Section #1 - MISP Protocol Identification	
Study Title:	Host mechanisms involved in achieving SVR using a fixed dose combination of grazoprevir and elbasvir in treatment of chronic hepatitis C genotype 1 in non-cirrhotic patients with chronic kidney disease after renal transplantation.
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Section #2- Core Protocol	
2.1 Objectives & Hypotheses	**2.1 Objectives and Aims** **Aims** 1. To evaluate the determinants of Sustained Virologic Response (SVR) in patients treated with grazoprevir and elbasvir in the presence of immunosuppression (post-renal transplant). **Primary Objective** 1. To determine the difference in the host immune response as measured by interferon stimulated gene (ISG) expression (IP-10 in particular), and HCV specific T-cell response in post-renal transplant patients compared to historical, non-transplant patients and its association with SVR. 2. To examine the efficacy of grazoprevir and elbasvir in patients post- renal transplantation compared to historical, non-transplant patients. **Secondary Objectives** 1. To evaluate the safety and adverse events of grazoprevir and elbasvir post-renal transplant patients compared to historical, non-transplant patients. 2. To describe the effects of chronic HCV treatment on two-year renal allograft outcomes in patients treated after renal transplantation. **2.1.1 Clinical hypotheses** **Primary Hypotheses** 1. Patients treated for chronic HCV with grazoprevir and elbasvir after renal transplantation will maintain a robust host immune response as measured by ISG expression (IP-10 in particular) and HCV specific T cell responses in the presence of immunosuppression. 2. The proportion of patients who achieve SVR12 will be greater than 90% in post-renal transplant patients. **Secondary Hypotheses** 1. The safety, tolerability, and adverse events of treatment with grazoprevir and elbasvir in patients with chronic kidney disease will not be significantly different in post-transplant patients compared to non-transplant patients. 2. The treatment of chronic HCV using grazoprevir and elbasvir will improve the renal allograft outcomes in terms of rejection or graft survival. **2.1.2 Clinical Impact** Chronic HCV in patients with CKD is associated with an increased risk of death as well as renal transplant graft failures (1). With the FDA approval of grazoprevir and elbasvir, we now have a regimen that can be used in these patients with severe CKD. However, it remains unknown if there is an immunologic advantage for both achievement of SVR 12 and renal allograft outcomes in treating these patients prior to their transplant compared to waiting until after transplantation when they will be immunosuppressed. And while we predict that transplant recipients will maintain high rates of SVR, it is uncertain whether these immunosuppressed transplant recipients will maintain robust host immune responses to HCV treatment.

2.2 Background & Rationale, Significance of Selected Topic & Preliminary Data

Background

Hepatitis therapy has evolved rapidly from an interferon based, prolonged regimen with moderate efficacy to oral, direct acting antiviral (DAA) based regimens with high efficacy. Recently, sofosbuvir/ledipasvir, as single tablet combination and Viekira pak, a combination pill of multiple DAAs, have all been approved for the treatment of hepatitis C in genotype 1 and 4 infected patients. However, limited data exist in the use of either of these regimens in patients with renal disease, particularly those with CKD stages 4 and 5.

Sofosbuvir enters the hepatocyte where it is metabolized to its active form, GS-461203. The downstream inactive nucleoside metabolite GS-331007 is almost exclusively eliminated from the body renally, mediated through a combination of glomerular filtration and active tubular secretion (2). Results of phase II and III clinical trials of sofosbuvir have previously excluded patients with serum creatinine levels greater than 2.5 mg/dL or creatinine clearance (CrCl) level less than 60 mL/min. In subjects with end stage renal disease (ESRD) (relative to subjects with normal renal function), sofosbuvir and GS-331007 AUC (0-inf) were 28% higher and 1280% higher, respectively, when sofosbuvir was dosed 1 hour before hemodialysis compared with 60% higher and 2070% higher, respectively, when sofosbuvir was dosed 1 hour after hemodialysis. No dosage adjustment is required for patients with mild or moderate renal impairment (CrCl 30 mL/min-80 mL/min) (2). Thus, the safety of sofosbuvir has not been established in patients with severe renal impairment (CrCl <30) or ESRD. Unlike with sofosbuvir, no clinically relevant changes in ledipasvir pharmacokinetics were found between volunteers with normal renal function and those with severe renal impairment (eGFR <30 mL/min by Cockcroft-Gault) after a single dose of 90 mg of ledipasvir was administered (3).

HCV-TARGET is an ongoing prospective observational cohort study characterizing the use of DAA agents across clinical practices in North America and Europe. The study reported adverse events and efficacy of sofosbuvir containing HCV treatment regimens in patients with variable degree of renal dysfunction (eGFR <30, 31-45, 46-60 and >60). The patients received different regimens that included sofosbuvir (peg/sof/rbv; sof+ sim ± rbv, sof/rbv). Overall, the regimens were well tolerated with no increased discontinuation among patients with low eGFR. The SVR12 rates were similar across the groups regardless of renal function. Notably, there were progressive deterioration of renal function and renal symptoms in the eGFR <30 ml/min suggesting the need for close monitoring of these patients. In summary, patients with low baseline renal function have a higher frequency of anemia, worsening renal dysfunction and SAEs, while the treatment responses remain high and comparable to those without renal impairment (4).

Viekira Pak consists of paritaprevir/ritonavir, ombitasvir and dasabuvir, all cleared by hepatic metabolism. Single dose pharmacokinetics of paritaprevir/ritonavir, ombitasvir and dasabuvir was evaluated in HCV negative volunteers with mild (eGFR 60-89 ml/min), moderate (eGFR 30-59ml/min) and severe (<30ml/min) renal dysfunction. The results concluded that changes in the pharmacokinetics were not considered to be clinically relevant in HCV infected patients (5). Thus, Viekira Pak remains an option for some patients with CKD. However, there are significant drug-drug interactions between the ritonavir in Viekira Pak, which is a CYP3A inhibitor and tacrolimus, which is a substrate of CYP3A and which a majority of renal transplant patients are on post-transplantation.

Twenty patients with HCV genotype 1 infection and CKD stages 4/5 (eGFR <30 without cirrhosis) were treated with AbbVie 3D ± RBV in a multicenter open-label phase IIb study. Notably, 70% of patients were African Americans and 65% had CKD receiving hemodialysis. Ribavirin (GT1a only) was dosed 4 hours before hemodialysis and monitored with weekly hemoglobin assessments. Though still ongoing, all patients thus far have achieved SVR4 (10/10) and SVR12 (2/2). Interestingly, 8 of 13 patients required

interruption of RBV due to a drop in hemoglobin and 4 of 8 patients also required erythropoietin during the first 7 weeks of therapy. Mean drug concentration (C_{trough}) of all drugs were measured which were within the range that was observed with previous pharmacokinetic studies in healthy volunteers (6). In summary, HCV genotype 1 non-cirrhotic patients treated with AbbVie 3D +/- RBV can result in viral suppression in most patients. However, increased frequency of ribavirin–induced anemia can occur frequently, requiring close monitoring of all patients and judicious dose reductions of RBV. In addition, the use of this ritonavir containing regimen is limited in the renal transplant population due to drug-drug interactions with calcineurin inhibitors.

Recently, the combination of elbasvir/grazoprevir has shown efficacy in both treatment naïve and null responders with chronic HCV genotype 1 infection with or without cirrhosis (7). Both grazoprevir and elbasvir are cleared by hepatic metabolism and hence, could be used in patients with impaired renal function, including renal transplant candidates or recipients. Grazoprevir is a weak inhibitor of CYP3A4 and thus the area under the curve for single-dose tacrolimus increased by approximately 40%, but decreased the C_{max} by approximately 40% (8).

Evaluation of host immune response to treatment

Persistent activation of host inflammation in the liver is partially mediated by endogenous interferons, which contributes to the pathologic development of hepatic fibrosis (9). This activated response is present in both acute and chronic infection; however, it is unable to eradicate HCV (10). Increased mRNA expression of these ISGs in liver biopsy samples is associated with failure of interferon based therapies (11). It has been previously demonstrated that host responses play an important role in achieving SVR in patients with chronic hepatitis C undergoing therapy using DAAs. Clearance of HCV during DAA treatment is associated with a downregulation of type II and III IFNs along with their respective receptors and ISGs and SVR was associated with an increased ISG expression in the liver and IFNA2, which could play a role in eliminating residual HCV (11).

IP-10, an interferon gamma inducible CXC chemokine targeting T lymphocytes and NK cells and monocytes, has been shown to be produced by hepatocytes. Elevated pre-treatment IP-10 levels have been associated with a poorer response to therapy and inability to achieve SVR (12).

In addition to the ISG expression mediated response, T cells also play a role in viral clearance. Enhanced polyclonal CD4+ T cell responses have been found in cleared acute HCV infections and this response persists in those who permanently clear infection (13) (14). In cleared chronic infection, polyclonal and persistent CD4 T-cell responses directed at a variety of different HCV-specific proteins have similarly been detected (13).

Rationale for the study

Host immunity is also likely a major determinant of achieving SVR renal transplant recipients with variable degree of immunosuppression. However, it is unclear what role immunosuppression may play in modulating this host immune response. The modulation of this response by immunosuppression in patients who are post-transplantation could have significant implications for clinical outcomes and achievement of SVR as well as the maintenance of SVR over time. In this regard, we would like to evaluate host response to DAA therapy using grazoprevir and elbasvir in patients with CKD by evaluating three previously well characterized host responses associated with favorable SVR outcomes. These include ISG expression (11), IP-10 levels (15) and HCV specific T-cell responses (16).

	A recent study (C-SURFER) evaluated the safety and efficacy of 12 weeks of grazoprevir and elbasvir for HCV genotype 1 patients with CKD stages 4/5. The study was designed to randomize eligible patients to either immediate or deferred treatment with grazoprevir and elbasvir. The delayed treatment arm received placebo and was treated with grazoprevir and elbasvir subsequently. The study participants were HCV genotype 1, CKD stages 4/5 (eGFR <30), and 76% were on hemodialysis, and 46% were African Americans. A small number of patients with compensated cirrhosis were allowed. The study reported an intention to treat (ITT) and modified ITT analysis of 94% and 99% for SVR12. There were no changes in hemoglobin or other adverse events or erythropoietin use in the immediate treatment group compared to placebo. Four percent of patients in the deferred treatment group discontinued treatment due to an adverse event, mainly headache, nausea or fatigue (17). In summary, a regimen of grazoprevir and elbasvir could be an effective regimen to treat HCV genotype 1 infection in patients with severely compromised renal function. While studies evaluating the safety and efficacy of this regimen in patients with chronic kidney disease have shown success with few adverse events, little is known about the efficacy of this, or any other, therapy in patients on immunosuppression after renal transplantation or whether it is optimal to treat hepatitis c before or after transplantation. Because waiting until after transplant to treat patients for HCV is advantageous by allowing those patients to receive HCV-positive donor organs, most centers are waiting to treat these patients. In order to observe whether there is any difference immunologically or clinically in treating patients before or after renal transplantation, we will compare our transplant cohort to a cohort of historical, non-transplant patients treated for HCV in whom we have stored blood samples from prior IRB-approved studies. **Significance** The prevalence of chronic hepatitis C in patients with CKD is much higher than that observed in the general population (18). This highlights the importance of finding a ribavirin free regimen that is not excreted through the kidneys. However, it is unclear what the timing of HCV therapy should be for these patients. Additionally, the effect of immunosuppression on the host response to HCV therapy is unknown. On the one hand, these patients could be treated for hepatitis C infection prior to renal transplantation to avoid the impact of immunosuppression as well as the impact of HCV-related liver and kidney disease on allograft function post transplantation. On the other hand, this approach will eliminate access to HCV positive kidneys (making the wait list time longer) and potentially reduce the efficacy of DAA therapy (due to drug-drug interaction between calcineurin inhibitors and HCV drugs). Recent studies have shown that grazoprevir is a weak inhibitor of CYP3A4 and may increase tacrolimus levels (approx. 40% above expected), though change has not been seen in elbasvir or grazoprevir levels (19) (8). There are real benefits in waiting to treat HCV in patients until after transplantation in that it could decrease the wait time for transplantation by not necessitating a delay in transplant while completing HCV therapy and by permitting the use of HCV-positive kidney donors. Showing the impact that immunosuppression has on the host immune response to HCV during therapy would enable clinicians to decide whether patients should be treated before or after transplant, especially in cases where the wait for a transplant may be quite long.
2.3 Study Design	**Experimental design** The study will be a pilot, prospective, single-center, open-label, non-randomized, non-controlled clinical trial. 25 HCV genotype 1 infected post-renal transplant patients will be enrolled in the study. Recruitment will be conducted through the renal transplant and nephrology outpatient clinics at the University of Maryland.

Study population

The study will involve a single cohort of post-renal transplant patients.

All patients will have HCV genotype 1 chronic infections with active viremia and have no cirrhosis at the time of enrollment. All patients will additionally have stable renal function.

The post-transplant patients will include renal transplant recipients of both living donor and deceased donor organs infected with HCV prior to their transplantation with stable GFRs and active HCV viremia. These patients will also be recruited from the University of Maryland's multidisciplinary transplant nephrology clinic.

Inclusion criteria

- At least 18 years of age at the time of screening
- Have stable renal function for one month (30 days) prior to enrollment
- Have Chronic HCV infection prior to transplantation
    - Documented HCV viremia ≥ 1,000 IU/ml at screening
    - Either documented HCV Ab positivity or HCV viremia ≥ 1,000 IU/ml at least 6 months prior to enrollment
- Documented genotype 1 HCV infection prior to enrollment and after their transplant in the post-transplantation cohort
- HCV disease staging within 12 months prior to enrollment by liver biopsy, transient elastography, or biochemical testing
- Be able to give informed consent and comply with study guidelines
- Women of childbearing age will be required to have a negative pregnancy test at enrollment and use birth control throughout the duration of treatment.
- Patients will have undergone renal transplantation no greater than five years prior to enrollment, and will be followed in our University's nephrology and infectious disease clinic. They will all have stable renal function at the time of enrollment.

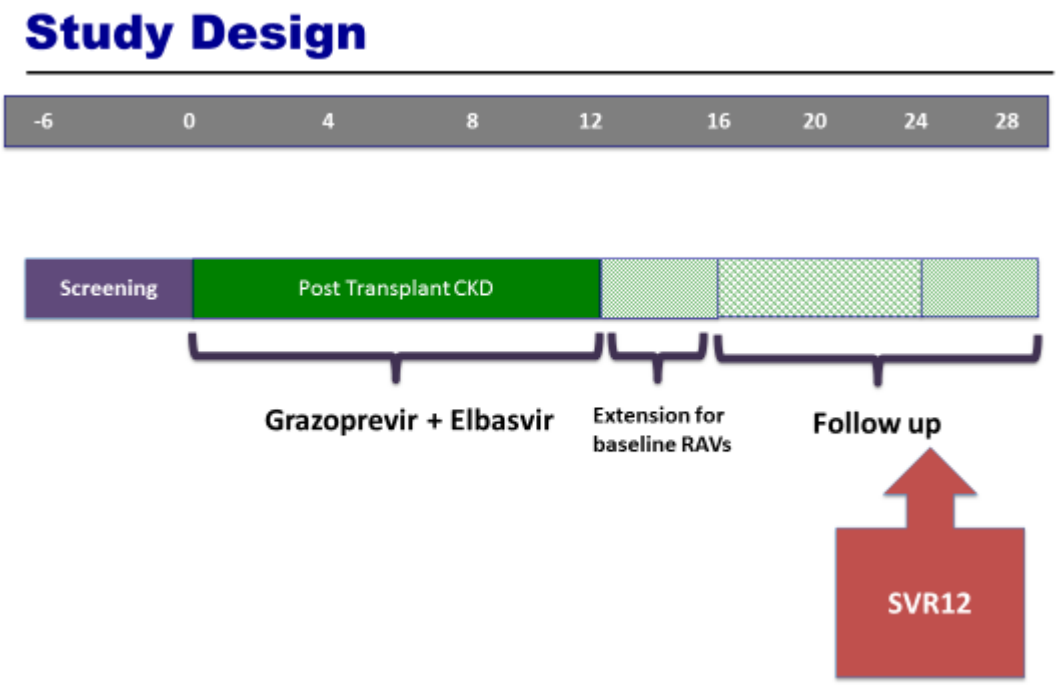
Exclusion criteria

- Documented positive HBsAg, and/or HBV DNA prior to enrollment
- Any prior exposure to HCV protease inhibitor therapy
- HIV co-infection if on a protease inhibitor, etravirine or efavirenz based regimen or elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate
- Increase in creatinine of 15% or greater within one month (30 days) of the screening visit
- Evidence of HCC at the time of enrollment
- Liver disease caused by an etiology other than HCV
- F4 or decompensated cirrhotic patients
- Child Pugh class B or C
- AST or ALT >350 within 6 months prior to enrollment
- Albumin < 3g/dL at the time of enrollment
- Platelet count < 75,000 at the time of enrollment
- History of clinically significant allergy or adverse event with protease inhibitors
- Evidence of the acquisition of HCV at the time of or after transplantation
- Pregnant or breastfeeding women
- Cyclosporine; St. John's Wort; Efavirenz;, Phenytoin; Carbamazepine; Bosentan; HIV Protease Inhibitors; modafinil; Ketoconazole; or Rifampin use within 7 days of enrollment
- Coadministration of more than 20 mg atorvastatin; 10 mg rosuvastatin; 20 mg of fluvastatin, lovastatin or simvastatin

2.4 Study Flowchart

Patient screening and enrollment

Figure 1. Study Design



Schedule of lab work
A full list is provided as Appendix 1.

Table 1: Schedule of labs by week (wk)

	Screen	Day 0	Wk 1	Wk 2	Wk 4	Wk 8	Wk 12/16*	Post-Wk 1	Post-Wk 2+	Post-Wk 4	Post-Wk 12
Exam	X	X			X		X				X
CBC/CMP	X	X		X	X	X	X			X	X
HCV VL	X				X		X				X
Tacrolimus		X	X	X	X	X	X	X	X+	X	
ELISPOT (PBMCs)		X									X
ELISA		X								X	
Paxgene DNA		X									
Paxgene RNA		X								X	
Stored Samples (Serum, Plasma, PBMCs)		X								X	

*Wk 16 will be completed instead of week 12 only for those on 16 weeks of therapy.
+ Post-Treatment Wk 2 will only be done if clinically indicated based upon Post-Tx Week 1 labs.

	RAV testing (for NS5A positions 28, 30, 31 or 93) will be performed for all patients with HCV genotype 1a infection at the time of enrollment as per clinical recommendations (20).
2.5 Study Procedures	**Subject identification** Patients will be identified for screening through the renal transplant clinic or nephrology clinics at the University of Maryland. **2.5.1 HCV Treatment Arm** **Screening** All patients will be screened at the IHV Clinical Trials Unit infectious disease, or renal transplant outpatient clinic at the University of Maryland. All patients will sign an informed consent as approved by our Institutional Review Board (IRB) prior to study participation. At this visit, all patients will have clinical and research screening labs drawn and a history and physical examination performed. Additional requirements will be genotype testing prior to enrollment, but after transplant for the post-transplantation cohort, and disease staging within 12 months of enrollment by liver biopsy, elastography, or biochemical testing. For those who do not have a genotype or disease staging within the specified time frame, genotyping and elastography will be repeated as part of the study screening work up. Eligibility will be determined based upon these results within 6 weeks of starting the study drugs. Treatment-naïve or treatment-experienced patients who failed prior therapy with pegylated interferon and ribavirin have a 5-10% incidence of having baseline resistance associated mutants (RAVs). Given the reduced efficacy of this regimen in patients with genotype 1a with the presence of baseline NS5A RAVs (58-91% depending upon the methodology vs. 100% for patients with no RAVs), we will screen patients for RAVs in patients with HCV genotype 1a at the time of enrollment (20). Any patient with genotype 1a HCV found to have NS5A RAVs will undergo 16 weeks of therapy according to current treatment guidelines. A complete panel of tests that will be performed during this visit is detailed in Appendix 1. **Starting therapy** Study drugs will be administered starting on day 0 after a history and physical examination is performed, eligibility is confirmed and clinical and research labs are collected. A complete panel of tests that will be performed during this visit is detailed in Appendix 1. **Study visits during treatment** Patients will be followed every 4 to 8 weeks while they are receiving study drugs. Patients will be advised about study adherence and monitored for adverse events. A complete panel of tests that will be performed during this visit is detailed in Appendix 1. Study medications will be distributed at visits. **Safety and adverse event monitoring** At each study visit, study staff will inquire about adverse events that may or may not be related to study drugs. Any unfavorable medical occurrences will be recorded, whether or not considered related to the patient's participation in the research, temporally associated with the patient's participation in the research. Adverse events (AEs) classified as grade 3 or higher will be reported to the principal investigator. Any grade 3 or 4 AEs and all SAEs will be reviewed as they occur by the study team (21). Any AEs occurring more frequently than expected will be reported to the IRB. Principal Investigator shall forward to Merck's Global Safety group, any SAE or Suspected Unexpected Serious Adverse Reaction (SUSAR), including, but not limited to, all initial and follow-up information involving any study subject in the study. Notification shall be in the

form of a completed CIOMS I/MedWatch within two (2) business days of learning of the information. SAE reports and any other relevant safety information are to be forwarded to Merck's Global Safety group facsimile number: 215-993-1220. SAE and Reportable New Information will be reported to the IRB as per University policy.

All reports of Study Drug exposure during pregnancy or lactation, whether associated with an AE or not, must be reported to Merck's Global Safety group in accordance with the timelines and contact information for an SAE. Principal Investigator shall follow pregnancies to term to obtain the outcome of the pregnancy. The outcome of the pregnancy shall be forwarded to Merck's Global Safety group.

Clinical labs will also be drawn at these visits. There is a known, defined drug interaction between grazoprevir and tacrolimus causing an increase in tacrolimus levels of approximately 40%, as documented in the package insert (22).Levels of immunosuppressive agents will also be determined at these visits as clinically indicated. The need for dose modification of the patient's immunosuppression in the time between visits will be recorded.

End of treatment visit
Patients will be seen 12 weeks after starting study drugs (and 16 weeks in the case of genotype 1a patients with baseline NS5A RAVs) for an end of study visit. Patients will also be counseled about study adherence and we will inquire about adverse events. A complete panel of tests that will be performed during this visit is detailed in Appendix 1.

Post treatment follow up visits
Patients will be followed 12 weeks after they complete treatment. A complete panel of tests that will be performed during this visit is detailed in Appendix 1.

Additionally, patients may be enrolled in an ongoing study titled "A prospective cohort study to assess treatment efficacy in chronic hepatitis C mono-infection and co-infection with HIV" (HP – 00063362), to monitor longer-term outcomes. Outcomes including graft survival and episodes of rejection will be monitored by periodic medical record abstraction.

Early termination or treatment discontinuation
Patients who discontinue HCV therapy prior to 12 weeks will be followed at post-treatment week 4 and post-treatment week 12. HCV VL, safety labs and hepatic panel will be performed at these visits. A complete panel of tests that will be performed during this visit is detailed in Appendix 1.

2.5.2 Host Response Measurement

Determination of Interferon stimulated gene expression associated with HCV suppression

PCR. ISG expression rapidly shuts down with initiation of DAA therapy in HCV infected subjects. We would like to evaluate the effectiveness of the host response to DAA therapy in pre- and post-transplant (immunosuppressed) patients by evaluating the changes in ISG expression observed in the two groups of patients before and 4 weeks after completion of HCV therapy.

Expression analysis of select genes will be performed with predesigned or custom TaqMan assays individually or assembled into custom-designed 96-well plates or 384-well microfluidic cards (Life Technologies). Total RNA isolated from paxgenes will be reverse transcribed using random primers with the High Capacity cDNA Reverse Transcriptase Kit (Life Technologies). 1–25 ng RNA will be used for each qRT-PCR reaction. Gene expression will be determined as Ct based on 40 PCR cycles. For statistical analysis,

	undetectable expression will be assigned a minimal detectable level with Ct of 40. Expression of *GAPDH* will be used as an endogenous control. Relative expression of targets normalized by *GAPDH* expression (ΔCt) will be calculated as $Ct_{GAPDH} - Ct_{target}$, with conversion and display relative to *GAPDH* expression by $2^{\Delta Ct}$. ΔΔCt values, used to calculate changes in expression between samples or groups of samples, will be calculated as $\Delta Ct_{sample\ A} - \Delta Ct_{sample\ B}$, then converted to a fold change by $2^{-\Delta\Delta Ct}$. Relative expression of individual ISGs FNs in blood will also be calculated as a percent of the sum of the total measured ISGs ([$2^{\Delta Ct}$ of each individual ISG/ total $2^{\Delta Ct}$ of all measured ISGs] × 100). 384-well microfluidic cards will be run on a 7900HT Fast Real-Time PCR System (Life Technologies). **Cytokine and Chemokine ELISA.** IP-10 is the most studied soluble ISG that has been associated with response to therapy. The expression of IP-10 is rapidly shut down with initiation of DAA therapy in HCV infected subjects and a higher pre-treatment IP-10 level has been associated with a poorer response to therapy. We would like to evaluate the effectiveness of host response to DAA therapy in pre- and post-transplant (immunosuppressed patients) by evaluating the fold changes in IP-10 and other proinflammatory/chemotactic cytokine expression observed in the two groups of patients before and 4 weeks after completion of HCV therapy. Whole blood will be allowed to clot in for at least 60 minutes prior to centrifugation at 1,700 *g* for 15 minutes. Serum will be collected and aliquoted prior to freezing at –80°C. After thawing, serum will be centrifuged at 1,450 *g* for 10 minutes, and the supernatant will be used for ELISA. Quantitation performed with multiplex (Chemokine 9-plex, Proinflammatory 9-plex) assays that include CXCL10 (MesoScale Discovery). Plate-to-plate variability of pooled samples will be used for normalization across experiments. All samples will be run as technical duplicates, and averages will be used for data analysis. **Determination of HCV specific CD4 and CD8 T cells by ELISPOT** We have previously described an augmentation of HCV-specific T-cell responses with DAA therapy being associated with achieving an SVR 12. HCV specific T-cell phenotype and function will be characterized as previously described and measured before and at SVR 12. In brief, peripheral T-cells will be isolated from PBMCs by Ficollhypaque density gradient separation and be stimulated with pooled HCV genotype specific peptides. For this experiment, we will use HCV 15-18-mer peptides with 11 or 12 amino acid overlaps spanning the entire HCV polyprotein and reconstituted in 5% sterile dimethylsulphoxide (DMSO), pooled consecutively into twenty-one groups and aliquoted until use. The number of HCV-responsive IFN-γ-producing PBMCs will be quantified by a standard ELISPOT assay (BD Biosciences), in which 96 well ELISPOT plates are coated with anti-IFN-γ biotinylated capture antibody and incubated overnight at 4°C. Plates will be blocked using a lymphocyte medium, and PBMCs will be allowed to rest for 6 hours at 37°C. PBMCs will then be plated between 250,000- 400,000 cells per well with either phytohaemagglutinin (PHA) as a positive control (5 mg/ml), DMSO as a negative control (0.05%), or pooled HBV peptides (3 mg/ml/peptide). All cultures will be performed in duplicate. After incubating for 12 hours at 37°C, cells will be removed, and plated with streptavidin detection antibody, enzyme conjugate, and substrate. The plates will then be air dried in the dark overnight, and developed spots will be enumerated using an ELISPOT plate reader.
2.6 Study Duration	We anticipate the entire study duration to be 24 months. The duration per study subject enrolled will be 30 to 34 weeks depending on their treatment duration. This will include 6 weeks of screening, 12 to 16 weeks of therapy, and 12 weeks of follow up to determine SVR 12 (Figure 1).

	After the initial 30-34 weeks, patients will be enrolled in our ongoing HCV treatment cohort study (HP – 00063362). Using this long term prospective cohort study, we will be able to follow our patients' longer-term outcomes through medical record abstraction.
2.7 Statistical Analysis and Sample Size Justification	Data analysis will be completed by the study biostatistician. **Primary Endpoints** **Primary Objective 1.** The primary endpoint for this objective will be the measurement of host immune responses using ISG expression including IP-10 levels, and HCV specific T-cell responses. ISG expression and IP-10 levels will be measured before and 4 weeks after completion of therapy. HCV specific T-cell responses will be measured before and during SVR 12 (12 weeks after completion of the study medications). **Primary Objective 2.** The primary endpoint for this objective will be the measurement of SVR 12. This will be measured at study week 24/28, or 12 weeks after completion of the study medications. **Secondary Endpoints** **Secondary Objective 1.** We will assess the safety of the medications using adverse event monitoring. The primary endpoint for this objective will be the presence of any severe adverse event. A secondary endpoint will be the early discontinuation of the study medications. This will be assessed at the study visits weeks 0, 4, 8, and 12. Based upon prior trials with these investigational drugs, we estimate rate of serious adverse events of about 15% (17). **Secondary Objective 2.** The primary endpoint for this objective will be episodes of rejection and graft survival for post-transplant patients after treatment and will be recorded via medical record abstraction as part of their enrollment in the ongoing prospective cohort study to assess treatment efficacy in chronic hepatitis C (HP – 00063362). Research data will be collected and managed using REDCap (Research Electronic Data Capture) hosted at the University of Maryland, which is a secure, web-based application to support data capture for research studies. All of these variables will be entered into REDCap by study staff at the end of each study visit based upon the labs obtained, patient questionnaires, and interim records reviews, and after the initial 30 to 34 weeks of the study based upon medical records reviews. <u>Statistical Methods</u> **Clinical treatment arm** All statistical analyses will be based on an intention-to-treat model, including all enrolled patients who received at least one dose of study medication. Both the primary efficacy and safety endpoints will be based on the proportion of patients with SVR 12 and severe adverse events, respectively, with point estimates and 95% confidence interval. Both primary efficacy and safety endpoints will be compared to a non-transplant, HCV-infected historical control group by using two-sided, exact one-sample binomial test at a significance level of 0.05. Demographic and baseline characteristics will be summarized using standard descriptive statistics overall and by patient cohort. Univariate logistic regression will be performed to identify baseline factors associated with SVR 12 at a significance level of 0.05. **Measurement of host immune response**

Mann-Whitney or Wilcoxon matched-pairs signed rank test will be used for 2-group comparisons, and a linear mixed-effects model will be used for multigroup longitudinal comparisons with fixed effects for group (SVR versus relapse), time, and group/time interaction. A significant group/time interaction would indicate that the 2 groups had different patterns over time. A class variable will be used for time to allow an arbitrary average time pattern within a group. In addition to fixed effects summarizing average group responses over time, the model will accommodate random, patient-specific deviations in intercept and time effects. Correlations will be assessed by nonparametric Spearman rank correlation. Spotfire S+ 8.2 (TIBCO), Prism 6.0 software (GraphPad), and SAS 9.3 (SAS Institute, Inc.) will be used for statistical analysis and data presentation using a p-value of 0.05 to indicate statistical significance.

Power/Sample Size:

This is a pilot, prospective clinical trial comparing the host immune response using ISG expression, including IP-10 levels, and HCV specific T-cell responses between a historical, non-transplant cohort of 25 patients from IRB approved studies previously conducted at the IHV and post-renal transplant (immunosuppressed) patients and its association with SVR 12. The study team will perform an interim data and safety evaluation for the efficacy, safety, and tolerability of grazoprevir and elbasivir therapy. Data analysis will focus on the clinical efficacy endpoint of SVR 12 with secondary analysis looking at the relationship of the host immune response markers with the primary endpoint of SVR 12 in both groups.

We anticipate enrollment of 25 patients in this arm of the study. This will be compared to a historical control group of 25 patients with stored blood samples from previous IRB approved clinical trials conducted at the IHV.

We anticipate a SVR 12 of greater than 90% in the grazoprevir plus elbasvir treatment group post-renal transplantation. With 50 participants (25 in this study and 25 historical controls), the study will be able to estimate the difference in SVR12 proportions to within ±0.18 (Table 2). Table 2 shows a substantial gain in precision by increasing the sample size from 15 per arm to 25 per arm; however, the effect diminishes beyond 25.

Table 2: Sample Size Calculation for SVR12

N per arm	15	20	25	30	50
Accuracy to within ±	0.24	0.20	0.18	0.17	0.13

2.8 Specific Drug Supply Requirements

We request study drug supply from Merck Inc. for all study subjects. The study drugs will be received and distributed by Lisa Langer, RPh, through the UMD investigational drug pharmacy. The study drugs will be stored under the conditions specified by the manufacturer. Packaging and labeling will be done by Merck. At the study's conclusion the remaining investigational product will be disposed of according to the ICH/GCP guidelines and our institution's policies.

2.9 Adverse Experience Reporting

2.9.1 Overview

Adverse experience reporting will be performed per study agreement at each study visit. During each clinical evaluation with the patients, information regarding adverse events and adverse reactions will be elicited. Any untoward or unfavorable medical occurrences will be recorded, whether or not considered related to the patient's participation in the research. Additionally, patients may be seen at unscheduled visits for a grade 3 or 4 adverse event or any unexpected adverse event or potential toxicity.

Adverse events (AEs) classified as serious or grade 3 or higher will be reported to the principal investigator. Any grade 3 or 4 AEs and all SAEs will be reviewed as they occur by the study team. Any AEs found to be occurring above the level expected by the study team will be reported to the IRB and study sponsor.

For this study, patients may have expected AEs related to transplantation in the post-transplantation arm or related to renal failure in the pre-transplantation arm. This protocol will record all AEs possibly related to the treatment of HCV as defined by the Office for Human Research Protections (OHRP) (21).

2.9.2 Definitions

Adverse Event (AE): For this study, an adverse event will include any untoward or unfavorable medical occurrence associated with the patient's participation in the research including, but not limited to:

- Worsening (a change in nature, severity or frequency) of medical conditions present at the onset of the study, including renal and liver dysfunction
- The development of new illnesses
- Any infections
- Abnormal laboratory values (significant shifts from the baseline values within the range of normal obtained at screening that the investigator considers clinically relevant)
- Clinically significant abnormalities in physical examination, vital signs, weight changes, and/or laboratory tests
- Subjective symptom reporting while on the study medication

Serious Adverse Event (SAE): An adverse event or adverse reaction (AR) is considered "serious" if, in the view of the investigator it results in any of the following outcomes (adapted from FDA regulations at 21 CFR 312.32(a).) (21):

- Death
- A life-threatening event: an AE or AR is considered life-threatening if, in the view of the investigator, it places the patient at immediate risk of death. This does not include AEs or ARs that, had it occurred in a more severe form, may have caused death.
- Inpatient hospitalization or prolongation of an existing hospitalization
- Persistent or significant disability or incapacity
- Congenital anomaly or birth defect
- Important medical events that may not fall into the above categories may be considered serious when, based upon appropriate clinical judgement, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the above listed outcomes

2.9.3 Grading and Attribution of Adverse Events

2.9.3.1 Grading Criteria

Adverse events will be graded according to the criteria set forth in the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 (NCI-CTCAE manual). Adverse events will be graded on a scale from 1 to 5 according to the following standards (23):

- Grade 1 = mild adverse event
- Grade 2 = moderate adverse event
- Grade 3 = severe and undesirable adverse event
- Grade 4 = live-threatening or disabling adverse event
- Grade 5 = death

2.9.3.2 Attribution Definitions
The relationship or attribution of an adverse event to participation in the study will initially be determined by the investigator. An AE will be defined as possible if the adverse event has a reasonable possibility of being related to study participation and/or there is evidence to suggest a causal relationship. An AE will be defined as definite if the adverse event is clearly related to study participation. An AE will be defined as unrelated if the adverse event is clearly not related to study participation and/or there is insufficient evidence to suggest a causal relationship (21).

2.9.4 Collection and Recording of Adverse Events

2.9.4.1 Collection Period
Adverse events data grade 1 and higher will be collected from the time or initiation of study drug until a patient completes study participation or until 30 days after the patient withdraws from the study. All Grade 3 or higher AEs will be reviewed by the investigator and reported to the sponsor and IRB if they are found to occur more frequently than expected by the study team and are related to study participation or the study drug.

2.9.4.2 Collecting Adverse Events
Adverse events may be identified during study visits through:

- Observation of the patient
- Interview
- Unsolicited complaints from the patient
- Abnormal clinical or laboratory values

2.9.4.3 Recording Adverse Events
At each study visit, the investigator will review all grade 1 or higher AEs and, if potentially related to study participation or the study drug, will report them to the study primary investigator. An AE or SAE will be followed until resolution with or without sequelae, or until the end of study participation or 30 days after participant withdrawal, whichever comes first.

2.9.5 Reporting of Serious Adverse Events and Adverse Events
All SAEs determined to be possibly or definitely related to study participation or the study drug will be reported to the IRB and the study Sponsor (Merck Inc.).

The IRB will be promptly notified of any unanticipated problems involving risks to participants or others, not otherwise reported as an adverse event.

2.9.6 Data Safety Monitoring Board
For this clinical trial, a Data Safety Monitoring Board (DSMB) led by an independent chair in collaboration with the PI and co-investigators will be utilized. The DSMB will review this trial for safety in both the pre-transplant and post-transplant groups. The first DSMB meeting will occur six months from the time of the enrollment of the first participant and then every six months thereafter. Additionally, the board may be convened ad hoc if an adverse event calls for immediate attention.

The DSMB will review the following information:

- Any adverse events
- Any safety related amendments
- Significant Protocol deviations
- Lab tests
- Enrollment
- Outcomes

	• Chart audit as needed The DSMB may require changes in the protocol and has the power to halt the study if a participant's safety is ever in question. Board members will be notified of any serious study related adverse events. The DSMB will be composed of at least four members: 1. An independent physician-investigator not involved in the study who will serve as chair 2. A physician-investigator with expertise in infectious diseases including hepatitis C treatment in renal transplant patients 3. A physician-investigator with expertise in transplant nephrology in hepatitis C infected patients 4. An epidemiologist with extensive training in biostatistics.
2.10 Itemized Study Budget	A refined itemized budget detailing the costs associated with the study was provided with the final protocol.
2.11 References	1. Bunchorntavakul C, Maneerattanaport M, Chavalitdhamrong D. Management of patients with hepatitis C infection and renal disease. World J Hepatol 2015;7:213-225. 2. Kirby BJ, Symonds WT, Kearney BP, and Mathias AA. Pharmacokinetic, Pharmacodynamic, and Drug-Interaction Profile of the Hepatitis C Virus NS5B Polymerase Inhibitor Sofosbuvir. Clin Pharmacokinet 2015; epub. 3. Harvoni [package insert]. Foster City, CA: Gilead Sciences, Inc.; 2015. 4. Saxena V, Koraishy FM, Sise M, et al. Safety and efficacy of sofosbuvir-containing regimens in hepatitis C infected patients with reduced renal function: real-world experience from HCV-TARGET [abstract LP08]. J Hepatology 2015;62:S267. 5. Viekira Pak [package insert]. North Chicago, IL: AbbVie Inc.; 2015. 6. Cohen D, et al. Ombitasvir/ABT-450/Ritonavir and Dasabuvir with or without ribavirin in treatment-naïve HCV genotype 1-infected adults with chronic kidney disease [abstract]. 50th Meeting of the International Liver Congress, April 22-26, 2015, Vienna, Austria. 7. Lawitz E, Gane E, Pearlman B, et al. Efficacy and safety of 12 weeks versus 18 weeks of treatment with grazoprevir (MK-5172) and elbasvir (MK-8742) with or without ribavirin for hepatitis C virus genotype 1 infection in previously untreated patients with cirrhosis and patients with previous null response with or without cirrhosis (C-WORTHY): a randomized, open-label phase 2 trial. Lancet 2015;385:1075-86. 8. Yeh WW, Feng H, Dunnington KM, et al. No clinically meaningful pharmacokinetic interactions between HCV inhibitors grazoprevir/elbasvir with tacrolimus, mycophenolate mofetil, and prednisone, but cyclosporine increases grazoprevir/elbasvir exposures in healthy subjects [abstract]. AASLD 2015, Boston, MA. 9. Rahermann B. Pathogenesis of chronic viral hepatitis: differential roles of T cells and NK cells. Nat Med 2013;19(7):859-868. 10. Wieland S, et al. Simultaneous detection of hepatitis C virus interferon stimulated gene expression in infected human liver. Hepatology 2014;59(6):2121-2130.

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2.12 Publication Plan	We anticipate submitting the results of the study for publication as soon as the final analysis of the primary outcomes is performed, within 3-6 months after the completion of the last study participant. We anticipate two publications, to be published in peer-reviewed journals. We also anticipate two abstract presentations, one likely after study enrollment is complete and another after the completion of the study and analysis.

2.13 Curriculum Vitae	Dated CVs for the PI, Co-investigators, and study coordinator were provided with the application materials.
2.13 Protocol Submission for Investigator-Initiated Studies	This protocol was submitted directly by the study PI.