Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Kwo PY, Mantry PS, Coakley E, et al. An interferon-free antiviral regimen for HCV after liver transplantation. N Engl J Med. DOI: 10.1056/NEJMoa1408921

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ABT-450 was identified as a lead compound by AbbVie and Enanta Pharmaceuticals.

Abbreviations: HCV, hepatitis C virus; RVR, rapid virologic response; EOTR, end-of-treatment response; SVR, sustained virologic response; HCC, hepatocellular carcinoma; ULN, upper limit of normal; LLN, lower limit of normal.

CORAL-I Investigators

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Eligibility Criteria

Main Inclusion:

1. Male or female between 18 and 70 years of age, inclusive, at time of enrollment.
2. The patient is a recipient of a cadaveric or living donor liver transplant no less than 12 months before screening, which was a consequence of HCV infection or of hepatocellular carcinoma occurring in the setting of HCV infection.
3. A qualifying liver biopsy with evidence of fibrosis not greater than F2 by Metavir scale (or equivalent score by a different scoring system) obtained at least 9 months post-transplant, and within 6 months of the Screening Visit, or during the screening period.
4. Female who is:
    - practicing total abstinence from sexual intercourse (minimum 1 complete menstrual cycle)
    - sexually active with female partners only
    - not of childbearing potential, defined as:
        - postmenopausal for at least 2 years prior to screening (defined as amenorrheic for longer than 2 years, age appropriate, and confirmed by a follicle-stimulating hormone [FSH] level indicating a postmenopausal state), or
        - surgically sterile (defined as bilateral tubal ligation, bilateral oophorectomy or hysterectomy) or has a vasectomized partner(s).
    - of childbearing potential and sexually active with male partner(s):
        - of childbearing potential and sexually active with male partner(s) currently using at least one effective method of birth control at the time of screening and two effective methods of birth control while receiving study drugs (as outlined in the patient informed consent or other patient information documents), starting with Study Day 1 and for 7 months after stopping study drug as directed by the local ribavirin label. (Note: Hormonal contraceptives, including oral, topical, injectable or implantable varieties, may not be used during study drug treatment.)
5. Females must have had negative results for pregnancy tests performed: at screening by serum specimen within 35 days prior to initial study drug administration; and at baseline (prior to dosing) by urine specimen.
6. Males who are sexually active with female partner(s) of childbearing potential must agree to use two effective methods of birth control over the relevant study time period. The contraceptive practices must be observed from Study Day 1 and must continue for 7 months after stopping study drugs or as directed by the local ribavirin label.
7. Currently taking an immunosuppressant regimen based on either tacrolimus or cyclosporine where doses of immunosuppressant drugs have not been increased for at least 2 months before the Screening Visit and no new immunosuppressant drugs have been added for at least 2 months before the Screening Visit. Corticosteroids such as prednisone or prednisolone are permitted as components of the immunosuppressant regimen providing the dose is no more than 5 mg/day.
8. Patients with liver transplantation as a consequence of hepatocellular carcinoma (HCC) in the setting of chronic HCV may be eligible if:

- The HCC is not known to have exceeded the Milan Criteria on pathologic examination of the explanted liver (or equivalent staging methodology)
    - Milan Criteria defined as either a single HCC lesion ≤ 5 cm or up to three separate HCC lesions none larger than 3 cm, and with no evidence of gross vascular invasion, and no regional nodal or distant metastases) and post transplantation there is no evidence of recurrence of HCC for a least 1 year; or
- The HCC is known to have exceeded the Milan criteria on pathologic examination of the explanted liver (or equivalent staging methodology), but the patient is without evidence of recurrence for at least 2 years post-transplant.

9. HCV interferon therapy treatment-naïve or treatment-experienced prior to liver transplantation. Prior treatment, if applicable, with interferon and/or peginterferon, with or without ribavirin, at any time prior to liver transplantation.
10. Screening HCV genotype testing indicating infection with genotype 1 HCV only. In a case where HCV genotype 1 subtyping results meet any of the following criteria, the patient will be recorded in IRT as having non-1b subgenotype:
    - other than 1a or 1b; or
    - could not be determined; or
    - demonstrate a mixture of genotype 1 subtypes
11. Patient has plasma HCV RNA level > 10,000 IU/mL at Screening.

Main Exclusion:

1. Use of everolimus or sirolimus as part of the stable immunosuppressive regimen within two months of the Screening Visit.
2. Recent (within 6 months prior to study drug administration) history of drug or alcohol abuse that, in the opinion of the investigator, could preclude adherence to the protocol.
3. Positive test result at screening for Hepatitis B surface antigen (HBsAg) or anti-Human Immunodeficiency virus antibody (HIV Ab).
4. History of re-transplantation of the liver or history of any other organ transplant in the addition to liver transplatation.
5. Recipient of a liver transplant from a donor with known HIV infection, HBV surface antigen-positive and/or HCV antibody-positive test results.
6. Documented history of post-transplant complications directly involving the hepatic vasculature, e.g., thrombosis of the portal vein, the hepatic artery and/or hepatic vein, which in the opinion of the investigator have not resolved at the time of Screening.
7. Documentation of gastro-esophageal varices, ascites and/or hepatic encephalopathy following liver transplantation and which are, in the opinion of the investigator, considered to have occurred as a consequence of hepatic impairment and that do not have an otherwise reasonable explanation.
8. HCV genotype performed during screening which indicates a mixed genotypic infection.

9. De novo HCV infection in the post-transplant period.
10. History of steroid resistant rejection of the transplanted liver at any time in the post-transplant period or a history of rejection (biopsy proven or presumed) treated with high dose steroids within 3 months of Screening.
11. Any cause of active liver disease in the post-transplant period other than chronic HCV infection, non-alcoholic steatohepatitis and/or steatosis but including following:

    - Hemochromatosis
    - Alpha-1 antitrypsin deficiency
    - Wilson's disease
    - Autoimmune hepatitis
    - Alcoholic liver disease
    - Drug-related liver disease

12. Screening laboratory analyses showing any of the following abnormal laboratory results:

    - ALT > 7 × Upper limit of normal (ULN)
    - Calculated creatinine clearance (using Cockcroft-Gault method) < 55 mL/min
    - Albumin < 3.3 g/dL Prothrombin time/International normalized ration (INR) > 1.5
    - Hemoglobin ≤ Lower limit of normal (LLN)
    - Platelets < 75,000 cells per mm^3
    - Absolute neutrophil count (ANC) < 1500 cells/µL or < 1200 cells/µL for patients of African descent who are black
    - Total bilirubin ≥ 3.0 mg/dL
    - Hemoglobin A1C level > 8%

Drug Kit Assignment

Patients who met the eligibility criteria were enrolled via the Interactive Response Technology (IRT) system on Study Day 1. For enrollment of eligible patients on Day 1, the site contacted the IRT system in order to receive a unique study drug kit number. The study drug kit numbers were assigned according to schedules computer-generated before the start of the study by the AbbVie Statistics Department.

Blinding

This is an open-label study.

Sample Size Determination

With a planned sample of 30 patients, the probability of observing at least one safety event occurring at

an incidence rate of 10%, 20%, or 30% was 0.042, 0.001, and <0.001, respectively. With a planned enrollment of 30 patients, and an observed sustained virologic response rate of 80%, the 2-sided 95% confidence interval at post-treatment week 12 would be 65.7 to 94.3%.

Collection of Samples for HCV RNA Measurement

Plasma samples for HCV RNA measurement were obtained at screening. Additional samples for HCV RNA measurement were obtained at each scheduled visits every 1-2 weeks through the final treatment visit or premature discontinuation. Following administration of the last dose of study drug, samples for HCV RNA measurement were collected at post-treatment weeks 2, 4, 6, 8, 12, 24, 36, and 48 or at the time of premature discontinuation.

Plasma samples collected at screening were also used to assess HCV genotype and subtype using the Versant® HCV Genotype Inno-LiPA Assay, version 2.0 or higher (LiPA; Siemens Healthcare Diagnostics, Tarrytown, NY), and for IL28B rs12979860 haplotype analysis.

HCV RNA Measurement

Plasma HCV RNA levels were determined for each sample collected by the central laboratory using the Roche COBAS TaqMan® real-time reverse transcriptase-PCR (RT-PCR) assay v2.0. The lower limit of detection (LLOD) is 15 IU/mL and the lower limit of quantification (LLOQ) is 25 IU/mL.

Virologic Failure Criteria

The following criteria were considered evidence of virologic failure leading to discontinuation of study drug for individual patients being treated with study drugs:

- Confirmed increase from nadir in HCV RNA (defined as 2 consecutive HCV RNA measurements > 1 $\log_{10}$ IU/mL above nadir) at any time point during treatment;
- Failure to achieve HCV RNA < LLOQ by Week 6;
- Confirmed HCV RNA ≥ LLOQ (defined as two consecutive HCV RNA measurements ≥ LLOQ) at any point during treatment after HCV RNA < LLOQ.

If any of the above criteria were met, the patient was to discontinue study treatment.

Patients who completed the treatment with HCV RNA < LLOQ at the end of treatment and who had a confirmed HCV RNA ≥ LLOQ (defined as 2 consecutive HCV RNA measurements ≥ LLOQ) at any point in the post-treatment period were considered to have relapsed.

Virologic Resistance Testing

HCV RNA was extracted from samples obtained at baseline and at the time of virologic failure. Only samples with an HCV RNA level of ≥ 1000 IU/mL underwent sequence analysis in order to allow accurate assessment of products of amplification. Therefore, if the HCV RNA level at the time of virologic failure was < 1000 IU/mL, the sample closest in time after the failure with an HCV RNA level ≥ 1000 IU/mL was used. The target genes were amplified by RT-PCR and then nested PCR using primers appropriate for

subtype 1a or 1b sequences encoding NS3/4A protease, NS5A, and NS5B polymerase. The nested PCR amplification product was used as the template for DNA sequencing of the population of amplified molecules. The DNA sequence from each baseline sample was translated into amino acid sequence and compared to the appropriate reference sequence (1a-H77 or 1b-Con1) in order to identify pre-existing resistance-associated variants. The DNA sequence from each post-baseline sample was translated into amino acid sequence and compared to the sequence from the corresponding baseline sample to identify resistance-associated amino acid variants that emerged as a result of treatment.

Ribavirin Dosing

Ribavirin is typically dosed based on weight, 1000 to 1200 mg divided twice daily per local label. For example, patients weighing < 75 kg, ribavirin may be taken orally as 2 tablets in the morning and 3 tablets in the evening which corresponds to a 1000 mg total daily dose. Patients weighing ≥ 75 kg, ribavirin may be taken orally as 3 tablets in the morning and 3 tablets in the evening which corresponds to a 1200 mg total daily dose.

However, in the post liver transplant setting, patients may be at an increased risk of ribavirin-related hemolytic anemia. Therefore, for this study, ribavirin dosing was at the investigator's discretion. Ribavirin dosing at study initiation was considered acceptable at a total daily dose of 600 to 800 mg, which is lower than the typical weight-based dosing, though ribavirin dosing up to 1200 mg daily was permitted. Patients were instructed to take study medication at the same time(s) every day. Patients were instructed to take all compounds together with food, including cyclosporine or tacrolimus. All cyclosporine or tacrolimus doses were to be taken with the morning doses of study drugs and with food, as taking the study drugs in combination with tacrolimus or cyclosporine at different times can significantly impact the levels of the immunosuppressant medications.

Investigators informed patients in advance of Study Day 1 of the plan for calcineurin inhibitor management during the study, (e.g., an investigator could instruct a patient not to take their morning calcineurin inhibitor dose prior to the site visit on Study Day 1.)

Calcineurin Inhibitor Dosing

Tacrolimus

Healthy volunteers phase 1 drug interaction studies between AbbVie direct-acting antiviral combinations and the immunosuppressive agent tacrolimus was carried out in healthy volunteers.

The pharmacokinetics of a single dose of 0.5 mg tacrolimus co-dosed with ombitasvir–ABT-450/r and dasabuvir, each at steady-state, was compared to a single dose of 2 mg tacrolimus in healthy volunteers in study M13-491. Based on the pharmacokinetic data from this study, a dose of 0.5 mg approximately every 7 days to maintain the tacrolimus levels within the therapeutic range was recommended to maintain desired trough concentrations of 5 to 9 ng/mL.

Investigators were instructed to observe tacrolimus trough levels during the study to determine the appropriate tacrolimus re-dosing interval with a 0.5 mg dose. Tacrolimus levels were anticipated to stabilize around week 4 of the study. At the investigator's discretion, extra blood draws for tacrolimus level testing could be performed at any time throughout the trial and were recorded as an unscheduled visit.

Cyclosporine

Healthy volunteers phase 1 drug interaction studies between AbbVie direct-acting antiviral combinations and the immunosuppressive agent cyclosporine was carried out in healthy volunteers.

The pharmacokinetics of a single 30 mg dose of cyclosporine in combination with ABT-450/r, ABT-267 and ABT-333, dosed to steady-state was compared to a single dose of 100 mg cyclosporine dosed alone in healthy volunteers in the Study M13-103. Based on the pharmacokinetic data from this study, a dose equivalent to one-fifth the pre-study dose of cyclosporine was recommended to be taken as a single daily dose with study drugs, in the morning with food, to maintain desired trough concentrations of 75 to 100 ng/mL.

At the beginning of Week 2 (Study Day 8), a further dose reduction (by approximately half) could be taken informed by the results of cyclosporine trough level testing scheduled at the Week 2 visit. Cyclosporine trough levels were expected to stabilize from approximately day 15 onwards. Subsequent modifications in cyclosporine dose while on direct-acting antivirals were guided by the scheduled cyclosporine trough level testing. At the investigator's discretion, extra blood draws for cyclosporine level testing could be performed at any time throughout the trial and were recorded as an unscheduled visit.

Calcineurin Inhibitor Summary

Drug-drug interactions with immunosuppressive calcineurin inhibitors were manageable with modified dosing during the treatment period. For this regimen, the suggested dosing modifications for tacrolimus (0.5 mg per week or 0.2 mg every 3 days) and cyclosporine (1/5 the daily pre-treatment dose given once daily) resulted in comparable trough concentrations before and during treatment.

The tacrolimus dose of 0.2 mg was only available to European investigators.

Serious Adverse Event Narratives

Two (5.9%) patients experienced treatment-emergent serious adverse events.

A 65-year old male experienced serious events of hypotension and tachycardia after initiating tamsulosin to treat urinary retention occurring in the setting of an elective carotid endarterectomy. The events were considered possibly related to the active regimen as a drug-drug interaction may increase tamsulosin exposure. Antiviral treatment was not interrupted in this patient, who went on to achieve a sustained virologic response.

Another patient experienced serious adverse events of peripheral edema and subsequent neuropathic pain in a lower extremity. This 54-year old diabetic woman had a history of peripheral edema, peripheral neuropathy, and chronic musculoskeletal pain. The event of peripheral edema was assessed to have a reasonable possibility of being related to ribavirin, but treatment was not interrupted. This patient experienced virologic relapse at post-treatment day 3.

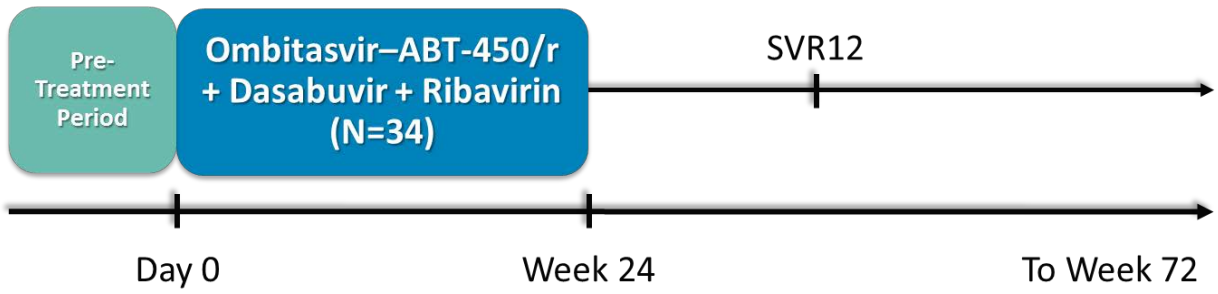

Figure S1. CORAL-I Study Design. M12-999 was a phase 2, open-label trial of 24-week treatment with ombitasvir–ABT-450/r and dasabuvir with ribavirin in liver transplant recipients infected with HCV genotype 1. Calcineurin inhibitor trough levels were assessed during a pre-treatment period (up to 14 days prior to study day 0) to allow for investigators to adjust calcineurin inhibitor dosing for commencement of study drug initiation.

Table S1. Baseline Variants in Direct-Acting Antiviral Targets.

Direct-acting antiviral target	Variant	Frequency, n/N (%)†	Fold Resistance‡
	Variants in HCV Genotype 1a		
NS3	V36L	1/29 (3.4)	2*
	V36M	1/29 (3.4)	2*
	Q80K	11/29 (37.9)	3*
	Q80L	1/29 (3.4)	2*
	Q80N	1/29 (3.4)	NA
NS5A	H58P	1/29 (3.4)	1*
	H58R	1/29 (3.4)	NA
NS5B	C316W	1/29 (3.4)	NA
	Y555F	1/29 (3.4)	NA
	Variants in HCV Genotype 1b		
NS3	None		
NS5A	L28M	1/5 (20.0)	2*
	L31M	1/5 (20.0)	1*
NS5B	C316N	1/5 (20.0)	5
	S556G	1/5 (20.0)	11

*Not considered clinically meaningful

†Total number of patients with a baseline sample containing variant at resistance-associated amino acid position (n) over the total number of patients with baseline samples sequenced (N).

‡Fold resistance conferred to ABT-450 (NS3 variants), ombitasvir (NS5A variants), or dasabuvir (NS5B variants).

NA = not available

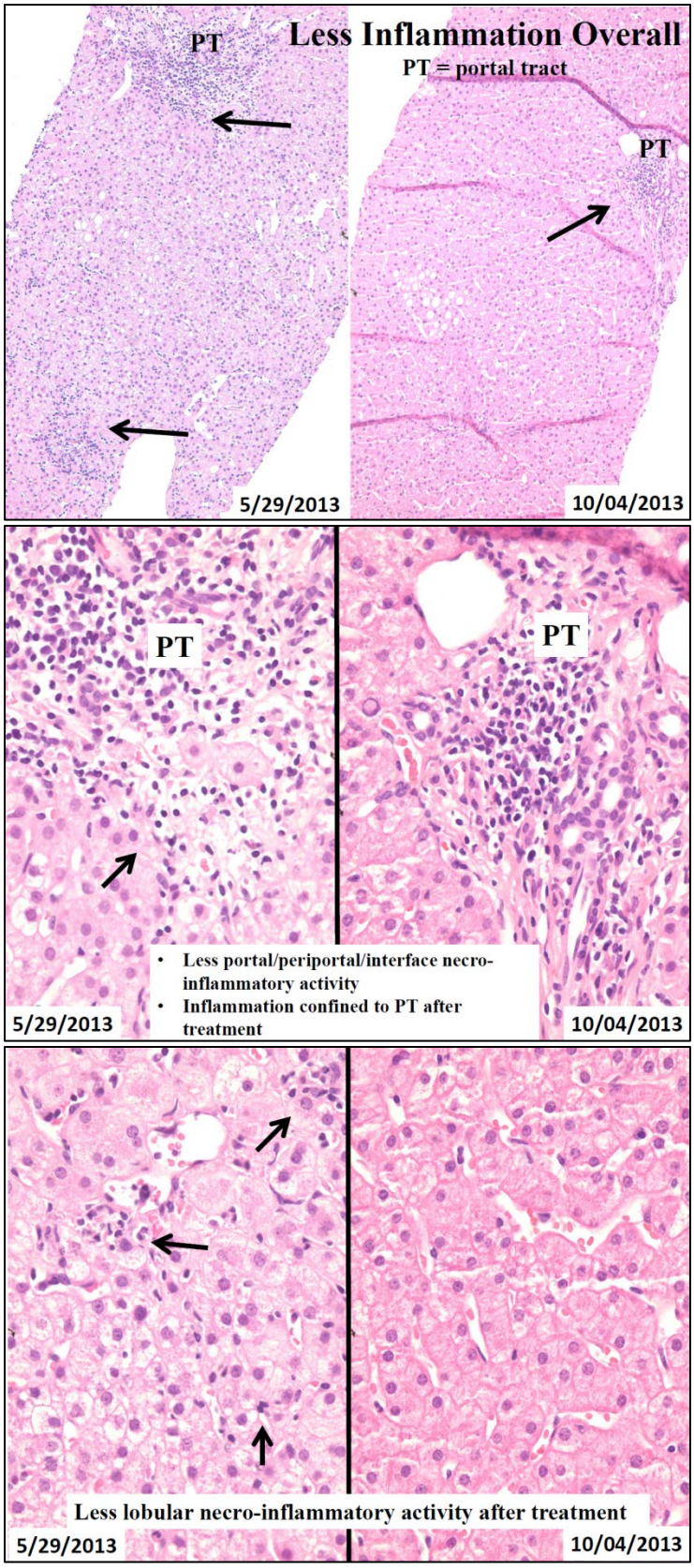

Figure S2. Liver Biopsy of Patient with Alanine Aminotransferase and Bilirubin Elevation.

A pre-treatment baseline liver biopsy (left panels) compared to on-treatment day 92 (right panels) indicated less necro-inflammation, thus ruling out graft rejection as a cause for increased alanine aminotransferase level >3X ULN coinciding with bilirubin elevation > 2X ULN.

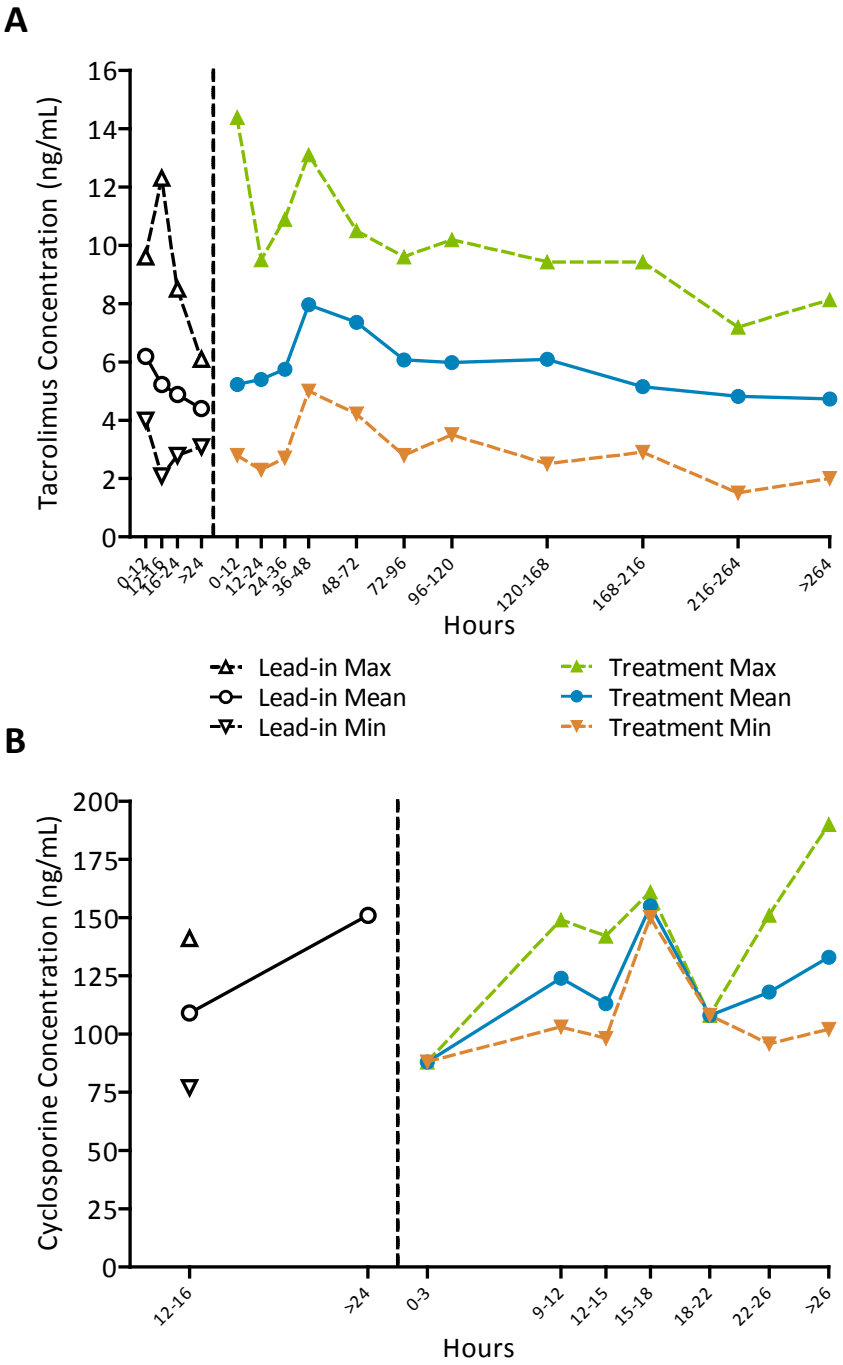

Figure S3. Geometric Mean Plasma Concentrations for Calcineurin Inhibitors. Geometric mean plasma concentrations of tacrolimus (A) and cyclosporine (B) binned by time after the last dose during the treatment period and pre-treatment period. Solid lines connect mean concentration and dashed lines connect the maximum or minimum concentration values by binned time period. Five patients with tacrolimus dosing errors were excluded from these analyses.

Table S2. Adverse Events Occurring in ≥10% of Patients, no. (%)	
MEDDRA 16.0 PREFERRED TERM	**Ombitasvir–ABT-450/r and dasabuvir with ribavirin (N = 34)**
Fatigue	17 (50.0)
Headache	15 (44.1)
Cough	11 (32.4)
Anemia	10 (29.4)
Diarrhea	9 (26.5)
Insomnia	9 (26.5)
Asthenia	8 (23.5)
Nausea	8 (23.5)
Muscle spasms	7 (20.6)
Rash	7 (20.6)
Back pain	6 (17.6)
Dizziness	6 (17.6)
Peripheral edema	6 (17.6)
Rhinorrhea	6 (17.6)
Abdominal pain	5 (14.7)
Pyrexia	5 (14.7)
Anxiety	5 (14.7)
Dyspnea exertional	5 (14.7)
Upper abdominal pain	4 (11.8)
Vomiting	4 (11.8)
Irritability	4 (11.8)
Urinary tract infection	4 (11.8)
Decreased appetite	4 (11.8)
Depression	4 (11.8)
Oropharyngeal pain	4 (11.8)

Table S3. Baseline and Post-Baseline Laboratory Abnormalities, N = 34		
Parameter	**Baseline no. (%)**	**Post-baseline no. (%)**
Alanine aminotransferase		
Grade 1	20 (58.8)	7 (20.6)
Grade 2	2 (5.9)	1 (2.9)
Grade 3	3 (8.8)	0
Grade 4	0	0
Aspartate aminotransferase		
Grade 1	18 (52.9)	6 (17.6)
Grade 2	5 (14.7)	1 (2.9)
Grade 3	0	0
Grade 4	0	0
Alkaline phosphatase		
Grade 1	9 (26.5)	17 (50.0)
Grade 2	0	0
Grade 3	0	0
Grade 4	0	0
Total bilirubin		
Grade 1	1 (2.9)	6 (17.6)
Grade 2	2 (5.9)	16 (47.1)
Grade 3	0	2 (5.9)
Grade 4	0	0
Hemoglobin		
Grade 1	0	11 (32.4)
Grade 2	0	9 (26.5)
Grade 3	0	1 (2.9)
Grade 4	0	0

No. indicates the number of patients with an increase from baseline or post-baseline nadir to grade 1, 2, 3, or 4 after the first nadir through study drug end day 2.

Table S4. Potentially Clinically Significant Laboratory Abnormalities During Treatment, no. (%)

Variable (criteria)	Ombitasvir–ABT-450/r and dasabuvir with ribavirin (N = 34)
Alanine aminotransferase (> 5 × ULN and ≥ 2 × baseline)	0
Aspartate aminotranferase (> 5 × ULN and ≥ 2 × baseline)	0
Alkaline phosphatase (> 1.5 × ULN)	5 (14.7)
Total bilirubin (≥ 2 × ULN)	7 (20.6)
Platelet count (< 50 x 10^9/L)	0
White blood count (< 2 x 10^9/L)	1 (2.9)†
Total neutrophils (< 1 x 10^9/L)	1 (2.9)†
Lymphocytes (< 0.5 x 10^9/L)	5 (14.7)‡
Activated partial thromboplastin time, sec (> 2 x ULN)	0
International normalized ratio (> 2 x ULN)	0
Creatinine (≥ 132.605 µmol/L)	4 (11.8)
Creatinine clearance, calculated (< 50 mL/m)	0
BUN (> 5 × ULN)	0
Uric acid (> 713.817 µmol/L)	4 (11.8)
Phosphate, inorganic (< 0.6 mmol/L)	0
Calcium (> 3.1 mmol/L)	0
Calcium (< 1.75 mmol/L)	0
Magnesium (> 1.23 mmol/L)	0
Magnesium (< 0.4 mmol/L)	0
Sodium (> 155 mmol/L)	0
Sodium (< 130 mmol/L)	0
Potassium (> 6 mmol/L)	1 (5.9)
Potassium (< 3 mmol/L)	0
Glucose (> 13.9 mmol/L)	2 (5.9)
Glucose (< 2.2 mmol/L)	0
Albumin (< 20 g/L)	0
Protein, total (< 50 g/L)	0
Cholesterol (> 10.34 mmol/L)	0
Triglycerides (> 5.7 mmol/L)	3 (8.8)

†Patient had low white blood cell count and neutrophil count occurring between days 85 and 113. The nadir white blood cell count was 1.2 × 10^9/L (day 92) and nadir neutrophil count was 500 × 10^9/L (day 113). This patient was taking mycophenolic acid, which was discontinued. The patient also received Neupogen® (filgrastim) from days 93 to 116.

‡Lymphocytes < 0.5 × 10^9/L were observed for 5 patients. These 5 patients had lymphocyte counts < 1000 × 10^9/L at screening and/or day 1.