Protocol Title: Ezetimibe as a Safe and Efficacious Treatment for Chronic Hepatitis C

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I. ABSTRACT

While Hepatitis C virus (HCV) infects ~2% of people in the United States (US), 1 in 10 Veterans are chronically infected. Hence, US Veterans are infected at a rate 5 times greater than the general population accounting for almost half of the HCV patients in the country. This is of major concern for the veteran population because not only does chronic HCV infection lead to liver steatosis, insulin resistance, chronic inflammation, and fibrosis, but increases in HCV cirrhosis have played a major role in the rise in hepatocellular carcinoma, accounting for the majority (~50-60%) of cases in the US. Hence, there is a practical need for more affordable effective HCV antivirals with high barriers to viral resistance and/or ways to shorten the current expensive treatment duration.

Viral entry into permissive cells is a recognized antiviral target. In addition, blocking cell-to-cell spread has been shown to enhance antiviral drug efficacy, limit viral escape that may arise against other antivirals within combination drug cocktails and enhance drug synergy. We and others have shown that HCV entry inhibitors can act synergistically with current HCV DAAs allowing for more rapid viral clearance. This combined with our recent proof-of-concept study showing the utility of mathematical modeling of early on treatment viral response to interferon-free HCV DAA therapy as a means to optimize the duration of treatment, provides confidence that improvements can be made.

As such, our long term goal is to develop affordable therapeutic and prophylactic regimens to prevent HCV entry/spread and test the efficacy of those inhibitors in patients. Toward this end, our laboratory has shown that the Niemann-Pick C1 Like-1 (NPC1L1) cellular cholesterol uptake receptor is required for HCV entry into hepatocytes. Furthermore, we have shown that ezetimibe (EZE) (Ezetrol®, Ezetimibe®, and Zetia®), a drug that inhibits NPC1L1-mediated cholesterol uptake and is FDA-approved as lipid lowering agent for treatment of hypercholesterolemia, blocks HCV entry in hepatoma cells in vitro and human hepatocytes transplanted into uPA-SCID mice. Furthermore, retrospective analysis of the National VA database using multivariable logistic regression models to control for age, sex, race, alcohol use, drug use, and other co-morbidities, we found HCV prevalence to be lower ($p < .001$) and early interferon/ribavirin treatment response kinetics to be better in patients taking EZE. Together all this suggests that EZE might lead to cure within a shorter DAA-based treatment window, increase the barrier to viral escape, and perhaps ultimately be useful as a prophylactic to prevent re-infection.

The *objective of this study* is to assess the efficacy of EZE as adjunct to DAAs for the treatment of chronic HCV. *We hypothesize* that when included in combination treatment regimens that EZE will augment 2nd phase HCV decline resulting in faster viral clearance (i.e. shorter/cheaper DAA therapy).

AIM. Assess the efficacy of EZE as an adjunct therapy in chronically HCV infected patients undergoing HCV DAA treatment.

Relevance to VA. (1) Veterans are not only the ideal study population, but also a population that would derive immediate benefit if the proposed study confirms that EZE shortens DAA treatment. (2) To provide a proof-of-concept regarding the importance of blocking viral cell-to-cell spread as part of an optimal antiviral strategy advancing knowledge about drug synergy and how to increase the barrier to viral escape, a critical concern for all emerging RNA viruses that might affect our troops.

II. BACKGROUND AND SIGNIFICANCE

HCV treatment. Recently we have seen the release of IFN-free, all oral HCV DAA treatment regimens with cure rates near 100%. While this is an amazing breakthrough in HCV treatment, caveats remain. First, although high sustained virological response (SVR) rates have been achieved, viral escape has been documented raising questions regarding the extent of viral resistance that might be encountered in real-life populations. The second issue is that the high cost of these drugs have left many around the world being denied treatment. Hence, there is a need to prevent viral escape and reduce treatment cost. Here we propose to investigate the re-indication of a readily available FDA-approved drug for the benefit to those chronically infected with HCV.

Clinical significance of viral entry/spread inhibitors. Viral entry represents a promising opportunity for drug discovery; however, no HCV entry inhibitors have been FDA-approved. In addition, evidence is accumulating that blocking not only cell-free virus entry, but also viral cell-to-cell spread is an important untapped area for antiviral intervention. Relevant to all RNA viruses, blocking spread has been documented experimentally to

prevent viral escape that may arise against other antivirals within a combination drug cocktail[1,2]. Even more broadly applicable (i.e. to any pathogen), we[3,4] and others[5] have shown that entry/spread inhibitors act synergistically with IFN and DAAs resulting in more effective and rapid viral clearance. Additionally, others suggest that because viral cell-to-cell confers higher per cell virus doses, that blocking this transmission can actually reduce the effective dose of drug required thus enhancing antiviral drug efficacy[6].

EZE inhibits HCV infection in vitro and in vivo. We have identified NPC1L1 as a factor required for *cell-free* HCV entry[7], and shown it is also required for HCV *cell-to-cell* transmission[3],. Consistent with this ezetimibe (EZE), an NPC1L1 internalization inhibitor [8-13], reduced HCV foci formation in a dose-dependent manner when present 6h prior to infection and then removed (*Pre*) or when present during the 12h virus inoculation period and then removed (*Co*); however consistent with an entry inhibitor, inhibition was inefficient when EZE was added post-infection (*Post*) (**Fig. 1d**). Likewise, EZE was able to inhibit HCV infection in a hepatic xenorepopulation murine model[14.15]. Specifically, uPA-SCID mice repopulated with human hepatocytes[16,17] were pre-treated with EZE (10 mg/kg/day; a dose that produces serum levels achieved in patients) or diluent for 2 wks prior to infection with HCV GT1b serum. While 100% of the diluent-treated mice were HCV$^+$ by RT-qPCR 1 wk following inoculation, 71% (5/7) of EZE-treated mice were HCV-negative (**Fig.1e**) despite the fact that drug treatment stopped at the time of infection.

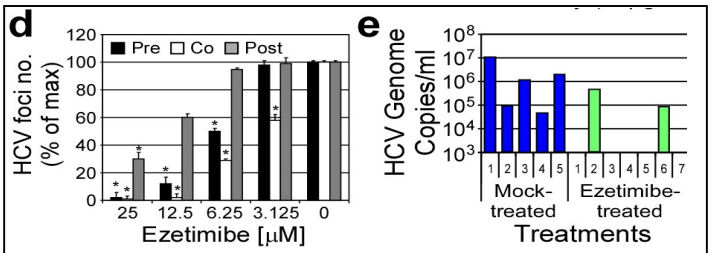

Fig. 1 EZE inhibits HCV infection. (d) Huh7 cells were treated with increasing doses of EZE infected with HCVcc and HCV RNA levels determined 72h p.i. by RTqPCR. **(e)** HCV RNA Copies/ml of serum in mock- and ezetimibe-treated chimeric mice was quantified by RTqPCR 1 week post inoculation with HCV+ genotype 1b serum. [* = ($p<0.05$)].

EZE act synergistically with HCV DAAs[4]. Importantly, EZE enhances treatment efficacy when used in combination with antivirals that block intracellular virus amplification (**Fig. 2**) Specifically, we have found that EZE synergizes with protease inhibitors (**Fig.2a**), polymerase inhibitors (**Fig.2b**), and NS5a inhibitors.

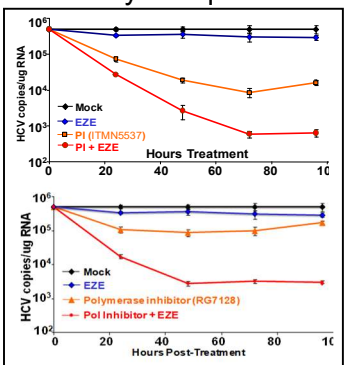

Fig. 2 Infected cells were treated as indicated. RNA was isolated from triplicates at indicated times and HCV copies were determined by RT-qPCR. (a) EZE enhances inhibition by the HCV protease inhibitor ITMN5537; (b) EZE enhances inhibition by the HCV polymerase inhibitor RG7128.

EZE impacts HCV outcomes. To confirm the clinical relevance of EZE, we performed retrospective analysis of the National Health and Nutrition Examination Survey (NHANES) and the National VA databases. In NHANES, we observed a significantly ($p=.02$) lower rate of HCV seroprevalence in people on EZE (0.2%) versus those not on EZE (1.7%). In the larger VA database where multivariable logistic regression models could be used to control for age, sex, race, alcohol use, drug use, and other co-morbidities, we again found HCV prevalence to be lower ($p <.001$) in patients on EZE supporting that EZE blocks HCV infection in people. Furthermore, analysis of treatment response revealed that despite the older age, larger percentage of African Americans, higher viral load and more severe liver disease (e.g. higher ALT/AST) of the population taking EZE, IFN/RBV treatment response was better (i.e. larger viral log reduction) in patients taking EZE. One caveat of this retrospective analysis, however, is that in the VA system EZE is almost exclusively prescribed as an add-on to statin treatment. Hence, while it was clear that statins alone had no effect on HCV treatment response (**Fig. 3a dark blue line**), the significantly better response kinetics seen in those taking EZE (**Fig. 3a, red line**) occurred in patients taking a combination of EZE and a statin.

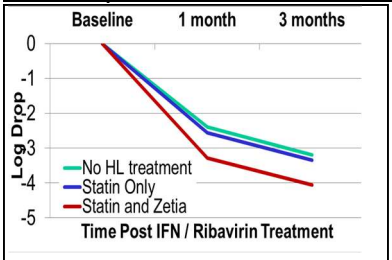

Fig. 3 EZE (Zetia©) enhances HCV treatment in patients. In the VA EZE is prescribed with a statin. Hence we used those taking a statin only as a control group. Shown is the average log HCV drop from baseline in each group. (HL = hyperlipidemia)

III. RESEARCH DESIGN AND METHODS

This is a prospective study in which we propose to compare HCV RNA inhibition in patient undergoing available HCV DAA treatment plus EZE or a placebo.

Subjects: Subjects will consist of patients at the Hines VA, who are scheduled to receive HCV DAA therapy and who meet the following specific inclusion and exclusion criteria:

Inclusion Criteria	***Exclusion Criteria***
1. Intent to treat with MAVYRET 2. Males/females 18 - 70 yrs of age. 3. Serum HCV RNA >2,000 IU/ml. 4. Other causes of chronic liver disease excluded by appropriate clinical, laboratory, or histologic evaluation 5. The following hematological criteria must be met: • Hemoglobin $\geq$ 12 g/dl. • Absolute neutrophil count (ANC) $\geq$ $1.0x10^9$ /L • Platelets $\geq$ 150 x 10^8 /L (*i.e normal*) 6. Serum creatinine <1.5 times the upper limit of normal (ULN) at screening. 7. Fasting blood sugar normal for non-diabetics or hemoglobin A_{1C} < 8.5% with diabetes. 8. Women of childbearing potential must have a negative pregnancy test prior to receiving treatment. Sexually active women must take adequate precautions to prevent pregnancy during the study. Pregnancy tests will be done at the final clinic visits and every 4 wks. 9. Patient provides written informed consent.	1. Evidence of liver disease other than HCV: • ANA >1:160 • Active alcoholic liver disease. • Hepatitis B surface antigen positive. • Hemochromatosis. • Wilson disease. • Alpha-1-antitrypsin deficiency. • Recent hepatotoxic drug exposure. • Cirrhosis with complications of portal hypertension including esophageal varices (> grade 1 by endoscopy), ascites, or hepatic encephalopathy, or bilirubin >2.0 mg/dl and/or overall Child-Pugh Score $\geq$ 7. 2. Use of oral cyclosporine, fibric acid, Fenofibrate, gemfibrozil or cholestyramine 3. Active substance abuse including, but not limited to alcohol or i.v./inhaled drugs 4. Use of chemotherapy or systemic steroid therapy within 30 days prior to enrollment. 5. Pregnancy, females who are breast feeding, or females of child bearing potential who are not using adequate birth control measures. 6. History of a medical condition that could interfere with participation or completion of the protocol. 7. Organ transplant recipient. 8. History of hypersensitivity to ezetimibe.

[Note: Based on currently available therapeutics the study will be performed with MAVYRET because this is the combination most frequently being prescribed in our liver clinics at the moment]

Sample Size: Power analyses are based on detecting a difference in 2nd phase decline between the DAA group and the DAA+EZE group. Pilot data of 20 patients receiving Harvoni therapy (SOF+ledipasvir) showed an average 2nd phase rate of decline (δ) of 0.40/day (SD=0.1). We conservatively estimate that addition of EZE will increase the 2nd phase viral decline slope by ~50% and assume a conservative common standard deviation of 0.2 in both groups. To achieve 80% power in detecting a significant difference in slopes, using a two-sample t-test with α=0.05, we need to enroll 19 individuals in each group after accounting for 10% attrition which may include drop out or rapid response preventing 2nd phase viral decline analysis.

Patient Screening: We will identify patients potentially eligible for the study during the course of routine clinical care in the Hines VA liver clinics. At that time, potentially eligible patients who express an interest would be introduced to the Clinical Research Coordinator (CRC). The CRC will further screen candidates assessing the inclusion/exclusion criteria listed in the Table above and explain the study in detail to the patient to determine if they wish to participate and give informed consent. If any of the inclusion/exclusion data is missing, blood will be drawn to confirm patient eligibility before their information is sent to the study designated HCV Clinical Pharmacy Specialist for randomization.

Study Design: Chronically HCV infected patients undergoing MAVYRET treatment as part of their routine clinical care will be randomized 1:1 to 3 arms (n=19) of 0, 20, or 40 mg per day of EZE for the duration of their prescribed standard of care treatment. All subjects will have routine laboratory monitoring for patients receiving MAVYRET (e.g.CBC, CMP, PT/INR, and HCV RNA) plus additional research blood draws and laboratory testing as needed. For all subjects, HCV RNA will be measured at baseline. Subjects will then receive EZE or a placebo for the duration of their prescribed treatment with frequent viral kinetic measurements. Post-treatment SVR12 will be obtained for clinical purposes and serve to assess for viral rebound. Safety assessments during therapy will include history with symptom assessment, physical examinations and laboratories including CBC, complete metabolic panel, creatine phosphokinase (CPK), Prothrombin Time and International Normalized Ratio (PT/INR), and fasting lipid panel.

Patients will be randomized 1:1 for treatment with 0, 20, or 40 mg per day of EZE

Group 1 –MAVYRET + placebo (n=19):

Group 2 –MAVYRET + 20 mg/day EZE (n=19); Subjects will receive a 10 mg oral dose of EZE twice daily

Group 3 –MAVYRET + 40 mg/day EZE (n=19); Subjects will receive a 10 mg oral dose of EZE twice daily

Laboratory Monitoring: All study required laboratory monitoring is indicated, however in some cases this might overlap with clinical care. All laboratory monitoring will be performed at Hines Outpatient Laboratory:

Baseline laboratories (i.e., at treatment initiation; t=0):

- CBC (e.g. RBC, WBC, hemoglobin, hematocrit, platelets, neutrophil count)
- Complete metabolic panel
  (e.g. Alb, BUN, Ca, CO2, Cl2, creatinine, glucose, K, Na, bilirubin, total protein, ALT, ALP, AST)
- Fasting lipid panel (e.g. Tot Cholesterol, triglycerides, HDL, LDL, VLDL)
- Creatine phosphokinase (CPK)
- Prothrombin Time and International Normalized Ratio (PT/INR)
- HCV RNA
- Urine pregnancy test for childbearing age women.

Serial laboratories:

- HCV RNA at:
  - 24, 48, 72 hours during treatment
  - 1, 2, 3, 4, 8 weeks
  - 3 and 6 months post-treatment
- Lipid testing at weeks: (*test added for research purposes*)
  - 4 and 8 weeks during treatment
  - 3 and 6 months post-treatment
- CBC, CPK, PT/INR and CMP
  - 4 and 8 during treatment (extra CPK also at 2 weeks)
  - 3 and 6 months post-treatment
- Urine pregnancy test every 4 weeks in women of childbearing potential
- Additional labs drawn based on clinical indications

Study Endpoints:
1) 2nd phase slope of viral decline;
2) Mean time to HCV RNA negativity
3) Number and severity of adverse events during EZE therapy.

Data Collection and Management. Study data will be collected using case record forms (CRFs) and subsequently entered into a secure online database (e.g., Research Electronic Data Capture, i.e., REDCap). Demographic data (e.g. age, sex, race), concurrent medication, HCV history, and information regarding relevant co-morbidities (e.g. cirrhosis, fibrosis, BMI, hyperlipidemia, hypertension) will be extracted from the patient electronic medical record. The collected data may also be transferred from REDCap to an encrypted SAS file for subsequent data analysis after all data entry is complete.

Patient tracking and Study Coordination. The precise details for patient tracking involve coordination among the CRC, the HCV Clinical Pharmacist Specialist, and the physician, but the overall tracking plan will include the following essential elements:

- Medications (The Hines research pharmacy has an SOP for drug management and record keeping. They have a dispensing form to keep a perpetual inventory and patients return unused study medication to assess compliance.)
  - Initial randomization (www.randomization.com)
  - Performing pill counts at each Pharmacy visit
  - Dispensing of study medication at each Pharmacy visit
  - Record keeping
- Clinical Visits
  - Scheduling visits
  - Making call reminders
  - Organizing research visit blood draws and filling out CRFs
  - Processing an aliquot of blood for onsite banking (for research laboratory tests)
  - Sending blood out for research-related clinical laboratory tests

Follow-up assessment

Patient will be seen in clinic and monitored clinically for signs of drug safety issues. The clinical assessment is based on symptoms, significant alterations in laboratories and efficacy of treatment. Symptoms of a general drug adverse events might include profound fatigue, muscle aches, headaches, insomnia, severe diarrhea, rash, altered mental status, or hepatic decompensation (i.e., ascites, hepatic encephalopathy or variceal bleeding). Specific indications for EZE discontinuation include:

- Intolerance to ezetimibe or a clinically significant therapy-related adverse event.
- ALT or AST increase to >3x baseline or >10x ULN
- Total bilirubin >3 mg/dl in absence of evidence of Gilbert's syndrome
- Muscle symptoms and a CPK level >5 x ULN
- An sustained increase in HCV RNA > 1 log
- Any unrelated hospitalization which the treating physician believes continued EZE use might be detrimental.

Data Analysis: Serum HCV RNA will be determined by clinical quantitative RNA testing. The mean log drop between groups at different time post-treatment will be compared using a two-sided Wilcoxon Rank Sum Test or Student T-test, as appropriate. To compare categorical variables, we will use two-tailed Fisher Exact and Pearson Chi-Square Tests. We expect a biphasic decline in viral load during DAA and DAA+EZE therapy. Based on univariable results, a multivariable (semi-parametric) linear mixed effects spline model, with change points modeled at time of intervention and/or transition to phase 2 via cubic smoothing splines, will be considered to assess decline in HCV RNA over time between the two groups. Moreover, Kaplan-Meier methods will be used to assess differences in time to HCV RNA negativity with individuals who do not achieve negativity in the study window right censored. Based on univariable results, a multivariable cox-proportional hazards model may be considered to adjust for important confounders. All analyses will follow the intent-to-treat principle and missing data will be assumed missing at random. Mathematical modeling: HCV RNA data including the first viral load below the limit of quantification (<15 IU/ml) or below the limit of detection will be used for model fits, using a population approach - subsequent observations (expected to be below the limit of detection) will be truncated. In order to accurately estimate the model parameters, we will exclude subjects with less than 2 samples above the limit of quantification during the 2nd phase. The viral clearance rate constant will be fixed to c=6/day as currently assumed in our other ongoing modeling efforts[18,19]. The population parameters: baseline viral load, ε and δ and their inter-individual variability (IIV) estimates will be obtained using a maximum-likelihood method implemented in MONOLIX version 4.3.2 (Lixoft, Orsay, France), which uses the SAEM algorithm. The model is fit to log10 viral load. Patients' baseline characteristics (such as patient type treatment naïve vs. non-responder) will be included as covariates in the model to study their effect on the parameters. Individual parameters will be estimated using the empirical Bayes method[20] .

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