A Multi-Center, Open-Label Study of Glecaprevir/Pibrentasvir to Treat Recipients of Transplanted Kidneys from Deceased Donors with Hepatitis C Virus (MYTHIC: Multi-center studY to Transplant Hepatitis-C InfeCted kidneys)

SUPPLEMENTARY DOCUMENT

Table of Contents

Supplementary Table 1: Inclusion and Exclusion Criteria for the MYTHIC Trial

Inclusion criteria for kidney transplant candidates at enrollment:

1. Age >21 and <65 years at the time of consent
2. Estimated glomerular filtration rate (eGFR) < 15 ml/min/1.73 m2 (calculated using the 4-variable Modification of Diet in Renal Disease [MDRD] equation) at the time of consent
3. Listed for an isolated kidney transplantation
4. Subjects must be able to understand and adhere to the study visit schedule and all other protocol requirements, and must voluntarily sign and date an informed consent
5. No available medically acceptable, compatible living kidney donor
6. Subject must agree to use an effective method of birth control as outlined in Appendix D, and must have implemented the method by the day of transplant
7. Assent from the patient's primary transplant nephrologist that participation would be reasonable for the patient
8. Attended an educational session on use of HCV seropositive allografts

Exclusion criteria for kidney transplant candidates at enrollment:

1. History of severe, life-threatening or other significant sensitivity to immunosuppressants utilized in kidney transplant.
2. Female who is pregnant, breastfeeding, or is planning to become pregnant during the study
3. Human immunodeficiency virus (HIV) RNA-positive or HIV antibody-positive
4. HCV RNA-positive
5. Hepatitis B Virus (HBV) surface Ag-positive or detectable HBV DNA
6. Primary focal segmental glomerulosclerosis (FSGS) or disease process with increased risk of causing early graft failure as assessed by the transplant nephrologist and/or investigator team
7. Presence of clinically significant liver disease evident after review of history, labs and fibroscan imaging:

   a) Persistently elevated liver enzymes (Alanine aminotransferase [ALT] >2 times upper limit of normal) of unknown cause

   b) Liver fibroscan result >8kPA, or >F2 on liver biopsy
8. Transplant candidate requiring antibody desensitization protocol for transplantation

9. Most recent calculated panel reactive antibody (cPRA) >80%. For this purpose, the cPRA assessed will be the cPRA most-recently reported to United Network of Organ Sharing (UNOS) at the time of wait listing.

10. Prior recipient of a non-renal solid organ transplant

11. Subject has any other medical condition that, in the opinion of the Investigator, would adversely affect the participant's participation in the study. Additionally, the investigator may exclude any patient with a pre-existing cancer that does not have a high probability of cure or control and as per local transplant center guidance.

12. Requirement for and inability to safely discontinue the medications or supplements listed in Table 7 of the protocol at least 2 weeks or 10 half-lives (whichever is longer) prior to the first dose of any study drug

13. Participation in another interventional study of any investigational agent or approved medication, or participation in another interventional study that the responsible investigator deemed to be an exclusion, from 6 months prior to screening to the last study visit.

Inclusion criteria for kidney donor and allograft*

1. Deceased donor organ with kidney donor profile index (KDPI) ≤0.85

2. HCV RNA-positive

Exclusion criteria for HCV RNA-positive kidney donor and allograft

1. Known prior HCV treatment with direct acting antiviral medication

2. HIV RNA-positive

3. HBV Surface antigen-positive or HBV DNA-positive

* Standard of care deceased or living donor kidney transplants were allowed to be accepted by enrolled subjects. For subjects who accepted a standard of care transplant, no study allograft criteria were applied. The trial also allowed acceptance of kidneys from HCV RNA-negative/HCV antibody-positive donors; those results will be presented in a different manuscript.

Supplementary Table 2: Summary of the visit schedule for recipients of HCV-viremic kidneys

	Visit	Research Visit Activities			Research Labs							G/P		Standard of Care					
Transplant, G/P treatment, & post treatment follow-up	**Visit[1]**	**Assess Adverse Events[12]**	**Current Medications**	**Confirm donor-recipient pair meet trial criteria[2]**	**G/P adherence**	**HCV Antibody**	**Urine Prot./Creat. Ratio**	**HCV RNA[3]**	**Archive blood samples[4]**	**DAA resistance**	**Donor blood sample[5]**	**Pregnancy test[6]**	**Study drugs dispensed**	**Vitals & PE[7]**	**Clinical Chemistry[8]**	**Hematology**	**HCV RNA[3]**	**Pregnancy test[6]**	**Tacrolimus level[9]**
Peri-transplant visit	V1	✓	✓	✓							✓			✓	✓	✓		As per transplant center standard of care	As per transplant center standard of care
Day 3[7]Post KT	V2	✓	✓			✓		✓	✓			✓		✓	✓	✓			
Day 1 of G/P treatment	V3	✓	✓									✓	✓	✓	✓	✓			
Treatment week 1±3 days	V4	✓	✓		✓			✓						✓	✓	✓			
Treatment week 2 ±3 days	V5 T5	✓	✓		✓														
Treatment week 4 ±3 days	V6	✓	✓		✓	✓	✓		✓			✓	✓	✓	✓	✓	✓		
Treatment week 6 ±3 days[10]	T7	✓	✓		✓														
Treatment week 8 ±7 days (EOT)	V8	✓	✓		✓		✓	✓	✓			✓		✓	✓	✓			
Post treatment week 4 ±7 days	V9 T9	✓	✓																
Post treatment week 12 ±14 days	V10	✓	✓				✓	✓	✓										
Post treatment week 24 ±28 days	V11	✓	✓				✓	✓	✓					✓	✓	✓			
1 year Post transplant ±28 days; End of study[11]	V12	✓	✓			✓	✓		✓					✓	✓	✓			
G/P treatment failure		✓						✓	✓	✓									
Premature discontinuation		✓					✓	✓	✓					✓	✓	✓			

G/P: Glecaprevir / Pibrentasvir

1 Visit types: V - in person visit to site; L - Lab visit; T - Telephone encounter

2 Includes evaluation of blood type and HLA compatibility

3 HCV RNA testing at V2, V4, V8, and V10 and V11 are study paid labs. HCV RNA testing at visit 6 is billed as SOC. All HCV RNA testing is done at local laboratory by a CLIA certified laboratory and with an FDA approved test.

4 Archived plasma samples (required) and PBMC (optional at Visits 8, 10, and 12) samples are shipped to the clinical coordinating center at Massachusetts General Hospital, see section 5.3.1.1

5 Lack of availability of donor blood sample will not preclude donor eligibility or transplant. HCV genotype is determined from the donor blood sample at local laboratory; Another portion of the donor sample is frozen for storage and potential testing for DAA resistance as needed. See section 5.3.1.1

6 If visit 2 and visit 3 are on the same day or within 14 days of each other only 1 serum pregnancy test need to be obtained. Visit 6 and Visit 8 pregnancy tests can be urine tests. Additional pregnancy tests not billed to the study are obtained as per transplant center standard of care.

7 Vitals and physical exam will be conducted and documented as per standard of care at transplant center

8 Liver function tests (ALT, AST, Alkaline Phosphatase, total bilirubin) will be ordered as a study lab if not standard of care as part of clinical chemistry labs at the local transplant center. See table 8 for clinical chemistry labs and hematology labs.

9 Includes Tacrolimus level and/or other utilized immunosuppressant that requires therapeutic drug monitoring as per transplant center standard of care;

10 Telephone call will be performed between clinic visits at treatment week 6 to assess subject adherence to G/P treatment.

11 Obtain information on donor specific antibodies as applicable per center's standard of care from

subject medical record.
12. Assess for clinical outcomes, including fibrosing cholestatic hepatitis, and extrahepatic manifestations of HCV at Visits 4-11.

Supplementary Table 3: Characteristics of 30 HCV-viremic deceased donor kidneys *

Characteristic	
Kidney Donor Profile Index (IQR)	53% (41—65%)
Age (years): Median (IQR)	33.5 (29.0—38.0)
Female	12 (40.0%)
Race:	
White	19 (63.3%)
African American	5 (16.7%)
Other	6 (20.0%)
Cause of death:	
Anoxia	25 (83.3%)
Cerebrovascular disease	1 (3.3%)
Head trauma	4 (13.3%)
Diabetes status:	
Yes	1 (3.3%)
No	25 (83.3%)
Unknown	4 (13.3%)
Hypertension	2 (6.7%)
Median terminal creatinine (mg/dl): median (IQR)	1.0 (0.7—1.5)
Donation after cardiac death	5 (16.7%)
Cold ischemia time (hours): median (IQR)	15 (11—19)
HCV genotype:	
1A	13 (43.3%)
2	1 (3.3%)
4	1 (3.3%)
Missing**	15 (50%)
Kidney Donor Profile Index: Median (IQR)	53% (41—65%)
Kidney Donor Profile Index recalculated as if donor was HCV RNA and antibody negative: Median (IQR)	33% (24 – 44)

* Results are presented for each of the 30 allografts. 25 total donors provided the 30 kidneys transplants

** One of the recipients of these kidneys had an HCV genotype sent, which was genotype 1a

Supplementary Table 4: Kidney biopsies in recipients of HCV-viremic deceased donor kidneys

Participant	Days since transplant	Main findings	Other notes	Exam performed	Indication
Cases of rejection					
1	124	Acute t-cell mediated rejection with endarteritis Banff 2b	C4d negative Transplant glomerulopathy 28 glomeruli, 4 sclerotic DSA negative	Light microscopy, immunofluorescence, electron microscopy	Elevated creatinine
2	97	Borderline T-cell mediated rejection;	C4d negative 24 glomeruli, 0 sclerotic Interstitial fibrosis and tubular atrophy grade 1 DSA negative	Light microscopy	Surveillance biopsy
3	180	Acute allograft rejection Banff 1A	C4d negative 50 glomeruli, 1 sclerotic DSA negative BK negative	Light microscopy	Elevated creatinine
Non-rejection					
1	26	Acute calcineurin inhibitor toxicity	10 – 20% interstitial fibrosis and 5% tubular atrophy 42 glomeruli, 1 sclerotic	Light microscopy, immunofluorescence, electron microscopy	Elevated creatinine
4	233	Mild interstitial inflammation with focal mild tubulitis, not meeting Banff criteria for acute or borderline acute cellular rejection	Mild acute tubular injury, mild vascular disease 5 – 10% interstitial fibrosis and tubular atrophy SV40 (BK virus) negative C4d negative	Light microscopy, immunofluorescence, electron microscopy	Elevated creatinine
5	90	Non-specific changes including mild increase inflammatory cells within glomeruli	Negative C4d 19 glomeruli (none sclerotic) 1 glomerulus with mesangial expansion and a double contour; uncertain significance	Light microscopy	Surveillance biopsy

6	34	Acute pyelonephritis, interstitial edema, moderate acute tubular injury	Interstitial inflammation, predominantly neutrophilic 4 glomeruli (none sclerotic) C4d negative	Light microscopy, immunofluorescence, electron microscopy	Elevated creatinine
7	14	Acute tubular injury, mild	11 glomeruli (none sclerotic) No significant interstitial fibrosis C4d negative	Light microscopy, immunofluorescence, electron microscopy	Elevated creatinine: Delayed graft function

Abbreviations: DSA = donor specific antibodies, BK = polyoma virus

Supplementary Table 5: Proteinuria among 30 recipients of HCV-viremic kidneys*

Participant	Maximum**	Most recent**	Cause of end-stage kidney disease	Biopsy performed
6	1.79	1.07	Hypertension	Yes *** • Indication: Elevated creatinine Findings: Acute pyelonephritis
7	1.11	0.27	Lithium toxicity	Yes *** • Indication: Elevated creatinine; Delayed graft function • Findings: Acute tubular injury
8	1.31	0.14	Polycystic kidney disease	No
9	1.03	0.12	Diabetic Nephropathy	No
10	1.28	0.17	Diabetic Nephropathy	No

* Per protocol, these recipients underwent surveillance for post-transplant proteinuria, estimated using the ratio of urine protein to creatinine in a spot urine sample

** The ratio of the concentration of urine protein/urine creatinine from a spot urine sample provides an estimate of 24 hour urine protein excretion in grams.

*** Additional details about biopsy results in Supplemental Table 4

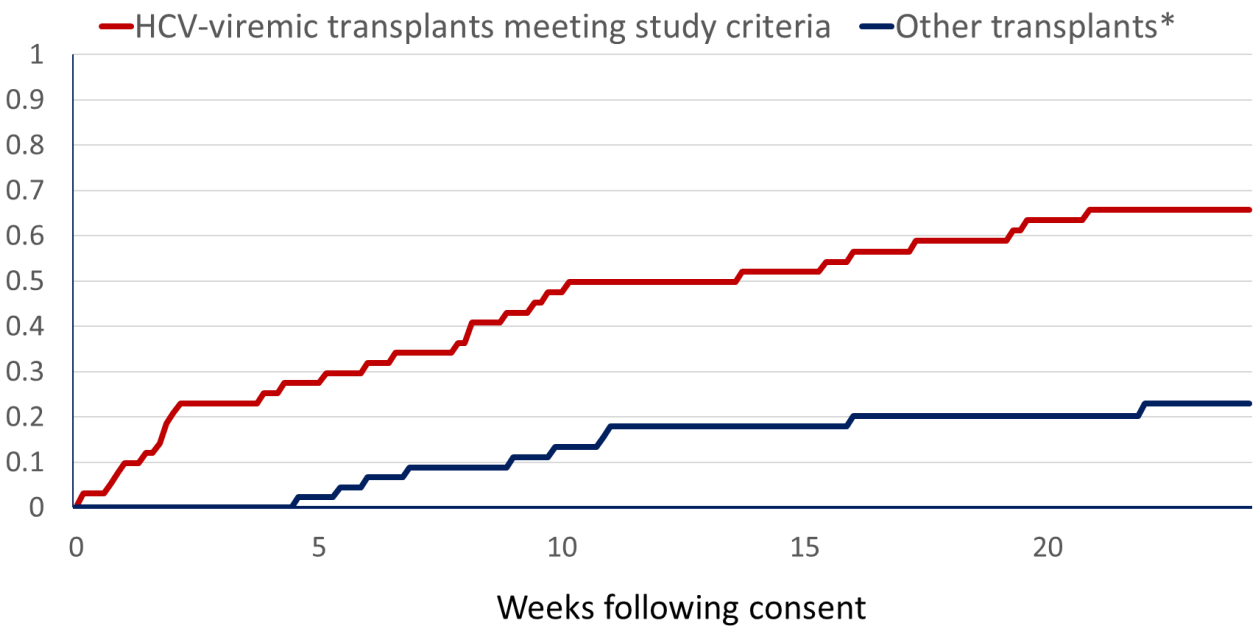
Supplementary Table 6: Serious adverse events among 63 patients consented for MYTHIC. Relatedness to HCV-infection or glecaprevir/pibrentasvir was determined by the primary investigator and reviewed by the steering committee and the data safety monitoring board. Severe adverse events deemed related to the transplant procedure itself were not considered "study-related".

Severe adverse events, by phase of study	
SAEs occurring among 30 recipients of HCV-viremic kidneys taking glecaprevir/pibrentasvir*	Count
Febrile neutropenia**	2
Infected peri-nephric fluid collection**	2
Syncope/Orthostatic hypotension	2
Delayed graft function	2
Urinary tract infection	2
ST elevation myocardial infarction	1
Gastritis	1
Pulmonary edema	1
Constipation	1
Bleeding from transplant biopsy site	1
Tacrolimus toxicity***	1
Ureteral leak	1
Cerebrovascular accident	1
Small bowel obstruction	1
Hyperglycemia	1
Fever/chills	1
SAEs occurring after completion of glecaprevir/pibrentasvir among 30 recipients of HCV-viremic kidneys	
Urinary tract infection**	2
Nausea/vomiting	2
Acute cellular rejection	2
Hydronephrosis requiring nephrostomy tube**	2
CMV enteritis	1
Death 9 months post-transplant in setting of hospitalization with MRSA bacteremia, acute kidney injury, cholangitis, liver failure, and CMV	1
SAEs occurring in waitlisted patients	
Hypertensive urgency/emergency****	3
Clotted dialysis access	2
Motor vehicle accident	1
Biliary obstruction	1
Orthostatic hypotension	1

Cardiovascular accident	1
Non-ST elevation myocardial infarction	1
Cellulitis	1
Rectal bleeding	1
Hyperkalemia	1

Legend: Delayed graft function was only considered a SAE if it prolonged hospitalization or led to re-hospitalization. As noted in manuscript, a total of 7 recipients of HCV-viremic kidneys had delayed graft function, defined as any dialysis in the first post-transplant week.
* Meeting MYTHIC trial donor criteria
** Both SAEs occurred in the same participant.
*** The patient who developed tacrolimus toxicity accidentally took 5 times prescribed dose of tacrolimus for multiple days
**** Two of the three cases of hypertensive emergency took place in the same participant
Abbreviations: SAE = severe adverse events, CMV = cytomegalovirus, MRSA = methicillin resistant staphylococcus aureus

Supplementary Figure 1: Cumulative incidence of kidney transplant in the MYTHIC trial



* Other kidney transplants were from donors that did not meet MYTHIC trial criteria for an HCV-viremic donor.

Supplementary Figure 2: Changes in serum HCV antibody status among among the 30 recipients of HCV-viremic deceased donor kidneys