Clinical Study Protocol

A Pilot Study to Evaluate Anti-HCV Effect of Maraviroc in HIV/HCV Co-infected Patients

MAraViroc Efficacy foR HepatItis C (MAVERIC)

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List of Abbreviations

AE	Adverse Event/Adverse Experience
Coinfection	Identifier for those with both HIV and hepatitis B or C
DAA	Directly Acting Antiviral
ART	Highly Active Anti-retroviral Therapy
HBV	Hepatitis B Infection
HCV	Hepatitis C Infection
HIPAA	Health Insurance Portability and Accountability Act
MAVERIC	Study short name
IHV	Institute of Human Virology
IRB	Institutional Review Board
N	Number (typically refers to number of subjects/sample size)
PI	Principal Investigator
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SAE	Serious Adverse Event/Serious Adverse Experience
SVR	Sustained Virologic Response 12 weeks following therapy
VL	Viral Load
WHO	World Health Organization

Protocol Summary

Full Title:	**A Pilot Study to Evaluate Anti-HCV Effect of Maraviroc in HIV/HCV Co-infected Patients**
Short Title:	MAVERIC
Conducted by:	Institute of Human Virology, University of Maryland
Principal Investigator:	Lydia Tang, MBChB
Sample Size:	N= 10
Accrual Ceiling:	15
Study Population:	Adults infected with viral hepatitis C and being treated for HIV infection
Study Design:	Single-site, longitudinal, open-label, interventional pilot study for evaluation of the effectiveness of Maraviroc on HCV viral levels in patients with HCV and HIV undergoing antiretroviral therapy. Participants will be seen at a IHV Clinic, Baltimore MD 21201.
Study Duration:	*Start Date:* August 1, 2016 *End Date:* August 1, 2017.
Primary Objective:	1. Determine the in-vivo antiviral efficacy of Maraviroc in HIV/HCV coinfected patients
Secondary Objectives:	1. Determine the viral kinetics of HCV in HIV/HCV infected subjects initiated with maraviroc. 2. Assess the safety and tolerability of adding maraviroc to ART in HIV/HCV coinfected subjects.
Exploratory Objectives:	Virologic and Immunologic assays to evaluate changes associated with CCR5 antagonism.
Endpoints:	*Primary Endpoint:* 1. Change in HCV VL from baseline to day 7. **Secondary Endpoints:** 1. Changes in HCV slope/log change in HCV VL between each patient 2. Changes in HCV slope between the two groups 3. Changes in HCV VL from baseline to day 2, day 3, day 5, week 1, week 2, 3, 4 4. Changes in CD4 and HIV viral load 5. Patient reports of adverse events 6. Percentage of patient with breakthrough of HIV

Précis

Hepatitis C virus (HCV) chronically infects over 170 million people worldwide.[1-3] Hepatitis C virus (HCV) is a leading cause of chronic liver disease and the leading indication for liver transplantation in the United States (US) with estimates of more than 5 million individuals infected in the US.[4] Standard therapy for HCV is undergoing significant change, moving away from interferon injections and ribavirin toward all oral directly acting antivirals (DAAs). It is expected that treatment rates will increase and success of treatment will continue to improve.[5]

HCV infection occurs more commonly in patients infected with HIV due to shared routes of transmission. HIV infection modifies the natural history of HCV infection considerably by increasing persistence, accelerating liver fibrosgenesis and historically is associated with poorer rates of responses. Although recent studies using DAA agents have been promising with high sustained virologic response (SVR, considered functional cure), widespread uptake of DAA agents in HIV/HCV coinfected patients is restricted due to unrecognized drug–drug interactions between HIV antiretroviral therapy (ART) and DAA agent. In this regard, treatment of HCV in HIV/HCV coinfected patients remains a complex issue.

Recently, in-vitro studies have demonstrated that maraviroc, and HIV ART that exerts anti-HIV effects through CCR5 antagonism, appears to have significant anti-viral effect on HCV replication comparable to sofosbuvir–a potent HCV NS5B inhibitor and the back bone to several combination anti-HCV DAA therapy currently approved. In this study, we propose to characterize the antiviral effect of Maraviroc in HIV/HCV coinfected patents naïve to anti-HCV DAA therapy. We intend to evaluate HCV viral kinetics after adding maraviroc to existing ART in 10 HIV/HCV coinfected patients by performing serial HCV RNA measurements. This study will provide the in-vivo evidence that CCR5 antagonists can be used to treat HCV and may offer prolonged effect in the HIV/HCV coinfection by assisting with modulation of hepatic regeneration pathways.

1 Background Information and Scientific Rationale

1.1 Epidemiology

Hepatitis C virus (HCV) is a major cause of chronic liver disease and is the leading indication for liver transplantation in the US. Approximately 4.1 million individuals (1.6%) in the US population have been infected with HCV.[6] Approximately 50% of HCV infected persons have been tested, with the remainder unaware of their diagnosis. Of those that reach care, 16% have been treated, and 9% have been cured of HCV.[7] [8] Among HIV-positive individuals, coinfection with HCV is relatively high, ranging from 15% to 30%.[9,10] Liver disease in HIV patients has become a major source of morbidity and mortality in the US and worldwide.[11] HIV/HCV coinfection is especially problematic in the US now that HIV patients are living longer on highly active antiretroviral therapy (ART), and are often dying of complications from liver disease primarily related to HCV. In addition, more than 75% of adults in the US infected with HCV were born between 1945 through 1965. As a result, the prevalence of liver disease resulting from HCV is likely to increase over time, adding a significant burden to the medical system. It is expected to continue to rise as more and more people get screened. The impact of chronic HCV on liver failure, HCC and mortality is enormous and continues to increase at an alarming rate. Recent advances in effective pill-only treatment regimens to treat and cure HCV is promising making it feasible to test, link and treat this population effectively.

1.2 Baltimore and Washington DC

Baltimore has been cited as a leading city in terms of injection drug use, ranking second in the nation for intravenous drug use (IVU) per capita in 2004[12]. A more recent study looking at patterns of drug use and infectious disease in Baltimore drug users found that 45% of recent users of cocaine or heroin were infected with hepatitis C and with nearly 8% co-infected with HIV[13].

1.3 Treatment

Until recently, a major limiting factor in expansion of treatment for HCV has been standard of care therapy with pegylated interferon, ribavirin, and more recently for genotype 1 disease, boceprevir and telaprevir. These medications have significant side effect profiles, response-based dosing regimens of over 6 months, and decreased efficacy in key groups including: those with genotype 1 disease, HIV coinfection, and cirrhosis. In particular the response rates observed in HIV/HV coinfected patients were much lower than those without HIV infection.[14,15] However, with new therapeutics becoming available, the picture is quite hopeful. There has been dramatic improvement using all oral DAA regimens with sustained virologic response (SVR) rates over 90%, short-duration therapies and well-tolerated, simple regimens including among those with HIV coinfection. Although the rates of SVR has been relatively high in limited studies that have evaluated these novel DAA regimens in HIV/HCV coinfected patients, it is unclear whether

achieving an SVR will result in regression of liver fibrosis. It is also not understood, at what rate the regression of fibrosis may occur and whether it would occur in all patients regardless of HIV status. In this regard, HIV/HCV coinfected patients have demonstrated a much more rapid progression of liver fibrosis over time. Hence, management of HIV/HCV coinfected patients offer several challenges that need to be addressed.

1.4 Rationale

CCR5 is a chemokine co-receptor best known for its central role in HIV binding to T-cells. However, CCR5 is expressed in hepatocytes, stellate cells and Kupffer cells within the liver[16] and may mediate liver fibrosis.[17-19] Interaction between HIV (or gp120) and CCR5 has been shown to upregulate HCV replication as well.[20] A recent study hypothesized that CCR5 blockade might modulate HCV replication.[21] They examined the effect of CCR5 blockade using two potent receptor antagonists, cenicriviroc (dual CCR2/5 antagonist) and maraviroc (CCR5 antagonist), on HCV replication in-vitro. Both agents are known to inhibit HIV replication and cenicriviroc is being evaluated in a Phase 2b trial in adults with NASH and hepatic fibrosis. Briefly, 100,000 Huh7.5$_{JFH1}$ human hepatocyte cells, which constitutively produce a cell-line adapted strain of HCV genotype 2 and express CCR5 were seeded in 24-well format plates. The next day, cells were incubated with 25 ug/ml, 0.25 ug/ml, or 0.0025 ug/ml of the antiviral drugs under investigation. These included maraviroc or cenicriviroc, sofosbuvir (an HCV polymerase inhibitor positive control) or raltegravir (an HIV integrase inhibitor negative control). The culture supernatants were collected after 24 hours of incubation with the drug. Cell number was evaluated to determine if cell viability affected HCV viral load production. HCV replication was monitored using a commercial HCV Core Antigen ELISA assay (Cell BioLabs, San Diego, CA). Negative strand HCV RNA was detected by a qualitative strand-specific RT-PCR assay as a measure of actively replicating virus . Positive strand HCV RNA was measured by qRT-PCR in the cell culture supernatant.

Collectively, CCR5 blockade with cenicriviroc & maraviroc decreased HCV core production, HCV negative strand production and HCV RNA released to supernatant to extents comparable with sofosbuvir, a known HCV NS5B inhibitor with extremely potent anti-HCV activity in-vitro and in-vivo.[22] The results were specific to R5 antagonism since Raltegravir (an HIV integrase inhibitor) did not lead to a similar effect on HCV replication. These results led us to believe that CCR5 blockade appears to modulate HCV replication in an *in-vitro* Huh7.5$_{JFH1}$ human hepatocyte cell system. This potent inhibition suggests that CCR5 may represent a new target for multidrug HCV therapy and warrants evaluation in HIV/HCV coinfected patients. In this regard, we intend to study a small group pf HIV/HCV infected patients.

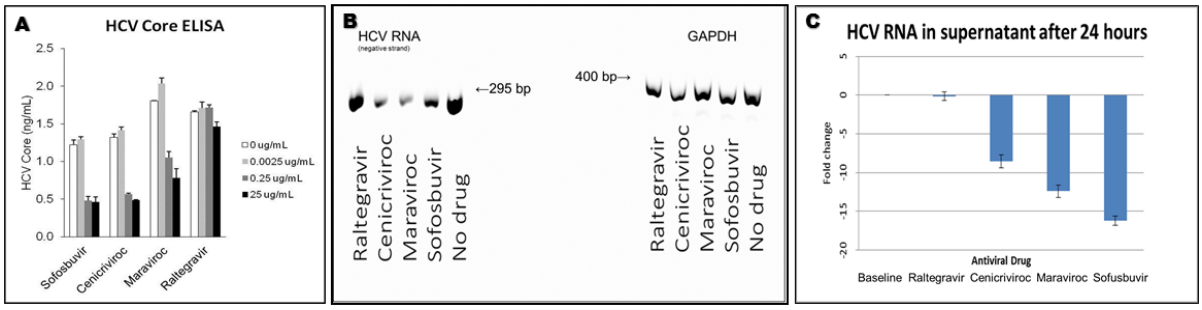

Figure 1A – Change in HCV Core Antigen concentrations

A dose-dependent response was observed for maraviroc, cenicriviroc, and sofosbuvir at the 0.25 ug/ml and 25 ug/ml concentrations but not at 0.0025 ug/ml. Raltegravir had no effect on HCV core antigen production, nor did DMSO buffer controls. Cell viability was not affected by any drug or by the DMSO buffer solution.

Figure 1B – Change in HCV Negative Strand RNA quantity

RNA from cell lysates was extracted using the mirVana miRNA Isolation Kit (Ambion) and resuspended in 50 uL of elution buffer. HCV RNA was detected by reverse transcription PCR and primers HCV-II sense (5'-CAC TCC CCT GTG AGG AAC T-3', nucleotides [nt] 38–56 of the 5'UTR) and HCV-I antisense (5'-TGG ATG CAC GGT CTA CGA GAC CTC-3', nt 342–320). Thirty cycles of PCR (94C for 30 sec, 58C for 1 min, and 72C for 2 min) were performed, and PCR products (295 base pairs in length) were visualized by gel electrophoresis. GAPDH (400 bp) was amplified as a control.

Figure 1C – Change in HCV Positive Strand RNA

HCV RNA was measured in the cell culture supernatants after 24 hours of drug incubation. Data shown is the fold change of RNA detected normalized and compared to HCV RNA at baseline.

1.5 Institutional Background

The Institute of Human Virology (IHV) is a world renowned institution dedicated to the study of natural history, pathogenesis, and therapeutics for human viral infection. The IHV has led the field in HIV and HCV pathogenesis and therapeutics for over the past two decades. IHV has spearheaded HIV therapeutics and as a single institution has initiated ART in over 1 million HIV-infected subjects globally. In this study, IHV is partnered with University of Cincinnati with Dr. Kenneth E Sherman, who is a leading hepatologist in the world and a pioneer in studies involving HIV and HCV coinfection. The preliminary data supporting this trial was performed in Dr. Sherman's laboratory.

2 Study Objectives

2.1 Primary Objective

Determine the in-vivo anti-HCV efficacy of Maraviroc in HIV/HCV coinfected patients.

2.2 Secondary Objectives

1. Determine the viral kinetics of HCV in HIV/HCV infected subjects initiated with maraviroc.

2. Assess the safety and tolerability of adding maraviroc to ART in HIV/HCV coinfected subject

2.3 Exploratory Objectives

Virologic and Immunologic assays to evaluate changes associated with CCR5 antagonism.

3 Study Design

This is a single-site, open-label, randomized, prospective, cross-over pilot interventional study for evaluating the anti-HCV effect if CCR5 antagonism in HIV/HCV coinfected patients receiving ART.

3.1 Study Drug

Maraviroc (trade name: **Selzentry**) is an antiretroviral drug in the CCR5 receptor antagonist class used in the treatment of HIV infection. It is also classed as an entry inhibitor. Maraviroc was approved for the treatment of HIV infection by the Food and Drug Administration in 2007. Specifically, maraviroc is a negative allosteric modulator of the chemokine CCR5 receptor, found on the surface of T lymphocytes. The chemokine receptor CCR5 is an essential co-receptor for most HIV strains and necessary for HIV to infect lymphocytes. The drug binds to CCR5 at a non-competitive site and alters gp120 binding site of CCR5, thereby blocking the HIV entry into the target cell.[23] Maraviroc is very safe and Phase III clinical trials did not show any difference in the incidence of adverse events in the placebo or maraviroc treated groups.

Dosing

Available as 150 or 300 mg tablets. As per labeling based on concomitant ART (see table 1 below).

Table 1. Maraviroc dosing for participants based on concomitant ART

Background Antiretroviral Therapy Regimen	Maraviroc dose
With NRTI's, tipranavir/ritonavir, nevirapine, raltegravir, and other drugs that are not potent CYP3A inducers nor inhibitors	300mg tablet twice a day
With potent CYP3A inducers including efavirenz (without a potent CYP3A inhibitor)	600mg (two 300mg tablets) twice a day
Boosted protease inhibitor-containing regimen	150mg tablet twice a day

3.2 Research Strategy

Study subjects will be selected and enrolled at a single site at IHV. HIV infected participants (coinfected with HCV and DAA-treatment naïve for HCV) who are well controlled on ART will receive Maraviroc for one month and serial measurements of viral and immune parameters will be performed. Rationale for not recruiting treatment naïve HIV/HCV coinfected participants is based on previous studies that have demonstrated an increase in HCV VL in HIV/HCV coinfected patients who initiated ART.[24] We believe this will interfere from characterizing the anti-viral effect, if any, of CCR5 antagonism. Hence, we plan to do an interventional study with frequent in-vivo viral kinetic analysis to evaluate anti-HCV effect of CCR5 antagonism.

Patients with HIV/HCV coinfection with HIV viral suppression and active HCV infection will be included. Patients will be randomized to one of 2 cohorts: immediate start of maraviroc (cohort 1), and delayed start (cohort 2). All patients will receive 4 weeks of Maraviroc (dose adjusted according to intake of concurrent CYP 3A inducers, see table 1) in addition to their regular ART. The immediate start group will receive maraviroc in addition to their HIV antiretroviral regimen from week 0 to 4. This will be followed by a wash out period of 4 weeks after cessation of Maraviroc. After the 4-week wash out period, patients will be crossed over the no-maraviroc arm. The delayed start group will receive no maraviroc from weeks 0 to 4, followed by a 4 week wash out period, before crossing over to the on-maraviroc arm. Figure 2 below illustrates the study scheme.

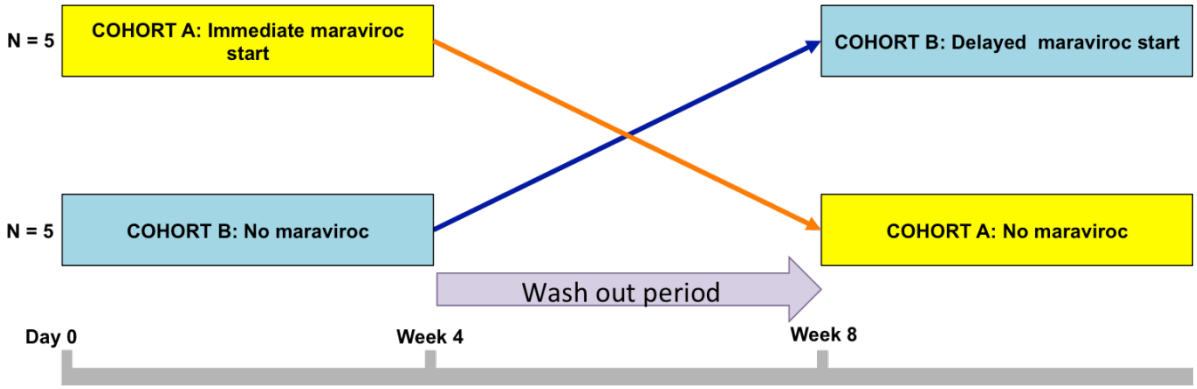

Figure 2: Study scheme

3.3 Primary Hypothesis

The primary hypothesis is that CCR5 antagonism will lead to lower HCV viral load. In addition, we hypothesize that CCR5 antagonism using maraviroc will demonstrate significantly lower HCV viral kinetics in the first 7 days of initiation of therapy.

4 Study Population

Patients will have HCV and HIV and receiving HIV therapy using ART as recommended by HIV treatment guidelines. These patients will be selected to receive one month of maraviroc and have serial measurements of viral, safety and immune parameters to evaluate the anti-HCV efficacy of CCR5 antagonism.

4.1 Rationale for Subject Selection

Subjects of both genders will be considered for inclusion in this study. There will be no racial, ethnic, or gender discrimination. Persons in jail or prison are not eligible for this study. Women will not have research procedures while pregnant.

4.2 Subject Inclusion Criteria

To be eligible for participation on this protocol, a participant must satisfy all of the following conditions:

1. Be ≥18 years old
2. HCV-infected without plans to undergo HCV treatment for duration of study
3. HIV infected
4. Currently receiving ART with HIV VL <50 IU/ml for ≥ 12 months
    a. One virologic blip ≤ 400 copies/ml permissible within the 12 months
5. CD4 T cell counts > 100 cells/mm3
6. Non-cirrhotics and cirrhotics can be included
7. Willing to sign informed consent

An HCV infected individual is defined as any individual with documentation of the following in the past:

- Positive HCV antibody and/or positive HCV RNA test (HCV RNA of 2,000 IU/mL or greater).

Anti-HCV DAA treatment naïve is defined as:

- No previous treatment for HCV with any DAA. Previous exposure to interferon and ribavirin are allowed.

An HIV infected individual is defined as any individual with documentation of the following:

- Positive Enzyme Linked Immunosorbent Assay followed by a positive Western Blot or detectable HIV viral load, or HIV viral <50 copies/mL with documentation this individual is currently on an active HIV ART.

Cirrhosis is defined as

- FibroTest® score >0.75 OR APRI (AST to Platelet Ratio Index) >2 AND FIB-4 (Fibrosis-4) >3.25 OR FibroScan with a result of >12.5kPa OR liver biopsy showing cirrhosis at any time

Absence of cirrhosis is defined as

- FibroTest® score ≤0.48 OR APRI ≤1 AND FIB-4 <1.45 performed within 1 year of screening OR FibroScan with result of ≤12.5kPa within 1 year of screening OR liver biopsy within 3 years of screening showing absence of cirrhosis.

4.3 Subject Exclusion Criteria

A participant will be ineligible to participate on this study if any of the following criteria are met:

1. Age < 18
2. Unable to comply with study visits, research study visits, or is planning to relocate during the study.
3. Have any condition that the investigator considers a contraindication to study participation
4. Pregnancy of breast feeding
5. Decompensated liver disease (Child-Pugh C)
6. Imminent treatment for HCV
7. Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >2.5 times the upper limits of normal
8. Concomitant use of drugs known to impact or be impacted in terms of pharmacokinetics or drug-drug interactions with either raltegravir, dolutegravir, or maraviroc. This includes:
   - Inducers of UGT1A1 (such as rifampin, phenytoin, phenobarbital rifabutin, St. John's wort)
   - CYP3A inhibitors (such as ketoconazole, itraconazole, clarithromycin, nefazodone, and telithromycin)
   - CYP3A inducers (such as rifampin, carbamazepine, phenobarbital and phenytoin)

4.4 Justification for Exclusion: Children and Pregnant Women

The study will be limited to adults ≥ 18 years of age as HCV is predominately a disease in adults. Children and pregnant or breast-feeding women will not be enrolled because of the lack of immediate benefit for the patient with the added risk of blood draws associated with the study.

5 Study Schedule

5.1 Screening /Enrollment

Screening will occur at the IHV. Subjects who consent to the study will be enrolled. A health history will be reviewed and a physical exam will occur prior to the baseline research lab draw. Outside labs performed within the last six months may be used to determine eligibility.

5.1.1 Procedures to be performed at Screening/Enrollment

- Complete Medical History
- Complete Physical Examination - including body weight, height, and vital signs (temperature, pulse, respiratory rate, and blood pressure) must be obtained.
- Complete Medication History – A complete review of all medications and supplements the patient is currently taking.
- Urine Pregnancy test for women of child bearing potential
    - Female subjects of childbearing potential will have a urine pregnancy test (HCG) performed prior to the study start date. Women who are found to be pregnant will be excluded from the study.
- Blood for Screening Labs obtained within 6 months of study entry –
    - CBC with differential
    - Serum Chemistry
    - LFT's (including AST, ALT, Total bilirubin)
    - Hepatitis C Quantitative RNA
    - HIV-1 RNA PCR
    - CD4 Cell Count

5.2 Randomization

Enrolled participants will be randomized to the immediate start or delayed start group in a 1:1 fashion at time of enrollment.

5.3 Day 0 to Day 7

5.3.1 Study visit, all subjects: Baseline Visit – Day 0

Once eligibility is known, *all subjects* will have baseline research sample collection of HCV VL, and PBMC, plasma, serum and Paxgene RNA and DNA for storage. Maraviroc will be initiated for participants randomized to the immediate start group.

5.3.2 Study Visits, immediate start group only: Days 1, 2, 4 and 7

HCV VL will be obtained at days 1, 2, 4 and 7. Day 7 labs can be completed up to 1 day later.

5.4 Study visit, immediate start group only: Day 14 (Week 2 visit)

HCV VL will be obtained. Safety assessment will be performed (CBC with differential and CD4 and HIV viral loads. Patient reports of adverse effects). Labs can be completed up to 2 days later.

5.5 Study visits, immediate start group only: Day 21/week 3

HCV VL will be obtained. Labs can be completed 2 days later.

5.6 Study visit, both groups: Day 28/week 4

HCV VL and PBMC, plasma, serum and Paxgene RNA for storage will be obtained. Labs can be completed 2 days later.

Immediate start group: Maraviroc will be discontinued at day 28/week 4.

5.7 Washout period, week 4 through to week 8

5.8 Study visit, both groups: Day 56/week 8

HCV VL and PBMC, plasma, serum and Paxgene RNA for storage will be obtained.

Delayed start group: A review of medical history and medication history will be performed to evaluate for any interim changes from screening visit. Physical exam will be performed. HCV VL and PBMC, plasma, serum, and Paxgene RNA for storage will be obtained. Maraviroc will be started.

5.9 Study visits, delayed start group only: Day 57, 58, 60, 63, 70 (week 10), 77 (week 11)

HCV VL will be obtained

Safety assessment at day 70/week 10 (on week 2 after initiating maraviroc).

Weeks 10 and 11 labs can be completed up to 2 days later.

5.10 Study visit, both groups: Day 84/Week 12

HCV VL and PBMC, plasma, serum. and Paxgene RNA for storage will be obtained. Visits can be completed up to 2 days later.

Delayed start group: Maraviroc will be discontinued. Safety assessment.

Immediate start group: **End of study participation**. Safety assessment.

5.11 Study Visit, delayed start group only: Day 112/week 16

HCV VL and PBMC, plasma, serum, and Paxgene RNA for storage will be obtained. Safety assessment. **End of study participation**. Visit can be completed up to 2 days later.

6 Study Procedures/Evaluations

6.1 Clinical Evaluations

Participants will be seen by a study team member at screening, and maraviroc start dates (day 0 for immediate start group, and day 56/week 8 for the delayed start group) for a review of health history.

Participants will also be seen by a study team member every weeks while taking maraviroc (day 0, week 1, week 2, week 3 week 4 for the immediate start group, and Day 56/week8, week 9, week 10, week 11, week 12 for the delayed start group) and every 4 weeks prior to starting, and after completing maraviroc (week 8 and week 12 for the immediate start group, and day 0, week 4, and week 16 for the delayed start group) for adverse event monitoring.

6.2 Laboratory Evaluations

Blood will be collected by standard blood drawing techniques. All laboratory assays will be done at University of Maryland Medical Center or at the IHV research lab facilities depending upon the assay. Stored samples will be processed and stored at the IHV research laboratories.

Screening: CBC with differential; creatinine and hepatic function tests including; albumin, alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT) will be collected or outside records showing eligibility will be used. Hepatitis serologies and viral loads will be completed if medically indicated.

Research Visits:

At the first study visit the following tests will be done:

- Baseline HCV VL
- Serum storage (10 ml)
- Plasma storage (6ml)
- DNA paxgene will be collected (8.5ml)
- RNA paxgene will be collected (2.5ml)
- Peripheral blood mononuclear cell (50 ml)

At all study visit including the first study visit the following **clinical laboratory** tests will be done:

- Hepatitis C viral load

After the first 2 weeks while on maraviroc (day 14/week 2 for the immediate start group; and day 70/week 10 for the delayed start group) the following **safety assessment** will be done:

- HIV VL, CBC with differential, and CD4 count (24.5 ml)
- Patient reports of adverse effects

Every week while taking maraviroc, and once every 4 weeks prior to starting, and after completing maraviroc, the following **clinical assessments** will be done:

- Adverse event monitoring (patient reports of adverse effects)
- Medication review

At study visits every 4 weeks including the first study visit the following blood specimens may be collected for **research laboratory** storage and tests (total 68.5 ml research, or about 4.6 tablespoons):

- RNA paxgene (2.5ml)
- Peripheral blood mononuclear cell (50 ml)
- Serum storage (10 ml)
- Plasma storage (6ml)

Outside Tests: Tissue may be requested from liver biopsies done for clinical purposes; imaging, FibroScan or other liver evaluation results may also be requested from outside providers with subject consent.

6.3 HCV and HIV Virologic Studies

We will study the viral and host immunity to HCV and HIV in all patients. The results will be used to characterize each individual with regards to immune status and chronicity of disease.

A] Full length HCV genome sequencing will be performed on plasma using the protocol as described and compared for variability of sequences.

B] Genome-wide transcriptional profiling Sequencing will be performed using RNAseq approach. This will provide the most comprehensive coverage of the genome, including empirically supported and predicted transcribed sequences,

enabling the discovery of previously unidentified novel events. We will analyze array data from samples from whole blood pax genes and will design a low-density PCR array of 20-30 genes of most interest that are highly representative of the analysis group for validation.

We will screen patient samples for miRNA panel that comprises of over 400 known, well-characterized miRNAs. The TaqMan Array Human MicroRNA Panel (Applied Biosystems, Inc.) is a fixed-set microfluidic card that simplifies the profiling of 400plus human miRNAs. After extracting RNA and making cDNA we will use the Multiplex RT and TaqMan MicroRNA Reverse Transcription Kit loaded on the micro-fluidic card, and run on the Applied Biosystems7900HT Fast Real-Time PCR System.

C] We will screen sera from all patients for differential expression of protein peaks that may be associated with liver fibrosis and HCC with the aid of a high-throughput approach using multiplex cytokine arrays, which has the capability of detecting several different cytokines and other biologically relevant proteins.

D] We will perform detailed phenotypic and functional evaluation of immune cell types in the periphery and liver (as available). This will help us in determining the nature of immune responses in patients who are chronically infected. Determination of specific immune defects in these individuals are important milestones in deciding future therapeutics. Exhaustion and activation markers on T, B and NK cells will be quantified. Specific immune responses against pooled HBV and HCV peptides will be performed using an ELISPOT assay and flow cytometry as previously described**.**

7 Potential Risks and Benefits

7.1 Potential Risks

The potential risks includes risks of phlebotomy which include: pain, bruising, fainting and very rarely, infection. These risks will be minimized by having trained staff perform the procedure.

There is potential for loss of confidentiality, which will be minimized by providing privacy during study visits. In addition, all laboratory specimens, and research records will be coded by a number to maintain subject confidentiality. All paper records will be kept locked with limited access. Electronic data will be password protected and will not include participant names or contact details.

Risks with Use of ***Maraviroc*** *(MVC, Selzentry™)*

Common side effects reported with maraviroc that may or may not be causally related include: cough, fever, colds/upper respiratory tract infections, rash,

muscle and joint aches, stomach pain, diarrhea, edema, sleeping problems, and dizziness.[25,26]

Serious side effects may include serious skin rash and allergic reactions, and elevations in liver function tests. People who are co-infected with hepatitis B or C might be at higher risk of having liver problems, although data from trials showed similar rates of liver function test abnormalities in patients on maraviroc compared to placebo groups.

Postural hypotension was reported in patients taking higher doses (than currently approved) of maraviroc in clinical studies.[25]

Post-marketing experience has reported skin and subcutaneous tissue disorders including Stevens-Johnson syndrome (a severe and sometimes fatal form of skin rash).

Maraviroc contains soya lecithin. Therefore if you have a medical history of allergy to soya or peanuts, you may develop an allergy reaction to maraviroc.

7.2 Potential Benefits

Participants will derive no direct clinical benefit from participation in this protocol. It is hoped that this research will be of benefit to society by aiding the understanding of immunological and virological aspects associated with the disease.

8 Stored Samples and Future Research

Future use of the specimens will be based on the scientific merit of the investigation and would be decided by the Principal Investigator. They would be related to the study of HCV and/or HIV. Other investigators may wish to study these samples and/or data. In that case, IRB approval must be sought prior to any sharing of samples and/or data. Any clinical information shared about the sample would similarly require prior IRB approval. If approved, and once a Material Transfer Agreement (MTA) has been established, the study team may send de-identified samples to the collaborators. Investigators will use stored samples only for research.

- **Intended Use:** Some tissue and blood samples may be stored for future analysis in this research study. These samples will be stored by the IHV researchers and may be used in future research to learn more about HCV and HIV, the immune system and/or other related medical conditions. The blood and tissue will be used over time to gain a better understanding of the pathogenesis of HCV and HIV, and especially the degree of liver disease progression. Genetic testing will be performed. Different analytical and laboratory methods will be employed including but not limited to: PCR, ELISA, ELISPOT and immunohistochemistry; RNA/DNA,

cytokine and antibody quantitation; viral particle detection and identification of genetic markers. The consent will include language for specimen storage.

- **Storage:** Samples will be kept in secure facilities with limited access. Samples and data will be stored using a unique identifier. Only investigators and study staff will have access to the samples and data.
- **Tracking:** Extra blood and tissue samples will be stored using a unique identifier that only the study team can trace back to the participants. These stored samples as well as a linkage file will be maintained in a database that will be managed by the Investigators and/or Study Coordinators and will be maintained on the IHV server. This database will also be stored on the password-protected computer of the Lead-Investigator/Study Coordinator in an encrypted and/or password-coded file.
- **Disposition at the Completion of the Protocol:** At the completion of the protocol (termination), samples and data will either be destroyed, or after IRB approval, transferred to another existing protocol. Final disposition of any samples cannot be done without written permission from the PI.
- **Reporting the Loss or Destruction of Samples/Specimens/Data to the IRB:**
    - Any loss or unanticipated destruction of samples or data resulting in a violation that compromises the scientific integrity of the data collected for the study will be reported to the IRB.
    - Participants may request at any point not to have their samples stored. In this case, the Principal Investigator will destroy all known remaining samples and report what was done to both the participant and to the IRB. This decision will not affect the subject's participation in any other protocols.

Generally, the results from the research stored samples will not be given to the participant's primary care provider or appear in the medical record. This is because the test results, unlike routine medical testing, may be experimental or preliminary. The relevance of these tests to direct patient care may be unknown. At the participant's request, the results of any research tests will be discussed with the primary care physician by the Investigators.

9 Assessment of Safety & Monitoring

As this is an observational study with intervention limited to blood collection, safety issues are not expected. Only those reportable Adverse Events (AEs) related to the research procedures will be reported. The PI will review the safety data and report any unexpected events to the IRB along with annual continuing review.

9.1 Recording/Documentation

At each contact with the patient (once a week while taking maraviroc, and then every 4 months prior to and after completing maraviroc), information regarding adverse events related to research procedures will be elicited by appropriate questioning and examinations and will be immediately recorded on a source document. Source documents will include: progress notes, laboratory reports and data collection tools. Source documents will be reviewed in a timely manner by the research team.

9.2 Definitions

Adverse Event: Any untoward medical occurrence in a human subject, including any abnormal sign, symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research.

Protocol Deviation: Any change, divergence, or departure from the IRB approved study procedures in a research protocol. Protocol deviations are designated as serious or non-serious and further characterized as:

- Those that occur because a member of the research team deviates from the protocol.
- Those that are identified before they occur, but cannot be prevented.
- Those that are discovered after they occur

9.2.1 Reporting Protocol Deviations, AEs and Deaths

Anticipated deviations in the conduct of the protocol will not be reported to the IRB unless they occur at a rate greater than anticipated by the study team. Expected adverse events will not be reported to the IRB unless they occur at a rate greater than that known to occur in those having phlebotomy procedures normally. If the rate of these events exceeds the rate expected by the study team, the events will be classified and reported. Deaths related to the natural history of hepatitis or co-infection will be reported at the time of continuing review.

9.2.2 Annual Reporting to the IRB

The following items will be reported to the University of Maryland IRB in summary at the time of Continuing Review:

- Serious adverse events or deaths that are not related to the research
- All adverse events associated with research including expected AEs Protocol deviations. This includes AEs related to phlebotomy within 48 hours of procedure.
- Serious, continuing, and minor non-compliance
- Any trends or events which in the opinion of the investigator should be reported

9.3 Type and Duration of the Follow-up of Subjects after Adverse Events

All SAEs and non-serious AEs identified in this study will be followed until resolution or until the PI judges the AE/SAE has stabilized and no more follow-up is required. This requirement indicates that the follow-up may be required for some events after the participant discontinues participation from the study. These events will be reported to the IRB annually.

9.4 Withdrawal of a Subject

A participant may withdraw from the study at any time. As part of their duties to ensure that research participants are protected, the IRB, IHV, or other government agencies may discontinue the study at any time. Voluntary withdrawal from the protocol may occur at any time at the request of either the participant or the PI. If a request is made to withdraw from the protocol, all further planned study procedures will be immediately cancelled and further follow-up will be terminated. Samples and data collected prior to this request will be stored and used as outlined in the protocol unless a specific request is made by the participant to remove all of their samples from the study. Data already obtained from the participant's samples will not be discarded. In addition, participants with poor compliance and/or failure to present for follow-up visits may be withdrawn from the study at the discretion of the PI.

If a patient becomes pregnant during the course of the study, the investigator, in consultation with the patient, should consider whether the potential benefit justifies the potential risk to the fetus of continuing the study therapy. If it is determined that the potential benefit does not outweigh the potential risk to the fetus, the patient should be discontinued from the study.

Should a subject develop evidence of HIV virologic breakthrough, or decline in CD4 to less than 200 (if above 200 at baseline) during the study they will be evaluated by their primary HIV provider and may be withdrawn from the study at the discretion of the PI.

Discontinuation of maraviroc will be considered in any patient with signs or symptoms of acute hepatitis, in particular if drug-related hypersensitivity is suspected or with increased liver transaminases combined with rash or other systemic symptoms of potential hypersensitivity (e.g. pruritic rash, eosinophila or elevated IgE).

Should a subject be started on anti-HCV therapy while in the study, maraviroc will be discontinued and the subject will be withdrawn from the study.

9.5 Return of Withdrawn/Removed Subjects

A participant who has withdrawn may not return to the study.

10 Remuneration Plan for Subjects

Participants will be remunerated with cash for each study visit time point completed to help cover the cost of travel and expenses.

Lab only study visits: participants will be renumerated $20 cash per visit
Study visits with physical exams: participants will be renumerated $50 cash per visit.
Viral Kinetics during initial addition of maraviroc: During the first week of maraviroc initiation, lab visits will occur more frequently, but will be very short. Participants will be renumerated on (and including) day 7 pro-rated per visit completed and will be renumerated an additional $50 cash on day 7 upon completion of the all lab visits during that time period.
Virologic and immunologic studies: participants will be renumerated an additional $100 cash for completing all lab study visits for sample collection for storage (not including maraviroc initiation visit).

11 Statistical Considerations

11.1 Sample size

Primary end point is change in week 7 HCV viral load from baseline.

11.2 Description of the Analyses

We estimate a 1 log drop in HCV VL in seven days is true.

The primary efficacy analysis will be intention-to-treat and include all patients who were enrolled into the study and received at least one dose of the study medication. Demographic and baseline characteristics will be summarized using standard descriptive statistics. Association of adverse events with treatment duration, HIV coinfection, fibrosis stage, and presence of cirrhosis will be explored. A 2-sided, exact, 1-sample binomial test will be used to test the statistical hypothesis described above at a significant level of 0.05. All analyses will be performed on SAS, version 9.3 (Cary, NC) or STATA version 13.0.

12 Ethics/Protection of Human Subjects

12.1 Informed Consent Process

Informed consent is a process where information is presented to enable persons to voluntarily decide whether or not to participate as a research subject. It is an on-going conversation between the human research subject and the researchers which begins before consent is given and continues until the end of the subject's involvement in the research. Discussions about the research will provide

essential information about the study and include: purpose, duration, procedures, alternatives, risks and benefits. Subjects will be given the opportunity to ask questions and have them answered.

The subjects will personally sign and date the informed consent document prior to undergoing any research procedures. The subjects may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the subjects for their records. Consenting process will be documented in the record. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

12.1.1 Illiteracy and Consenting

As the majority of the patient populations from which the study participants are drawn are literate, written consent will be obtained. Oral short form consent will be obtained in the case of illiterate participants with the written consent being presented orally by the person obtaining consent and witnessed by an impartial third party who will also sign the consent. The participant will be asked to sign the form or thumb-print the form if unable to sign

12.2 Subject Confidentiality

All records will be kept confidential to the extent provided by federal, state and local law. The study monitors and other authorized representatives of the Sponsor may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records. Records will be kept locked and all computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by IRB, FDA, the Joint Commission or the HHRP.

Appendix A: Scientific References

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Appendix B: Schedule of Procedures/Evaluations

Study Day/Week	Screen[a]	D0	D1	D2	D4	D7 W1	D14 W2	D21 W3	D28 W4	D35 W5	D42 W6	D49 W7	D56 W8		D59		D63 W9	D70 W10	D77 W11	D84 W12	D91 W13	D98 W14	D105 W15	D112 W16
IMMEDIATE START GROUP																								
Study Visit		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**				**9**							**10**				
Administrative Procedures																								
Informed Consent	X																							
Inclusion/ Exclusion Criteria	X																							
Medical History	X																							
Interim Medical History		X																						
Clinical Procedures/ Assessments																								
Complete Physical Exam	X																							
Targeted Physical Exam		X																						
Height	X	X																						
Vitals and Weight	X	X																						
Adverse Event Monitoring		X				X	X	X	X				X							X				
Laboratory Proceures/ Assessments																								
Hepatitis C VL	⊗	X	X	X	X	X	X	X	X				X							X				
HIV VL							X													X				
CD4							X													X				
CBC with Diff	⊗						X													X				
Creatinine	⊗																							
Hepatic panel	⊗																							

FibroTest or FibroScan	⊗																							
Pregnancy Testing (if applicable)	**(X)**																							
Research and Storage																								
Pax RNA (2.5ml red paxgene)		**X**							**X**				**X**							**X**				
Pax DNA (8.5ml blue paxgene)		**X**																						
Plasma storage (6ml purple top)		**X**							**X**				**X**							**X**				
Serum storage (10ml SST)		**X**							**X**				**X**							**X**				
PBMC storage (50ml green top)		**X**							**X**				**X**							**X**				
DELAYED START GROUP																								
Study Day/Week	Screen[a]	D0	D2	D3	D5	D7 W1	D14 W2	D21 W3	D28 W4	D35 W5	D42 W6	D49 W7	D56 W8	D57	D58	D60	D63 W9	D70 W10	D77 W11	D84 W12	D91 W13	D98 W14	D105 W15	D112 W16
Study Visit		**1**							**2**				**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**				**11**
Administrative Procedures																								
Informed Consent	**X**																							
Inclusion/ Exclusion Criteria	**X**																							
Medical History	**X**																							
Interim Medical History													**X**											
Clinical Procedures/ Assessments																								
Complete Physical Exam	**X**																							
Targeted Physical Exam													**X**											
Height	**X**												**X**											
Vitals and Weight	**X**												**X**											
Adverse Event Monitoring		**X**							**X**				**X**				**X**	**X**	**X**	**X**				**X**

Laboratory Proceures/ Assessments																								
Hepatitis C VL	⊗								X				X	X	X	X	X	X	X	X				X
HIV VL																		X						X
CD4																		X						X
CBC with Diff	⊗																	X						X
Creatinine	⊗																							
Hepatic panel	⊗																							
FibroTest or FibroScan	⊗																							
Pregnancy Testing (if applicable)	**(X)**																							
Research and Storage																								
Pax RNA (2.5ml red paxgene)		X							X				X							X				X
Pax DNA (8.5ml blue paxgene)		X																						
Plasma storage (6ml purple top)		X							X				X							X				X
Serum storage (10ml SST)		X							X				X							X				X
PBMC storage (50ml green top)		X							X				X							X				X

[a] Screening and day 0 visit can occur on the same day.

ⓍLabs from outside records from the last 6 months can be used. HCV VL at screening will only be done if no HCV VL available from outside records from the last 12 months. FibroTest at screening will only be done if no fibrosis staging available from outside records (see section 4.2 Subject inclusion criteria, definitions of cirrhosis and absence of cirrhosis)

Period of Maraviroc exposure for immediate start group

Period of Maraviroc exporsure for delayed start group