a difference in ELF of 1 point can be estimated with a 95% confidence interval from 0.6 to 1.4. Assuming an attrition rate of 33%, a target of 30 individuals will be recruited per group [9].

4.4 Framework

Superiority

4.5 Statistical interim analyses and stopping guidance

4.5.1 Interim analyses

None planned

4.5.2 Early stopping guidelines

Not applicable

4.6 Timing of final analysis

March 2022

4.7 Timing of outcome assessments

Excerpt from protocol paper, Bradshaw et al. 2020. [10]

Table 1 Summary of trial procedures

	Screening visit (–42 days)	Baseline	Week 4* ±2 days	Week 24 ±7 days	Week 48 ±7 days	Week 72 ±7 days	Week 96 ±7 days	Early termination visit
Informed consent	X							
Demographic data and medical history, including full ART history and alcohol assessment	X							
Randomisation		X						
Vital signs	X†	X†	X†	X†	X†	X†	X†	X†
Physical examination, including height, weight and waist circumference	X‡	X‡§		X‡§	X‡§	X‡§	X‡§	X‡§
ECG	X							
Urine dip¶ and pregnancy test (for WOCBP)	X	X		X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X
HIV-associated conditions	X	X		X	X	X	X	X
Symptom and AE review	X	X	X	X	X	X	X	X
Diet and exercise history**		X			X		X	X
CLDQ:NAFLD, SF-36 and WPAI:SHP questionnaires		X			X		X	X
ELF score		X			X		X	X
CD4/CD8 T-cell count	X				X		X	X
HIV-1 RNA level	X		X	X	X	X	X	X
Proviral DNA tropism††		X						
Haematology‡‡	X	X	X	X	X	X	X	X
Routine chemistry§§	X	X	X	X	X	X	X	X
Fasting chemistry¶¶		X			X		X	X
Additional chemistry***	X				X		X	X
HIV, HBV and HCV serology†††	X							
Full liver screen‡‡‡	X							
Ultrasound liver§§§	X							
Fibroscan¶¶¶	X				X		X	X
CT liver:spleen attenuation ratio****		X					X	
Drug dispensation††††		X	X	X	X	X		

*Week 4 visit only for individuals receiving MVC. Blood is unfasted.
†Heart rate, respiratory rate, temperature, BP, lying and standing BP (postural BP at screening only and to be repeated if history indicates).
‡Height only at screening; weight and waist circumference at every visit except week 4.
§Symptom directed physical examination only.
¶Point of care urine dip for haematuria, proteinuria, glycosuria, leucocytes and nitrites.
**Dietary history will be daily intake of olive oil, fruit, vegetables or salad, legumes, fish, wine, meat, white bread, rice and whole-grain bread.[29] Exercise history will be the number of times per week exercise is undertaken, number of minutes of exercise per episode and type of exercise.
††If no result within the preceding 24 weeks.
‡‡Haemoglobin, white cell count and differential, eosinophils and platelets.
§§Sodium, potassium, chloride, creatinine, urea, alanine aminotransferase, aspartate aminotransferase, bilirubin, alkaline phosphatase, gamma glutamyltransferase, albumin, phosphate, creatine kinase, glucose (screening, weeks 4, 24 and 72), lipids (total cholesterol, HDL, LDL and TGs) (weeks 24 and 72 only).
¶¶Fasting glucose and fasting lipids (total cholesterol, HDL, LDL, TGs).
***Haemoglobin A1c.
†††HCV antibody, HCV RNA or HCV antigen, HBsAg; if no prior record of result: HBcAb. HIV antibody-antigen only if no previous documented result.
‡‡‡If no previous record of result: INR, ferritin, caeruloplasmin, copper, thyroid function, alpha-1 antitrypsin, antimitochondrial antibodies, antinuclear antibodies, antismooth muscle antibody, antiliver/kidney/microsomal antibodies-1 and coeliac serology.
§§§If no previous imaging (ultrasound, CT or MRI) result confirming fatty liver in the preceding 24 weeks.
¶¶¶Includes both median stiffness and controlled attenuation parameter scores, to be performed within 7 days of the study visit.
****Optional. To be performed within 7 days of the study visit. Preference is for the 7 days prior to baseline.
††††Only for individuals assigned to the MVC group.
AE, adverse event; ART, antiretroviral therapy; BP, blood pressure; CK, creatine kinase; CLDQ:NAFLD, chronic liver disease questionnaire for non-alcoholic fatty liver disease; ELF, enhanced liver fibrosis; HBsAb, hepatitis B core antibody; HBsAg, hepatitis B surface antigen; HDL, high-density lipoprotein; INR, international normalised ratio; LDL, low-density lipoprotein; MVC, maraviroc; SF-36, 36-Item Short Form Survey; TG, triglycerides; US, ultrasound; WOCBP, women of childbearing potential; WPAI:SHP, Work Productivity and Activity Impairment: Specific Health Problem Questionnaire.

5. Statistical principles

5.1 Confidence intervals and p-values

Confidence intervals (95%) will be presented for estimated proportions and for differences in means.

5.2 Adherence and protocol deviations

Self-reported adherence to the study drug will be calculated in two ways:

(a) Proportion of study medication dispensed that is returned at the final study visit
(b) Mean number of doses taken that were prescribed

Protocol deviations that will be reported include visits that were performed out-of-window [as per 4.7 above].

5.3 Analysis populations

Available cases following intention-to treat-principles

6. Study population

6.1 Screening data

Reasons for being screened out will be summarised in the CONSORT flowchart, according to the 2010 Statement extension to pilot and feasibility studies. [11]

6.2 Eligibility criteria

6.2.1 Inclusion criteria

(1) Aged 18 years and older

(2) HIV-1 infected with durably suppressed (≥ 6 months) HIV VL (<50 copies/ml)
NB. One HIV VL blip (50-200 copies/ml) is allowed in the 6 months prior to screen.
(3) Has evidence of NAFLD on hepatic imaging (USS, CT or MRI) or liver biopsy either at screen or in the 6 months prior to screen

(4) Provides written, informed consent to participate

(5) Is willing to comply with the protocol requirements

(6) If female and of childbearing potential, is using effective birth control methods (as agreed by the investigator) and willing to continue practicing these birth control measures during the trial and for at least 30 days after the end of the trial.

Note: Women who are postmenopausal for least 2 years, women with a total hysterectomy, and women who have a tubal ligation are considered of non-childbearing potential

(7) If male, and sexually-active with female partners of child bearing potential, is using effective barrier contraception, and willing to continue using this during the trial and for at least 30 days after the end of the trial

6.2.2 Exclusion criteria

(1) Severe cardiovascular disease including known angina or history of myocardial infarction

(2) History of postural hypotension, defined as a reduction in the systolic blood pressure of ≥ 20mmHg after standing for at least one minute

(3) Individuals previously exposed to MVC

(4) HIV viral load detectable (≥50 copies/ml). One blip (VL 50-200 copies/ml) within 6 months prior to screen is allowed.

(5) Current HCV or HBV (HBcAb-positive, HBsAg-negative is permitted; anti-HCV Ab positive with HCV RNA or HCV antigen negative for ≥ 6 months following treatment or spontaneous clearance is permitted)

(6) Other chronic liver disease including but not exclusively: cirrhosis, alcohol-related liver disease, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, haemochromatosis, Wilson's disease, alpha-1 antitrypsin deficiency, non-cirrhotic portal hypertension, drug-induced as deemed by a hepatologist
Note: alcohol-related liver disease includes liver disease in the presence of excess alcohol intake as defined according to EASL guidelines 2016 (i.e. >20g/day or >17 units/week for women and >30g/day or >26 units/week for men).
(7) ALT or AST > 5x the ULN (where ULN is defined as 41 IU/L)

(8) Severe renal insufficiency (creatinine clearance < 30 mL/min)

(9) HIV-2 infection

(10) Known allergy or intolerance to MVC or its constituents including hypersensitivity to peanuts or soya

(11) If female, pregnancy or breastfeeding

(12) Individuals currently taking medications or herbal agents that are contraindicated with MVC including St John's Wort.

6.3 Recruitment

Refer to the published protocol paper (page 8) [10]

6.4 Withdrawal/follow up

We will report both withdrawal from prescribed medication and withdrawal from follow-up assessments

Timing of withdrawal will be shown on a CONSORT flow-diagram

The reasons for withdrawal/loss to follow up will be summarised as follows:

Moved away, not contactable, clinician decision (including adverse reaction), participant decision (including pill burden, burden of extra visits, shielding), death

6.5 Baseline participant characteristics

The following sets of baseline characteristics (demographic and clinical) will be summarised by treatment group and overall:

► Age.
► Per cent male.
► Per cent of each ethnicity.
► Duration of HIV infection.
► Nadir CD4 count.
► Baseline CD4 count.
► Per cent with undetectable HIV viral load.
► Per cent receiving PI-based cART versus non-PI-based cART.
► Per cent receiving integrase inhibitor (II) based cART versus non-II-based cART
► Per cent receiving NNRTI based cART versus non-NNRTI-based cART
► Per cent receiving concomitant lipid-lowering therapy.
► BMI.
► Waist circumference.
► Weight.
► Blood pressure.
► Fasting glucose.
► HbA1c.
► Bilirubin.
► ALT
► AST
► Fasting TG
► Fasting LDL.
► Fasting HDL.
► Fasting total cholesterol.
► Fasting HDL:cholesterol ratio.
► Baseline ELF score.
► Baseline Fibroscan stiffness result.
► Baseline Fibroscan CAP score.
► % with a CT liver:spleen attenuation ratio < 1.0.
► Baseline diet score.

Due to the large number of fields above, a full list of individual variables will be presented in the Stata syntax file "HEPMARC.do" which will be added as an appendix to this SAP.

7. Analysis

7.1 Outcome definitions

Proportion of eligible individuals approached who were successfully recruited = #consented/#eligible

Monthly participant recruitment rate = #participants consented/#days study open to recruitment per site per month

Participant retention in the study at 48 weeks = #participants at week 48/#randomised

Participant retention in the study at 96 weeks = #participants at week 96/#randomised

Proportion of participants reporting adverse events at 48 weeks = #participants reporting ≥1 AE since randomisation/#Participants at week 48

Proportion of participants reporting adverse events at 96 weeks = #participants reporting ≥1 AE since randomisation/#Participants at week 96

Level of self-reported adherence to the study drug at 48 and 96 weeks in those allocated to the maraviroc group = 1 - #missed doses/#total doses
1-#pills returns/#pills dispensed

SF36 score will be calculated using the instructions on: https://www.rand.org/health-care/surveys_tools/mos/36-item-short-form/scoring.html [last accessed 24/3/22]

7.2 Analysis methods

All analyses will consist of simple descriptive statistics, by treatment group. Normally distributed variables will be described by their means and standard deviations, skewed continuous variables by their medians and interquartile ranges, and categorical variables by the frequency and percentage in each category. In addition, we will present 95% confidence intervals for the following:

Proportion of eligible individuals approached who are successfully recruited

Participant retention in the study at 48 & 96 weeks

Level of self-reported adherence in the maraviroc group

For each of the secondary outcomes, we will estimate the difference in means (transforming data to normal, as appropriate) between groups and the 95% confidence interval around this difference.

7.3 Missing data

The proportion of missing data per variable will be reported but no imputation will be performed

7.4 Additional analyses

Not applicable

7.5 Harms

Information on adverse events/reactions will be tabulated by treatment group for body system, severity, expectedness, and causality according to section 9 of the protocol (version 5.0, 13/9/19).

7.6 Statistical software

All analyses will be performed using Stata version 17.0 [12]

8. References

1. Vuille-Lessard E, Lebouche B, Lennox L, Routy JP, Costiniuk CT, Pexos C, et al. Nonalcoholic fatty liver disease diagnosed by transient elastography with controlled attenuation parameter in unselected HIV monoinfected patients. Aids. 2016;30(17):2635-43. doi: 10.1097/QAD.0000000000001241. PubMed PMID: 27603289.

2. Maurice JB, Patel A, Scott AJ, Patel K, Thursz M, Lemoine M. Prevalence and risk factors of non-alcoholic fatty liver disease in HIV-monoinfection: a systematic review and meta-analysis. Aids. 2017. doi: 10.1097/QAD.0000000000001504. PubMed PMID: 28398960.

3. Tafesh Z, Verna, EC. Managing nonalcoholic fatty liver disease in patients living with HIV. Current opinion in infectious diseases. 2017;30:12-20.

4. Review T, LaBrecque DR, Abbas Z, Anania F, Ferenci P, Khan AG, et al. World Gastroenterology Organisation global guidelines: Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Journal of clinical gastroenterology. 2014;48(6):467-73. doi: 10.1097/MCG.0000000000000116. PubMed PMID: 24921212.

5. Thompson M, Saag M, DeJesus E, Gathe J, Lalezari J, Landay A, et al. A 48-week randomized phase 2b study evaluating cenicriviroc versus efavirenz in treatment-naive HIV-infected adults with C-C chemokine receptor type 5-tropic virus Aids. 2016;30:869-78.

6. Ratziu V et al. Cenicriviroc Treatment for Adults with Nonalcoholic Steatohepatitis and Fibrosis: Final Analysis of the Phase 2b CENTAUR Study. Hepatology 2020 Sep;72(3):892-905. doi: 10.1002/hep.31108.

7. Zeb I, Li D, Nasir K, Katz R, Larijani VN, Budoff MJ. Computed tomography scans in the evaluation of fatty liver disease in a population based study: the multi-ethnic study of atherosclerosis. Academic radiology. 2012;19(7):811-8. doi: 10.1016/j.acra.2012.02.022. PubMed PMID: 22521729; PubMed Central PMCID: PMC3377794.

8. Irvine KM, Wockner LF, Shanker M, Fagan KJ, Horsfall LU, Fletcher LM, et al. The Enhanced liver fibrosis score is associated with clinical outcomes and disease progression in patients with chronic liver disease. Liver international : official journal of the International Association for the Study of the Liver. 2016;36(3):370-7. doi: 10.1111/liv.12896. PubMed PMID: 26104018.

9. Martinez-Gonzalez MA, Fernandez-Jarne E, Serrano-Martinez M, Wright M, Gomez-Gracia E. Development of a short dietary intake questionnaire for the quantitative estimation of adherence to a cardioprotective Mediterranean diet. European journal of clinical nutrition. 2004;58(11):1550-2. doi: 10.1038/sj.ejcn.1602004. PubMed PMID: 15162136.

10. Bradshaw D, Gilleece Y, Verma S, Abramowicz I, Bremner S, Perry N. 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 alone. BMJ Open 2020;10:e035596. doi:10.1136/bmjopen-2019-035596

11. Eldridge SM, Chan CL, Campbell MJ, Bond CM, Hopewell S, Thabane L, Lancaster GA; PAFS consensus group. CONSORT 2010 statement: extension to randomised pilot and feasibility trials. BMJ. 2016 Oct 24;355:i5239. PMID: 27777223

12. StataCorp. 2021. *Stata Statistical Software: Release 17*. College Station, TX: StataCorp LLC.

9. Appendix 1: Subset of variables for analysis (*if applicable*)

Variable name	Variable type	Range/categories	Description

10. Appendix 2: Derived variables (*if applicable*)

Variable name	Variable type	Range/categories	Derivation	Description