Maraviroc Add-On Therapy for Steatohepatitis in HIV

The MASH study

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MAIN SPONSOR: **Imperial College London**

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Funder

This study is being funded by Viiv Healthcare, and they are also providing unlabelled IMP (Maraviroc).

This trial will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), amended regulations (SI 2006/1928) and the International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines. It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

GLOSSARY OF ABBREVIATIONS

ALT	Alanine aminotransaminase
AMA	Anti- mitochondrial antibody
ANA	Anti- nuclear antibody
ANCA	Anti- neutrophil cytoplasmic antibody
ASMA	Anti- smooth muscle antibody
AST	Aspartate aminotransferase
BMI	Body mass index

CPA	Collagen proportionate area
CT	Cholesterol
CTA	Clinical Trials Authorisation
CYP3A	Cytochrome P3A
DDI	Drug-to-drug interaction
EOT	End of treatment
FBC	Full blood count
FPA	Fat proportionate area
HBs Ag	Hepatitis B s Antigen
HCV	Hepatitis C Virus
HCV Ab	Hepatitis C Virus Antibody
H&E	Haemotoxylin and Eosin
HOMA	Homeostasis model assessment method index
HTA	Human Tissue Act
IL6	Interleukin-6
IMP	Investigational Medicinal Product
GGT	Gamma- glutamyltransferase
HDL	High density lipoprotein
LDL	Low density lipoprotein
LKM	Liver-kidney-mitochondria antibody
LSM	Liver stiffness measurement
MVC	Maraviroc
NAFLD	Non- alcoholic fatty liver disease
NASH	Non- alcoholic steatohepatitis
PBMC	Peripheral blood mononuclear cell

sCD14	Soluble cluster of differentiation 14
sCD163	Soluble cluster of differentiation 163
SmPC	Summary of Product Characteristics
sTNFR1/2	Soluble tumour necrosis factor receptor 1/2
TG	Triglycerides
ULN	Upper limit of normal
USS	Ultrasound Scan

KEYWORDS

NAFLD; steatohepatitis; HIV; maraviroc; fibrosis

STUDY SUMMARY

TITLE Maraviroc Add-On Therapy for Steatohepatitis in HIV

DESIGN Open label, single arm, multi-centre study to evaluate the role of maraviroc in HIV- associated NASH.

POPULATION Patients with well controlled HIV-1 mono-infection and biopsy- confirmed NASH.

TREATMENT Maraviroc add-on therapy 300mg BD (150mg bd with CYP3A inhibitors, 600mg bd with CYP3A inducers) for 48 weeks.

STUDY DURATION 56 weeks

1. Background

Nonalcoholic fatty liver disease (NAFLD) has become one of the most frequent causes of chronic liver disease worldwide.[1] NAFLD is defined by liver steatosis, the accumulation of triglycerides in the hepatocytes, in the absence of a secondary cause such as excessive alcohol consumption. The condition encompasses a spectrum of diseases from nonalcoholic fatty liver (NAFL) to nonalcoholic steatohepatitis(NASH), fibrosis and cirrhosis. In non-HIV patients NAFLD is principally the hepatic manifestation of the metabolic syndrome defined by central obesity, high blood pressure and impaired glucose and lipid homeostasis.[2]

Hepatic fibrosis is the key prognostic indicator for increased liver- related morbidity and mortality in patients with NAFLD.[3] The progression of fibrosis is more rapid in patients with NASH, which is therefore believed to be a key development in the pathogenesis of chronic liver disease in NAFLD.[4] Currently the gold standard for diagnosing NASH and fibrosis is liver biopsy. There have been significant advances in the development of non- invasive markers of liver fibrosis (e.g. measures of liver stiffness such as fibroscan, or serological markers such as NAFLD Fibrosis Score)[5], but there is currently no recommended non-invasive test for the diagnosis of NASH.[6] Therefore, given the importance of NASH in fibrosis development and the slow evolution of fibrosis, most clinical trials in NAFLD investigate the impact of interventions on NASH using histopathological scores (e.g. NAFLD Activity Score) of liver biopsy tissue as a surrogate for reducing fibrosis progression.[7]

In HIV-monoinfected patients on antiretroviral therapy (ART), 10-20% are found to have persistent elevated alanine aminotransferase (ALT) levels (Swiss Cohort) [8] and around 35% have hepatic steatosis on radiological examination.[9] Studies including liver biopsies are scarce and small in participant numbers, but have suggested that a substantial number (around 50%) of HIV mono-infected patients who have a liver biopsy suffer from non-alcoholic steatohepatitis (NASH) and about 20% have clinically significant fibrosis (≥ F2) [9] putting them at risk for cirrhosis and its complications including hepatocellular carcinoma.

In HIV subjects, the pathophysiology of NAFLD/NASH is a complex interaction between chronic HIV viral infection and antiretroviral drug exposure with obesity and the metabolic syndrome [10][11]. A recent study has highlighted the importance of insulin resistance and the metabolic syndrome in the development of adipose- derived inflammation and liver fibrosis in HIV mono-infected patients.[12]

Currently there are very few options for the treatment of NASH both in non-HIV nor in HIV-infected individuals.[13] Life style changes (diet and exercise) remain the recommended treatment of NAFLD/NASH but are often difficult to obtain in practice.[14][15] Some patients with advanced fibrosis may also have some irreversible liver damage not amenable to lifestyle alone. Therefore in response to these issues and the growing prevalence of the disease, an expanding number of drugs are in development in Phase II and

III trials for the general population, which are expected to transform management of the disease in the next 5 years.[7] HIV infection is invariably an exclusion criteria in these trials.

The C-C chemokine receptor type 5, also known as CCR5 or CD195, is found on multiple immune cells including T cells, B cells and macrophages. The HIV-1 gp120 of R5 tropic viruses binds to the CCR5 co-receptor to enter human cells. Physiologically, the CCR5 ligand CCL5 (RANTES) is a chemokine that guides lymphocytes to sites of tissue injury, and CCR5 is expressed on cells that promote fibrogenesis (pro-inflammatory monocytes, Kupffer cells, hepatic stellate cells).

CCL5 is upregulated in patients with liver fibrosis and cirrhosis, [16][17] and its role in the pathogenesis of liver fibrosis has been now well established.[18] The CCR5-CCL5 axis may also have a role in the pathogenesis of NASH through the recruitment of inflammatory cells to diseased adipose tissue (contributing to insulin resistance) and the liver,[19] and mouse models have shown CCL5- induced hepatic inflammation may contribute to the development of hepatocellular carcinoma.[17]

Maraviroc (MVC) is a potent selective CCR5 antagonist that has been approved for the treatment of CCR5-tropic HIV-1 infection in treatment-experienced subjects in the European Union [20] with a favourable hepatic safety profile. [21] In a murine model MVC reduced hepatic steatosis,[22] and in endothelial cells MVC reduced the production of inflammotory cytokines suggesting antiinflammtory effects.[23] Only one small study has investigated the potential beneficial effects in the liver of MVC, in HIV-HCV coinfected patients (published in abstract form), indicating an improvement of liver enzymes and markers of liver fibrosis with MVC.[24] The benefits of MVC have never been assessed in HIV-infected patients with NASH. However, the combined CCR2/CCR5 antagonist Cenicriviroc has demonstrated promising anti-fibrotic effects in Phase II trials for NASH, and is currently being assessed in the Phase III AURORA trial (https://clinicaltrials.gov/ct2/show/NCT03028740).

We propose here to assess whether a MVC add-on therapy has beneficial effects in HIV-infected patients with histologically-proven NASH. An add-on strategy will allow us to maintain patients on their effective HIV regimen, will not require the need for CCR5-tropism testing and will not exclude patients infected with predominant CXCR4-tropic HIV strains.

The aim of this study is to conduct a proof of concept trial which investigates whether Maraviroc reduces the inflammatory infiltrate in the liver of patients with HIV-NASH. If successful the study will inform the first phase III trial in HIV-associated NASH with larger size and longer duration.

2. Research Plan

2.1 Aims and Objectives

Primary Objective: To assess whether 48 weeks of Maraviroc (MVC) add-on antiretroviral therapy changes the hepatic immune cell infiltrate in HIV-1 monoinfected patients with NASH.

Secondary Objectives:

1) To assess the impact of MVC add-on therapy on biochemical metabolic parameters at the end-of-treatment (EOT)
2) To assess the impact of MVC add-on therapy on circulating inflammatory cytokines and adipokines at the EOT
3) To assess whether MVC reduces the NAFLD activity score (NAS) by a minimum of 2 points with at least one point improvement in more than one category and no worsening of fibrosis at EOT [25]
4) To assess whether MVC improves liver fibrosis using liver histology and non-invasive markers of fibrosis (Fibroscan, APRI, Fib-4, NAFLD Fibrosis Score) at EOT
5) To assess the impact of MVC on liver enzyme levels during the course and at the EOT

2.2 Study Design

Design: Multicentre single arm open label trial.

Population:

Inclusion criteria

1. HIV-1 infected individuals (males and females) aged 18-75 years
2. No clinical concern for viralogical failure defined by HIV RNA ≤200 copies/mm^3 for at least 6 months prior to the date of inclusion.
3. CD4 count ≥ 200 cells/mm^3
4. Histological evidence of NASH based on liver histology performed within 12 months prior to visit 1 (Week 0) with a NAFLD activity score (NAS) ≥ 4 with a score of at least 1 in each component (steatosis, lobular inflammation, and hepatocyte ballooning)[26] and <10% weight loss since the time of liver biopsy (see appendix 2 for criteria for offering liver biopsy)
5. Consent to second liver biopsy after 48 weeks treatment with MVC.
6. Patient able to understand and sign a consent form

Exclusion criteria

1) Liver co-morbidities:

1. Positive HBs antigen (HBsAg)
2. Positive HCV antibody (HCVAb), with the exception of subjects with the presence of HCVAb but negative hepatitis C virus RNA without treatment (i.e. spontaneous clearance following acute infection).
3. Underlying acute or chronic liver disease including non- B non- C viral hepatitis (A & E), autoimmune liver disease, biliary disease, hemochromatosis, Wilson´s disease, alpha-1-antitrypsin deficiency.

4. History of decompensated cirrhosis including ascites, hepatic encephalopathy, or variceal bleeding.
5. Suspicion of drug-related toxicity defined by abnormal LFTs following the recent introduction of a new medication.

2) Additional co-morbidities:

1. Active, serious infections that require parenteral antibiotic or antifungal therapy within 30 days prior to screening visit.
2. Active AIDS-defining disease other than oesophageal candidiasis.
3. Any active life- threatening disease
4. Active malignancy (except for early dysplastic lesions eg anal dysplasia)
5. Congestive cardiac failure
6. Severe renal impairment with CrCl<30mL/min

3) Contra-indications to liver biopsy

1. Platelet count <100x10^3 cells/mm^3.
2. INR > 1.4
3. Extra-hepatic biliary duct dilatation
4. Ascites

4) Lifestyle:

1. Excessive alcohol consumption during the last 6 months prior inclusion defined by more than 14 units/week for women or 21 units/week for men

5) Concomitant medications:

1. Patients actively treated with Maraviroc or having received Maraviroc over the last 12 months.
2. Weight reduction through bariatric surgery in the past 5 years or planned during the conduct of the study.
3. Current or anticipated treatment with radiation therapy, cytotoxic chemotherapeutic agents or immunomodulating agents.
4. Receiving any experimental medications within 30 days prior to screening or anticipated use during the trial.
5. Patients receiving pioglitazone, rosiglitazone, vitamin E 800 IU/day and/or ursodeoxycholic acid since these drugs may have confounding effect on efficacy of MVC

6) Others

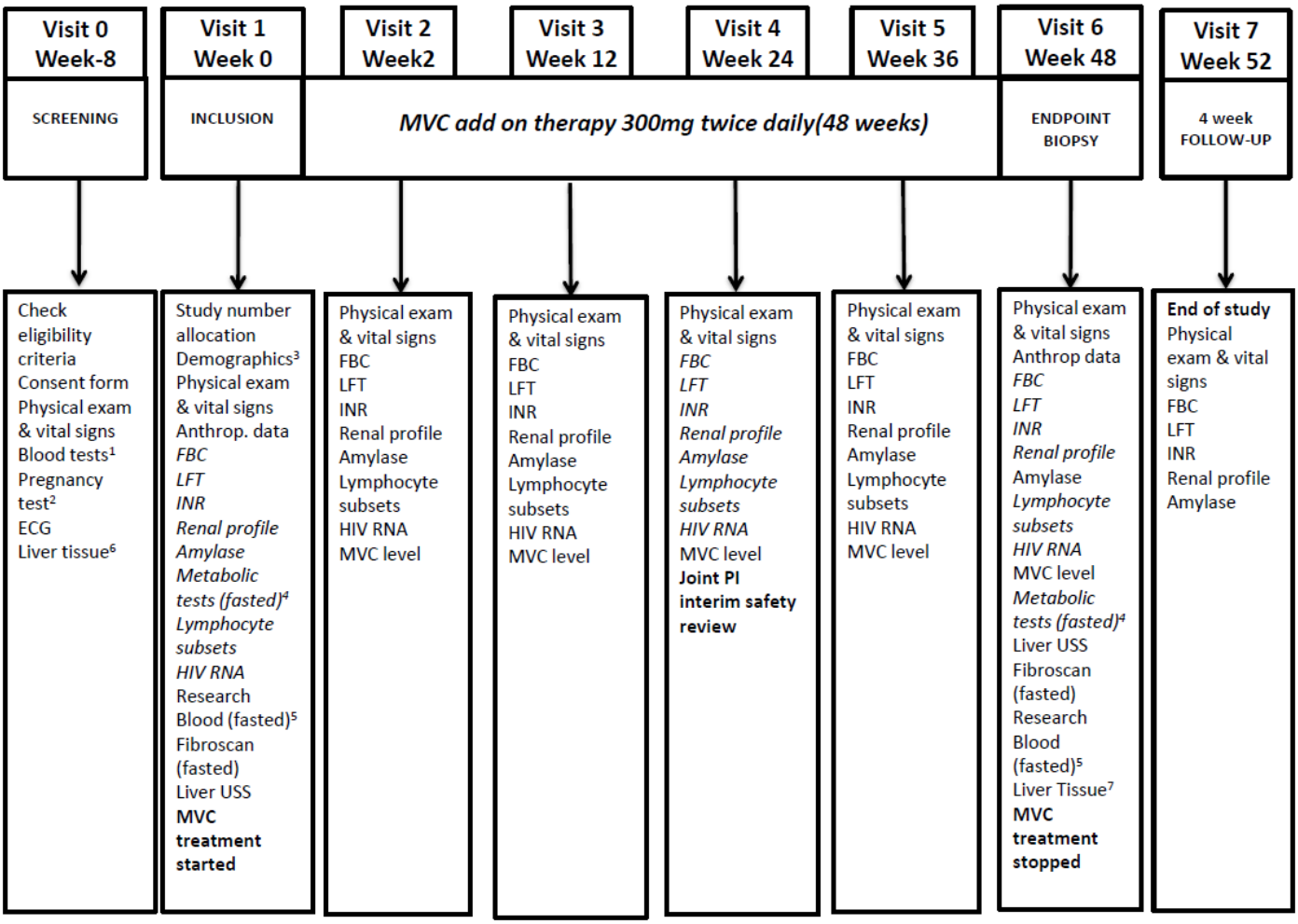
1. Females who are pregnant or breastfeeding
2. Allergy to the study drug or its components (including peanut and soya)
3. Participation in any other clinical trial at Screening without approval from the Sponsor

Intervention:

Following informed consent, patients will receive Maraviroc 300mg BD for 48 weeks. This dose will be adjusted for drug-to-drug interactions (DDI, see section 4.2).

2.3 Summary of Investigations, Treatment and Assessments

Figure 1 and table 1 summarise the study procedure and sample collection at each visit. Subjects will sign an informed consent form and be screened. Subjects with clinical features of NASH but without a liver biopsy within the last 12 months will have a screening liver biopsy. Subjects who have had a liver biopsy within 12 months that meets eligibility criteria and have not lost >10% body weight since the procedure will not require a repeat screening biopsy. **Patients with a screening biopsy that does not meet inclusion criteria will not proceed with the study,** and those who meet inclusion criteria will be invited to return within about 8 weeks of the screening visit to start the trial procedures. Following that baseline inclusion visit, there will be 6 further study visits, which will include a physical examination and blood tests. The end-of-treatment visit at week 48 will also include bedside liver scans and a liver biopsy. The MVC treatment will be discontinued after 48 weeks. During the final visit at week 52, the patient will be reviewed, the results of the liver biopsy and other clinical tests will be discussed with the participant, and appropriate ongoing clinical care arranged. There will be a +/- 7-day treatment window for visit procedures to be performed.



1 Full blood count (FBC, including differential white cell count), liver function tests (LFT), renal profile, amylase, INR, HBV/HCV serology, HCV RNA if HCV serology positive, HIV RNA, lymphocyte subsets, autoimmune liver serology, immunoglobulins, alpha 1 antitrypsin, copper and caeruloplasmin, thyroid function tests, haematinics including ferritin and transferrin saturation

2 serum beta-HCG in women in age of childbearing

3 Includes basic demographics, smoking, recreational drug use, alcohol consumption, medical history, concomitant medications

4 Fasting glucose, insulin, triglycerides, total/ HDL/ LDL cholesterol, ferritin

5 About 35ml blood (15ml lithium heparin, 15ml serum, 5ml whole blood). Whole blood only at week 0.

6 Screening liver biopsy performed within 12 months of baseline visit.

7 Endpoint liver biopsy on all participants at week 48.

Abbreviations: Anthrop: Anthropometric; BP: blood pressure; FBC: full blood count, LFT: liver function tests; MVC: maraviroc; USS: ultrasound

Italicized blood tests at M0, M6 and M12 are standard of care. Where indicated, fasting is overnight.

Fig. 1: Study Procedure

	Visit 0 Week -8 Screening	Visit 1 Week 0 Inclusion	Visit 2 Week 2	Visit 3 Week 12	Visit 4 Week 24	Visit 5 Week 36	Visit 6 Week 48 EOT	Visit 7 Week 52 Final visit/ Premature D/c visit
Study Entry								
Check eligibility criteria	x							
Consent form for study	X							
Clinical Data								
Past medical history	X							
Demographic data		X						
Physical exam and vital signs	X	X	X	X	X	X	X	X
Weight, height, Waist and hip circumference		X					X	
ECG	X							
Imaging								
Fasting fibroscan		X					X	
Liver USS		X					X	
Clinical Blood Tests								
Pregnancy test (serum Beta HCG)	X							
Liver function tests (ALT,AST, ALP, GGT, Total Bili)	X	X	X	X	X	X	X	X
Full blood count (including differential white cell count)	X	X	X	X	X	X	X	X

INR	x	x	x	x	x	x	x	x
Renal profile	x	x	x	x	x	x	x	x
Amylase	x	x	x	x	x	x	x	x
HCV serology	x							
HCV RNA if HCV Ab +ve	x							
HBsAg	x							
HIV RNA	x	x	x	x	x	x	x	x
Lymphocyte subsets	x	x	x	x	x	x	x	x
Transferrin saturation, ferritin	x	x (ferritin only)					x (ferritin only)	
Liver autoantibodies (ASM, ANA, AMA, LKM, ANCA) and immunoglobulins	x							
Alpha-1 Antitrypsin	x							
Serum copper & caeruloplasmin	x							
Fasting glucose, insulin, TG, HDL/LDL, cholesterol		x					x	
Research Blood Tests								
Serum & Plasma for IL6, leptin, adiponectin, usCRP, sCD14, sCD163, TNFR1/2		x					x	
Whole blood (DNA extraction for PNPLA3 and TM6SF2 Genotyping)		x						
MVC Level			x	x	x	x	x	
Liver Histology								

Liver biopsy	x*						x	
Study Drug								
Dispense medicine		x		x	x	x		
Adherence check			x	x	x	x	x	
Safety Monitoring								
Interim adverse safety review					x			
Data Entry								
eCRF data entry	x	x	x	x	x	x	x	x

Table 1: Study visits. * ≤12 months before baseline visit (Week 0).

2.3.1 Liver Biopsy

Eligible subjects with suspected NASH (Appendix 2) who have consented to take part in the study will have a screening liver biopsy. Subjects with liver histology that meets eligibility criteria will continue with the study (Figure 1) but subjects with liver histology outside of eligibility criteria will not continue with the study and proceed to the premature discontinuation visit.

In cases where liver biopsy was performed prior to study consent as part of routine clinical care which confirms subject eligibility, a repeat screening liver biopsy will not be required and we will seek consent to use this liver tissue obtained before enrolment for research purposes in the study.

All patients enrolled in the study will also have an endpoint liver biopsy after 48 weeks treatment with Maraviroc as part of the study protocol.

The liver biopsies will be formalin fixed and paraffin embedded for diagnostic histopathological analysis and for further determination of the immune infiltrate. If there is sufficient liver tissue (appreciation of the clinical investigator) and local resources permitting, a small liver sample (1-2 mm) will be stored in liquid nitrogen locally and transported to the Imperial College Gastroenterology and Hepatology BioBank for future molecular analysis.

Slides will be stained with specialist stains and analysed by immunohistochemistry to identify immune cell sub- types within the liver, specifically CD3+, CD4+, CD8+, T-bet+, CD56, CD68, CD163 and

myeloperoxidase positive cells. Cells will be enumerated under x400 magnification in the baseline liver tissue and compared with liver tissue following 48 weeks treatment with MVC.

Paraffin embedded liver samples will be stained with H&E safran and sirius red. Liver specimens will be analysed by a trained liver pathologist (Prof. R. Goldin, UK) blinded to the clinical, biological and radiological results. Liver steatosis, ballooning and inflammation will be reported using a semi-quantitative scale and used to calculate the non-alcoholic fatty liver disease activity score (NAS) according to Kleiner et al. [26] Fibrosis stage will be reported according to Brunt.[27] The severity of NASH will be graded using the NAS. The recent SAF score incorporating steatosis, inflammation and fibrosis will be also systematically analysed.[28]

CPA (collagen proportionate are) and FPA (fat proportionate area) will be performed at the Royal Free Hospital on liver tissue stained with sirius red at baseline and EOT using validated, automated computer software.[29]

2.3.2 Blood tests

Clinical blood tests, including basic haematology, biochemistry, HIV viral load and CD4 cell count will be collected at each visit. A MVC level will also be measured, and the patients will therefore be asked to refrain from taking the MVC until after blood tests on the day of each visit. Research bloods will be collected at baseline and EOT to measure changes in inflammatory cytokines, adipokines and markers of macrophage activation. Whole blood will be collected at baseline for DNA extraction and genotyping for variants associated with NASH (PNPLA3 and TM6SF2).

2.3.3 Imaging and other clinical tests

Bedside imaging of the liver, including ultrasound and Fibroscan, will be performed at baseline and week 48. ECG will be performed at screening and then if clinically indicated at subsequent visits.

2.4 Patient recruitment and Timelines

We will recruit 30 patients with paired biopsies (see Sample Size Calculation, section 6), about 5 from each centre.

Table 2 summarises the study timeline. We expect the last patient to be included at month 10 after the start of the study and therefore final clinical assessment post EOT liver biopsy at month 22. Full analysis of data will be conducted at month 22-24, with abstracts drafted and submitted to international conference on liver diseases (EASL or AASLD) and on HIV infection (CROI) at months 23-24. Publication of the data will be then submitted to international peer-reviewed journals.

Timing (Month)	Year 1						Year 2						Year 3						Year 4	
	M 2	M 4	M 6	M 8	M 10	M 12	M 14	M 16	M 18	M 20	M 22	M 24	M 26	M 28	M 30	M 32	M 34	M 36	M 38	M 40
Protocol preparation & Ethics approval	x	x	x																	
CRF & data base development	x	x	x																	
Screening & Recruitment				x	x	x	x	x	x	x	x									
Treatment period, data collection & followup				x	x	x	x	x	x	x	x	x	x	x	x	x	x			
Full data analysis																	x	x	x	
Preparation of Abstracts to conferences and paper publication																			x	x

Table 2: study timeline. We will aim to conduct recruitment in a 12-14 month period..

2.5 Study Endpoints

Primary endpoint: a change in the number of hepatic immune cells- including CD3+, CD4+, CD8+, T-bet+, CD56, CD68, CD163 and myeloperoxidase positive cells identified using immunohistochemistry-, following 48 weeks MVC therapy.

Secondary endpoints:

1. Improvement in biochemical (fasting glucose, lipids and HOMA index) metabolic parameters at EOT as compared to baseline.
2. Modification of circulating inflammatory cytokines, adipokines and markers of macrophage activation (high sensitive IL6, sTNFR1/2, sCD14, sCD163, hsCRP, Leptin, Total and High molecular weight adiponectin) at EOT as compared to baseline.
3. Number of subjects with a reduction in the NAS score by ≥2 points without worsening of fibrosis

at EOT as compared to baseline.

4. Number of subjects with a reduction in the degree of liver steatosis, inflammation and/or ballooning at EOT as compared to baseline
5. Number of subjects with a reduction of at least one stage of liver fibrosis in patients with fibrosis at EOT as compared to baseline
6. Number of subjects with a reduction of Fibroscan® values and biochemical markers of fibrosis (APRI, Fib-4, NAFLD Fibrosis Score[5]) from at EOT as compared to baseline
7. Number of subjects with normalization of Fibroscan values at the EOT
8. Number of subjects with a reduction in liver transaminases (ALT and AST) levels during the course and the EOT

3. Withdrawal Criteria

Patients wishing to be withdrawn from the study at any stage will be clearly advised that they can do this and they will be told that this will not affect them receiving further standard best care.

If consent is withdrawn, data and stored human tissue so far collected will be used in the study, unless the patient requests otherwise. If, during the course of study drug administration, the subject prematurely discontinues (D/C), the procedures outlined for the applicable Premature D/C Visit should be completed as defined in Table 1. Ideally this should occur on the day of study drug discontinuation, but no later than 2 days after their final dose of study drug. However, these procedures should not interfere with the initiation of any new treatments or therapeutic modalities that the investigator feels are necessary to treat the subject's condition. Following discontinuation of study drug, the subject will be treated in accordance with the investigator's best clinical judgment. The last dose of any study drug and reason for discontinuation will be recorded in the eCRF system.

If a subject is discontinued from study drugs or during the Post-Treatment Period with an ongoing adverse event or an unresolved laboratory result that is significantly outside of the reference range, the investigator will attempt to provide follow-up until a satisfactory clinical resolution of the laboratory result or adverse event is achieved.

4. Dispensing, Dosage, Drug Adherence and Accountability

4.1 Dispensing:

Viiv Healthcare will provide unlabelled drug bottles containing 60 tablets of either 150mg or 300mg MVC. The bottles will display batch number and date of manufacture only. Drug bottles will then be labelled by pharmacy at the Royal Free Hospital (Dr Alan Wong) in both English and German.

Dispensing will take place at Baseline following informed consent, and at week 12, 24 and 36. Patients will be manually allocated 3 (or 6 if taking the 600mg bd dose) labelled drug bottles (60 tablets per bottle) at each of these visits.

4.2 Dosage and administration:

Following informed consent, patients will receive either Maraviroc (MVC) 150mg BD, 300mg BD or 600mg BD for 48 weeks according to DDIs with pre-existing medication:

- **MVC 150mg BD:** patients co-prescribed CYP3A inhibitors (e.g. ritonavir- boosted protease inhibitors, cobicistat, clarithromycin),
- **MVC 300mg BD:** patients co-prescribed medications with no known DDIs with MVC.
- **MVC 600mg BD:** patients co-prescribed CYP3A inducers (e.g. efavirenz)

Members of the research team and study participants should refer to the latest EU SmPC for Maraviroc (Celsentri) in discussions about potential side effects (see appendix 1). All medications taken by the patients should be checked for potential DDIs on the University of Liverpool HIV Drug Interactions website (http://www.hiv-druginteractions.org/) prior to enrollment, and any concerns discussed with the PI. Clinical care will continue to be guided by the responsible physician.

4.2.1 Maraviroc Pharmacokinetics

Patients will be asked to refrain from taking the study medication on the day of study visits until after blood tests have been sent (at about 9am). A blood sample will be sent for measuring MVC trough level (Figure 1 and Table 1).

4.3 Study drug adherence and accountability:

Subjects must be instructed to bring back all remaining bottles of study medication (full or partially used) at every clinic visit through to the end of treatment. Study medication will be reconciled using medication pill count at every visit after discharge by the investigator or designee to monitor the subject's adherence with the medication regimen.

5. Pharmacovigilence

5.1. Definitions:

Adverse Event (AE): any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. *An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product (IMP), whether or not considered related to the IMP.*

Adverse Reaction (AR): all untoward and unintended responses to an IMP related to any dose administered. *All AEs judged by either the reporting investigator or the sponsor as having reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.*

Unexpected Adverse Reaction: an AR, the nature or severity of which is not consistent with the applicable product information (eg investigator's brochure for an unapproved investigational product or summary of product characteristics (SmPC) for an authorised product*). When the outcome of the adverse reaction is not consistent with the applicable product information this adverse reaction should be considered as unexpected. Side effects documented in the SmPC which occur in a more severe form than anticipated are also considered to be unexpected.*

Serious Adverse Event (SAE) or **Serious Adverse Reaction:** any untoward medical occurrence or effect that at any dose

- **Results in death**
- **Is life-threatening** – *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe*
- **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation**
- **Results in persistent or significant disability or incapacity**
- **Is a congenital anomaly or birth defect**

Medical judgement should be exercised in deciding whether an AE/AR is serious in other situations. Important AE/ARs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

Suspected Unexpected Serious Adverse Reaction (SUSAR): any suspected adverse reaction related to an IMP that is both unexpected and serious.

5.2. Causality:

Most adverse events and adverse drug reactions that occur in this study, whether they are serious or not, will be expected treatment-related toxicities due to the drugs used in this study. The assignment of the causality should be made by the investigator responsible for the care of the participant using the definitions in the table below.

If any doubt about the causality exists the local investigator should inform the study coordination centre who will notify the Chief Investigator. The pharmaceutical companies and/or other clinicians may be asked to advise in some cases.

In the case of discrepant views on causality between the investigator and others, all parties will discuss the case. In the event that no agreement is made, the MHRA will be informed of both points of view.

Relationship	Description
Unrelated	There is no evidence of any causal relationship
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant's clinical condition, other concomitant treatment).
Possible	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the participant's clinical condition, other concomitant treatments).
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
Definitely	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
Not assessable	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

5.3 Reporting procedures:

Recording of AEs/ SAEs will begin as soon as subjects sign a study consent form. Subjects will receive detailed information about common and serious known adverse reactions to MVC (See PIS, and SmPC in Appendix 1), and instructed to contact the study team immediately should they develop any symptoms of concern. The week 2 visit will primarily assess tolerability to the drug and include a physical examination and blood tests. An interim analysis of any safety concerns will be conducted 6 months after recruitment of the first patient via teleconference between all the study PIs.

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the study coordination centre in the first instance.

5.3.1 Non serious AR/AEs

All such toxicities, whether expected or not, should be recorded in the toxicity section of the relevant case report form and sent to the study coordination centre within one month of the form being due.

5.3.2 Serious AR/AEs

All SAEs and SUSARs should be reported to the sponsor within 24 hours of the local site becoming aware of the event. Under no circumstances should the reporting of SAEs and SUSARs to the sponsor be made more than 24 hours after the event. The SAE form asks for nature of event, date of onset, severity, corrective therapies given, outcome and causality (i.e. unrelated, unlikely, possible, probably, definitely). The responsible investigator should sign the causality of the event. Additional information should be sent within 5 days if the reaction has not resolved at the time of reporting.

5.3.3 Pregnancy

There is no proven teratogenicity with MVC. However, in animal models exposed to high doses of MVC there is some reproductive toxicity (Appendix 1, MVC SmPC). Therefore, subjects will be advised not to become pregnant and to avoid sperm donation from study Day 1 until 10 days after stopping MVC.

In the event that a positive result is obtained on a pregnancy test for a subject or a subject reports becoming pregnant during the Treatment Period, the administration of MVC to that subject must be discontinued immediately.

Pregnancy in a study subject must be reported to the sponsor within 1 working day of the site becoming aware of the pregnancy. The sponsor will then in turn inform ViiV.

Information regarding a pregnancy occurrence in a study subject and the outcome of the pregnancy will be collected for pregnancies through the duration of the study. Pregnancy in a study subject is not considered an adverse event. However, the medical outcome of an elective or spontaneous abortion, stillbirth or congenital anomaly is considered a serious adverse event and must be reported to the sponsor within 24 hours of the site becoming aware of the event.

5.3.4 Hepatotoxicity

Differentiating drug- induced liver injury from the underlying disease process under investigation can be challenging. This study will use recently published guidance to guide the response to fluctuations in liver function tests during the course of the trial (Appendix 3)[30].

SAEs

An SAE form should be completed and faxed to the study coordination centre for all SAEs within 24 hours. However hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs. The study coordination centre will notify the Sponsor of all SAEs occurring during the study within 24 hrs of becoming aware of the event.

SUSARs

In the case of suspected unexpected serious adverse reactions, the staff at the site should:

Complete the SAE case report form & send it immediately (within 24 hours, preferably by fax), signed and dated to the study coordination centre together with relevant treatment forms and anonymised copies of all relevant investigations.

Or

Contact the study coordination centre by phone and then send the completed SAE form to the study coordination centre within the following 24 hours as above.

The study coordination centre will notify the MHRA, REC and the Sponsor of all SUSARs occurring during the study according to the following timelines; fatal and life-threatening within 7 days of notification and non-life threatening within 15 days. All investigators will be informed of all SUSARs occurring throughout the study.

Contact details for reporting SAEs and SUSARs

Fax: 020 7724 9369, attention Dr Maud Lemoine

Email: m.lemoine@imperial.ac.uk/JRCO.CTIMP.Team@imperial.ac.uk

Please send SAE forms to: Dr Maud Lemoine & Sponsor

Tel: 020 7594 9022 (Mon to Fri 09.00 – 17.00)

6. Statistics and Sample Size

Assuming that Maraviroc may reduce the number of inflammatory cells from 10.88 to 3.76[31] within the same individual when the standard deviation of the difference is 2.0, recruitment of 30 persons will provide a >95% power of demonstrating a difference before and after the treatment at a one-sided significance level of 5%.

Statistical analysis will be conducted by a trained statistician using the anonymised data collected on the eCRF (see section7) on appropriate software. The primary endpoint is the change in inflammatory cell infiltrate between baseline and end-of-trial liver biopsies. A numerical value will be obtained as the average number of inflammatory cells per high power field over 3 fields counted by two pathologists blinded to the biopsy sequence. A paired t test will be used to determine whether observed changes are significant.

7. Data and Sample Storage

InForm software will be used for eCRF data entry across all study sites. ITMTM (Integrated Trial Management) System is a web-based data entry system which builds an Oracle database for each individual clinical trial. This will ensure the data can be collected accurately and stored securely. InForm is widely used across the pharmaceutical industry and Imperial College was the first academic organisation in the UK to adopt it. The study may be subject to inspection and audit by Imperial College London under their remit as sponsor, the Study Coordination Centre and other regulatory bodies to ensure adherence to GCP.

Research samples will be shipped into Imperial College and stored in -80 degree freezers in category 3 laboratories at Imperial College. These freezers are fully alarmed, serviced and HTA compliant. Access is

strictly limited to authorised personnel and all samples are labelled in an anonymised format and their storage and movement documented.

Data and all appropriate study documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period.

7.1 Future biomedical research

The consent form will seek permission for any research samples remaining at the end of this study to be transferred into Imperial's Hepatology and Gastroenterology Biobank (REC reference 16/SC/0021) in category 3 laboratory -80 degree freezers, and permission therefore for these samples to be used in any future research deemed appropriate by the Department's Biobank Governance Committee.

Where a patient has also consented to their remaining research samples being transferred into Imperial College's Hepatology and Gastroenterology Biobank at the end of the study, a copy of the patient's consent form will be provided to the Department's Biobank Governance Committee to prove permission for those samples to be kept and used where appropriate for future research. These copies will be stored in locked and designated Biobank filing cabinets and will be made available only to HTA or HTA delegated inspectors. They may also be referred to in the instance where a patient, in the future, withdraws consent for further research use and further storage by the Biobank. These samples will then be destroyed. HTA regulation and Data Protection legislation will be complied with at all times.

8. Monitoring and Audit

Imperial College will be the sponsor of this study. Participating sites will be expected to conform to GCP and protocol compliance. The Clinical Research Fellow for this study will visit participating sites regularly for monitoring purposes and Imperial College and the main regulatory bodies may at any time ask to monitor and audit at participating sites. InForm software will be used for the eCRF.

9. Regulatory Issues

9.1 CTA:

This study has Clinical Trials Authorisation from the UK Competent Authority; MHRA**. Reference: 19174/0382/001-0001**

9.2 Ethics and HRA approvals:

The study has been approved by the **East of England- Cambridgeshire and Hertfordshire Research Ethics Committee** under REC ref. **17/EE/0387.**

The three study sites in Germany will be required to apply for separate ethical approval through their own national system.

The study has also been submitted to the HRA for approval. Following the HRA initial assessment sites in the UK can be contacted to seek feasibility (capacity and capability). The coordinating centre will provide each participating site with a validated submission package (Statement of Activities, Schedule of Assessments, study documents, and letter of provisional agreement, followed by full permission). Each participating site must await full HRA approval and management permission (confirmation of capacity and capacity) from each R&D office (local site) before recruitment of patients can begin at that site.

The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

9.3 Consent:

Consent to enter the study will be sought from each participant only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed participant consent will be obtained. The right of the participant to refuse to participate without giving reasons will be respected. After the participant has entered the trial the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so will be recorded. In these cases the participant will remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

The consent form will also seek permission for any samples remaining at the end of this study to be transferred into Imperial's Hepatology and Gastroenterology Biobank (REC reference 16/SC/0021), and permission therefore for these samples to be used in any future research deemed appropriate by the Department's Biobank Governance Committee. If the patients do not consent to this their samples will be destroyed appropriately.

9.4 Confidentiality:

Participants' identification data will be required for the registration process. The Study Coordination Centre will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

9.6 Indemnity:

Imperial College holds standard negligent harm and non-negligent harm insurance policies which apply to this study.

9.7 Sponsor:

Imperial College London will act as the main Sponsor for this study.

9.8 Funding:

This study will be funded by Viiv Healthcare.

9.9 Audits and inspections:

The study may be subject to inspection and audit by Imperial College London under their remit as Sponsor, the Study Coordination Centre and other regulatory bodies to ensure adherence to GCP.

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Appendix 1: See attached SmPC for Celsentri (brand name of Maraviroc)

Appendix 2

It is standard practice for patients meeting the following criteria to be sent for liver biopsy with a clinical suspicion of NASH[6]:

Radiological evidence of hepatic steatosis

And

Fasting transient elastography (Fibroscan®) ≥7kPa [32] (or serological test indicating significant fibrosis)

And/or

Persistant elevated ALT >x2 upper limit of normal

And

Absence of contra-indication to liver biopsy: platelet count <100x10^3/mm^3, INR>1.4, extra-hepatic biliary duct dilatation, ascites.

And

Patient able to understand the procedure and sign a consent form for liver biopsy

Appendix 3

Treatment-Emergent ALT	Treatment-Emergent Bilirubin	Liver symptoms	Action
Normal baseline: ALT > 5× ULN **Elevated baseline: ALT > 3× baseline or > 300 U/L (whichever occurs first)**	Normal For patients with Gilbert's syndrome: No change in baseline TBL	None	Repeat ALT, AST, ALP, TBL, in 2–5 days Follow-up for symptoms.
Normal baseline: ALT > 8× ULN	Normal Patients with Gilbert's	None	Interrupt study drug. Initiate close monitoring and workup for competing

Elevated baseline: ALT > 8× baseline or > 500 U/L (whichever occurs first)	syndrome: No change in baseline TBL		etiologies. Study drug can be restarted only if another etiology is identified and liver enzymes return to baseline.
Normal baseline: ALT > 5× ULN **Elevated baseline: ALT > 3× baseline or > 300 U/L (whichever occurs first)**	TBL > 2× ULN For patients with Gilbert's syndrome: Doubling of direct bilirubin	None	Interrupt study drug. Initiate close monitoring and workup for competing etiologies. Study drug can be restarted only if another etiology is identified and liver enzymes return to baseline.
Normal baseline: ALT > 5× ULN **Elevated baseline: ALT > 3× baseline or > 300 U/L (whichever occurs first)**	Normal or elevated	Severe fatigue, nausea, vomiting, right upper quadrant pain	Interrupt study drug. Initiate close monitoring and workup for competing etiologies. Study drug can be restarted only if another etiology is identified and liver enzymes return to baseline.

Table taken from recently published position paper on drug toxicity in NAFLD trials.[30] ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBL, total bilirubin; ULN, upper limit of normal.