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ORIGINAL ARTICLE

An evaluation of the safety and preliminary efficacy of peri- and post-operative treprostinil in preventing ischemia and reperfusion injury in adult orthotopic liver transplant recipients

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Abstract

Background: Orthotopic liver transplantation (OLT) is the only treatment option for various end-stage liver diseases. Ischemia and reperfusion (I/R) injury is one of the unavoidable complications/conditions in OLT. In 2019, a total of 8896 livers were transplanted of which >94% organs were procured from deceased donors. An increase in the use of extended criteria donor (ECD) livers for transplantation further unraveled the role of hepatic I/R injury on short-term and long-term graft outcomes. Despite promising outcomes with the use of antioxidants, free radical scavengers, and vasodilators; I/R-mediated liver injury persists and significantly influences the overall clinical outcomes. Treprostinil, a synthetic prostacyclin I_2 (PGI_2) analog, due to its vasodilatory property, antiplatelet activity, and its ability to downregulate pro-inflammatory cytokines can potentially minimize I/R injury.

Aim: We investigated the safety and preliminary efficacy of continuous intravenous infusion of treprostinil in liver transplant recipients in a prospective, single-center, non-randomized, interventional study.

Material and methods: This was a dose escalation (3 + 3 design) phase 1/2 study. Deceased donor liver transplant recipients received 5 ng/kg/min for two days, or 2.5, 5, and 7.5 ng/min/kg for 5 days as a continuous infusion. Multiple blood samples were collected for biochemical parameter assessment and for measuring treprostinil levels. Indocyanine green plasma disappearance rate was used as a measure of hepatic functional capacity.

Results: Subjects tolerated continuous infusion of treprostinil up to 5 ng/kg/min for 120 h with no occurrence of primary graft non-function (PNF), minimized need for ventilation support, reduced hospitalization time, 100% graft and patient survival, and improved hepatobiliary excretory function comparable to normal healthy adults.

Discussion: Treprostinil can be administered to liver transplant patients safely during the perioperative period.

Conclusion: Based on this phase 1/2 study, further efficacy studies of treprostinil in preventing I/R injury of liver should be conducted to potentially increase the number of livers available for transplantation.

KEYWORDS
ischemia reperfusion injury (IRI), liver transplantation, treprostinil

1 | INTRODUCTION

Orthotopic Liver Transplantation (OLT) is the only treatment option for patients with various end-stage liver diseases (ESLD). In 1965, Thomas E. Starzl performed the first successful liver transplantation (LTx).[1-3] Currently, more than 14 000 patients are listed as active recipients awaiting livers and <50% of them are expected to be transplanted.[4,5] Livers for transplantation are primarily obtained from deceased donors (DD) accounting for >95% of livers transplanted, with the rest coming from living donors (LD).[5] All the deceased donor livers undergo a period of warm ischemia during procurement, cold ischemia during preservation in the UW solution, and subsequently warm ischemia and warm reperfusion. Both ischemia and reperfusion (I/R) can cause significant alterations in the cellular architecture and function of the liver. Hepatic injury post-LTx is apparent with a rapid rise in serum levels of bilirubin and aminotransferases during the first 24 h following transplantation.[6] There is strong evidence to support I/R injury as a leading cause of primary graft non-function (PNF), and it is the primary cause for early organ dysfunction observed in nearly 10% of the patients.[7-12] In recent times, there has been a significant increase in the use of extended criteria donor (ECD) livers for transplantation.[13] Extended criteria livers are more susceptible to I/R injury, and the resulting graft damage impacts both the short- and long-term clinical outcomes.

Although advances in surgical techniques, standard of care for organ transplantation, and novel immunotherapies have significantly improved transplantation outcomes, no pharmacological therapy is currently approved for prevention of I/R injury associated with transplantation. Protective effects of prostaglandin E_1 (PGE_1) and prostacyclin (PGI_2) analogs against liver I/R injury have been investigated in preclinical and clinical studies.[14,15] These compounds elicit protection against I/R injury by enhancing the microcirculatory blood flow, suppressing thromboxane A_2, increasing PGI_2, inhibiting platelet aggregation, decreasing sinusoidal endothelial cells (SECs) apoptosis, and downregulating pro-inflammatory cytokines by decreasing the activation and infiltration of leukocytes.[16] The odds ratio for the PNF of the allografts in a compiled analysis of ten studies, which included 652 patients, randomized to PG versus control was 0.55 (95% CI: 0.23–1.33). Additionally, PG significantly decreased the risk of acute kidney failure that requires dialysis with an odds ratio of 0.37 (95% CI: 0.18–0.75) indicating potential renal protection of PG as well.[17] A randomized placebo-controlled study that included 160 subjects showed that PGE_1 significantly reduced the hospital and ICU stays by 23% and 40%, respectively, and plasma bilirubin levels on 2 and 10 days post-transplantation.[18] Barthel et al[19] have shown that 7 day continuous infusion of iloprost decreased the PNF incidences from 20% in the placebo group to 5% in the iloprost-treated group (P = .087) and improved allograft synthetic function. However, one of the major challenges in using PG as standard of care in LTx patients is its chemical instability, short half-life, and high cost. Treprostinil, a stable synthetic PGI_2 analog, has potent anti-inflammatory activity with a half-life of ~3–4 h and can be administered intravenously, subcutaneously, orally, or by inhalation.

In orthotopic rat liver transplant model, we have previously shown treprostinil to prevent I/R injury.[20] Treprostinil (36–106 ng/kg/min) has been previously used in liver transplant patients with pulmonary hypertension without any adverse outcomes.[21] We hypothesized that patients who undergo orthotopic liver transplantation can be safely dosed with IV treprostinil. The aim of this prospective, single-center, open-label, dose-escalation, pilot study in liver transplant recipients was to investigate the safety of continuous IV infusion of treprostinil and document preliminary efficacy against IR injury during the first-week post-transplantation.

2 | METHODS

2.1 | Study design

This was a prospective, pilot, single-center, open-label, non-randomized, dose-escalation (3 + 3 design) phase I/II study, in liver transplant patients, with outpatient follow-up for up to 180 days. A signed informed consent approved by the University of Pittsburgh Institutional Review Board (IRB; protocol ID Number: PRO19100094; ClinicalTrials.gov Identifier: NCT01481974) was obtained from all subjects before any study-related procedure was initiated. Inclusion and exclusion criteria are presented in the Supplementary Materials. Treprostinil was administered as a continuous IV infusion at a dose of 5 ng/kg/min for 2 days (First 3 subjects-group 1), and then at a dose of 2.5 ng/kg/min (next 3 subjects-group 2), 5 ng/kg/min (following 3 subjects-group 3), 7.5 ng/kg/min (following 1 subject-group-4), and 5 ng/kg/min (following 3 subjects -group 5) for a period of approximately 120 h. Figure 1 shows the time flow of the study design for each participant. Treprostinil infusion was started perioperatively for group 1 and continued for 48 h; for groups 2–5, the infusion was initiated after the patient was hemodynamically stable after surgery and within 24 h after surgery and continued for 120 h. During infusion, hemodynamic parameters were recorded, and blood samples were

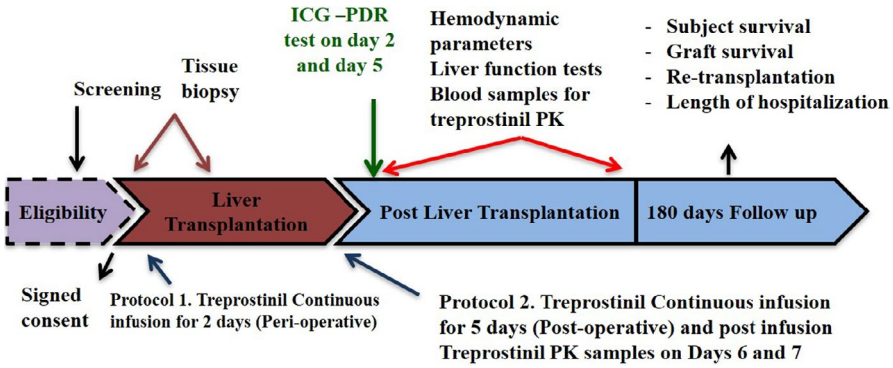

FIGURE 1 Study design flowchart. The study starts with actively screening for new subjects, approaching them, describing the benefits and risks related to the study, and obtaining signed informed consent from the subject. Initially, treprostinil infusion was initiated after induction of general anesthesia (protocol 1). Subsequently, treprostinil infusion starts once the patient was hemodynamically stable and continued for up to 120 h (Protocol 2). During infusion, hemodynamic parameters and blood samples were collected and indocyanine green plasma disappearance rate (ICG-PDR) test was conducted on days 2 and 5. Blood samples for post-infusion treprostinil PK were collected on days 6 and 7. Subjects were followed up for approximately 180 days post-transplantation to document graft and subject survivals

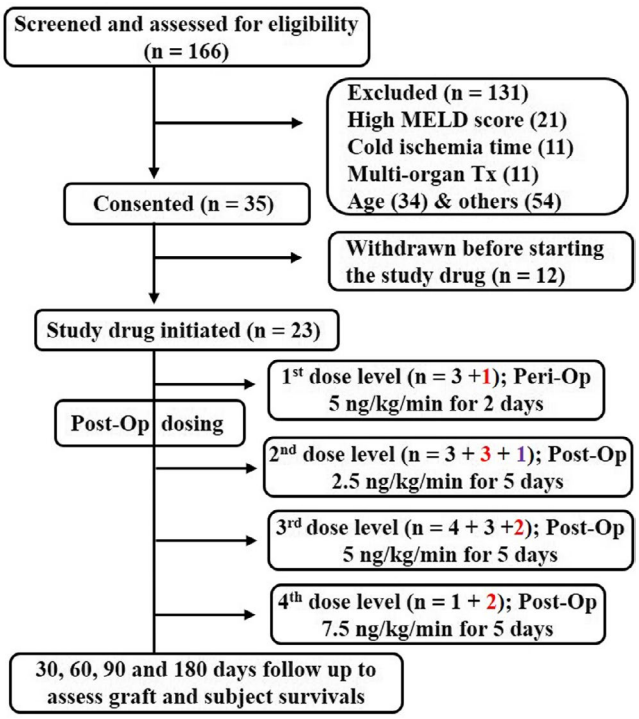

FIGURE 2 Study enrollment flowchart for the clinical study. This flowchart describes the patient enrollment in Remodulin clinical study at Montefiore hospital-UPMC. The flowchart shows number of subjects screened and assessed for eligibility (*n* = 166). Subjects consented (*n* = 35) were evaluated throughout the study and were enrolled to three dose levels in a 3 + 3 dose escalating phase I design. Subjects were followed for up to 180 days post-transplantation. * numbers in parenthesis [black (completed study), red (discontinued), and purple (early discharge)]

collected for measurement of different biochemical markers and for measurement of plasma concentrations of treprostinil. Additionally, the hepatobiliary excretory function was evaluated by the measuring indocyanine green plasma disappearance rate (ICG-PDR) on day 2 and 5 post-LTx. After stopping the treprostinil infusion, serial blood samples were collected from the patients for up to 48 h (post-transplantation days 3 and 4 for group 1 and days 6 and 7 for groups 2–5) for functional assessment and for measuring plasma concentrations of treprostinil. The clinical outcomes of the subjects were followed for approximately 180 days post-transplantation to document graft and subject survivals. Figure 2 describes the enrollment and disposition of consented subjects in the study and the dose-escalation procedure. The detailed dose-escalation regimens are presented in Supplementary Materials.

2.2 | Study objectives

2.2.1 | Primary endpoints

The primary endpoints of this study were to evaluate the safety and preliminary efficacy of a two-day perioperative or five-day post-operative course of treprostinil intravenous infusion. The safety profile was assessed in terms of tolerability of the drug, hemodynamic parameters, and need for inotropes. All adverse events were monitored and evaluated by attending physicians. Hemodynamic measurements (mean pulmonary arterial pressure (mPAP, mmHg), cardiac output (CO, L/min), cardiac index (CI, L/min/m^2), heart rate (HR, bpm), systolic blood pressure (SBP, mmHg), and diastolic blood pressure (DBP, mmHg)) were recorded up to 7 days post-transplantation, whenever available. Serum bilirubin concentrations were assessed over seven days post-transplantation as a marker of primary preliminary efficacy.

2.2.2 | Secondary endpoints

The secondary endpoints were biochemical parameters (alanine aminotransferase (ALT) and aspartate aminotransferase (AST) for seven

days after transplant), clinical parameters, and ICG-PDR data on days 2 and 5 post-transplant. Serum creatinine (SCr) levels were also measured. Prothrombin time (PT) was used as blood coagulation biomarker. Primary allograft non-function defined by patient death or need for re-transplantation within 30 days due to liver failure was monitored. The duration of ventilator support in the intensive care unit (ICU) and during the initial hospitalization days was documented. Subject and graft survivals were followed up for up to 180 days after drug infusion.

2.2.3 | Data analysis

The effect of intervention on the hemodynamic parameters was evaluated by fitting a linear regression for observed change (compared to time zero (baseline value)) for each parameter (HR, SBP, DBP, mPAP, CO, and CI) through the course of study. The area under the plasma concentration-time curve (AUC) for ALT, AST, total bilirubin, and serum creatinine was calculated using linear trapezoidal method. The AUCs for each parameter were compared using Mann-Whitney non-parametric test between patients who received treprostinil (n = 14) and those who did not receive treprostinil (n = 7) during the study. A $P < .05$ was considered statistically significant.

3 | RESULTS

3.1 | Patient recruitment and baseline demographics

One hundred and sixty-six patients who were selected to receive liver transplantation between December 2012 and May 2019 at UPMC were screened. Around two-thirds of the transplant recipients did not qualify to participate in the study primarily due to higher MELD score than inclusion criteria (16%), age (26%), or cold ischemia time outside the limits of IRB-approved protocol at that time. The eligibility criteria for recipient and donor organ are listed in Supplementary Materials. Table 1 shows the details of subjects excluded from the study. Thirty-five subjects signed the informed consent form (ICF) to participate in the study. Fourteen subjects completed the study (5 ng/kg/min for 2 days (n = 3), 2.5 ng/kg/min (n = 3), 5 ng/kg/min (n = 7), and 7.5 ng/kg/min (n = 1) for 5 days). Table 2 summarizes the status of all patients who consented to participate in the study.

All patients who received treprostinil were Caucasian and 19 out of 23 were male. The subjects were between 32 and 67 years (median = 58) with median weight of 92 kg. More than half (56%) of the recipients needed LTx because of hepatocellular carcinoma (HCC) and/or Hepatitis C Virus (HCV) infection. Median cold ischemia and warm ischemia (liver on field to portal vein unclamping) times were 447 (323–717) min and 35 (14–51) min, respectively. Patients who signed ICF and received liver transplantation but did not receive treprostinil were designated as controls. In the control group, all the subjects were male with median age and weight 55 years (43–63) and 98.15 kg (70–131), respectively. Cold and warm ischemia times were 404 (282–811) and 33 (9–45) min, respectively. All demographics summarized in Table 3 were similar between the control and treprostinil groups.

TABLE 1 List of reasons for patients who were excluded before consenting

	Reason	Times of occurrence	Percentage (%)
1	Age	34	26.0
2	MELD	21	16.0
3	Cold ischemia < 5 h	10	7.6
4	Multiple organ transplant	11	8.4
5	Conflicting clinical study	4	3.1
6	Failed LTx within 180 days	3	2.3
7	Not eligible for LTx	5	3.8
8	Cardiovascular history	2	1.5
9	HCV donor	2	1.5
10	Fulminant hepatic failure	2	1.5
11	Split liver	2	1.5
12	Refused study	4	3.1
13	Needed proxy consent	2	1.5
14	Hemophilia—bleeding risk	1	0.8
15	HIV positive	1	0.8
16	Allergic to iodine	1	0.8
17	International patient	1	0.8
18	Staff insufficiency	1	0.8
19	Cold ischemia > 12 h	1	0.8
20	Pulmonary hypertension	1	0.8
21	Liver declined	17	13.0
22	On contraindicated medication	5	3.8

3.2 | Safety assessment

Cardiovascular parameters observed in all patients who received treprostinil were within the normal range and were similar to those reported for healthy subjects.[22] Participants received inotropes as per the clinical need during the transplantation and immediately postop but did not need additional inotropes during the study period. Data obtained from subjects who received treprostinil and discontinued from the study was evaluated separately for subject safety (data not shown). At the end of infusion, HR observed in all patients was within the normal range. The slope of regression lines calculated for change in HR over time for treprostinil-treated and control groups was close to zero and was comparable (data not shown). HR values for control and different treprostinil groups are plotted in Figure 3.

TABLE 2 Status of consented patients

	Patient status	Number
Patients completed the study		14
Dose level 1 (Group 1) (perioperative infusion)	Completed 5 ng/kg/min for 2 days	3
Dose level 2 (Group 2) (post-operative infusion)	Completed 2.5 ng/kg/min for 5 days	3
Dose level 3 (Groups 3, 5) (post-operative infusion)	Completed 5 ng/kg/min for 5 days	7
Dose level 4 (Group 4) (post-operative infusion)	Completed 7.5 ng/kg/min for 5 days	1
Patients discharged earlier		1
A	Completed 2.5 ng/kg/min for 4 days only	1
Patients withdrawn or excluded from the study		13
A	Hemodynamically unstable; did not initiate the infusion	5
B	Hemodynamically unstable after starting the infusion (not related to study intervention)	6
C	Central line removed after ~32 h before completing the study	1
D	Infusion stopped for > 24 h by mistake	1
Patients excluded from the study after consenting		7
F	Patient died in the OR, before receiving treprostinil	1
G	Cold ischemia < 5 h or >12 h	2
H	Macrosteatosis > 40% & Metastasis	4

TABLE 3 Patient demographics and baseline characteristics

	Treprostinil (*n* = 14)	Control (*n* = 7)
Gender		
Male	92.8%	100%
Female	7.2%	0%
Race		
Caucasian	100%	100%
Age (Years)	58 (32–67)	55 (43–63)
Pre-transplant Body weight (Kg)	92 (63–136)	98.15 (70–131)
Reason for Liver transplantation		
HCC	33%	40%
HCV	23%	40%
Alcohol cirrhosis	44%	60%
Cold ischemia time (min)	447 (323–717)	404 (282–811)
Warm ischemia time (min)	35 (14–51)	33 (9–45)
MELD Score	28.5 (14–36)	36 (14–40)
Total Bilirubin (mg/dl)	4.9 (1.3–9.1)	8.5 (1.5–24.3)
Platelets ($\times 10^9$/L)	52 (34–126)	72 (15–156)
SCr (mg/dl)	1 (0.7–1.8)	1.2 (0.9–2.8)

Note: Data are presented as median (range).

Systolic arterial blood pressures (SBP) obtained from patient charts showed no significant changes in both treprostinil and control groups (Figure 3, panels C and D). Diastolic blood pressure (DBP) data presented in Figure 3, Panels E and F show a trend with an increase in DBP over time in both treprostinil and control groups but were close to within the normal range.

Other hemodynamic parameters presented in Figure 4 show that any observed differences were clinically insignificant. Most of the subjects had relatively high mean pulmonary arterial pressure, cardiac output, and cardiac index prior to treprostinil administration. mPAP and CI remained stable and did not show significant changes in both control and treprostinil groups. Cardiac output (CO) and cardiac index (CI) in both control and treprostinil groups were similar over the course of study (Figure 4, panels C–F). Any change in any parameters measured could not be attributed to treprostinil administration.

3.3 | Liver function assessment and clinical outcomes

Data presented in Figure 5, panel A and C show the time courses of ALT and AST in control and different treprostinil dose groups, respectively, and panels B and D show median levels of ALT and AST in control and all treprostinil groups. Highest levels of both AST and ALT (median values: 2534 vs. 2199 IU/L and 1365 vs. 1089 IU/L, respectively) were observed immediately after transplant. Plasma levels of ALT and AST recovered to normal in both treprostinil and control groups. Total bilirubin levels are presented in Figure 5. Median total bilirubin concentration in plasma immediately after surgery was 8.5 mg/dl and 4.9 mg/dl in control and treprostinil groups, respectively. The total bilirubin levels continued to be high during the first week in the control group but were normal (<1.5 mg/dl) in the treprostinil group within three days.

Serum creatinine, platelets, and prothrombin time to assess renal function and synthetic functions of the liver are presented in Figure 6. The median serum creatinine concentration was sustained between 1 and 1.5 mg/dl in treprostinil-treated subjects indicating normal renal function. Platelet counts were low in all our patients during the study. The prothrombin times were prolonged for both

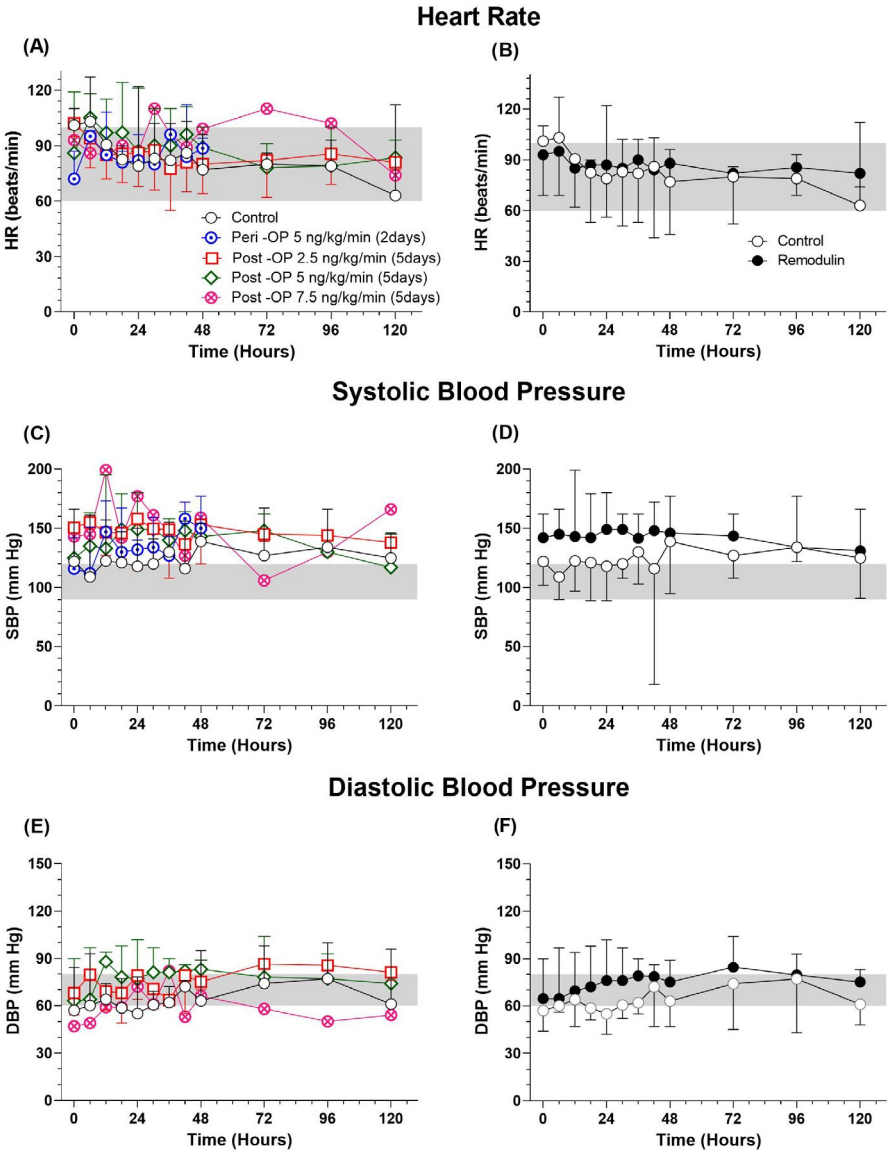

FIGURE 3 Heart rate and blood pressure parameters observed for 5 days in LTx patients. Panels A, C, and E depict HR, SBP, and DBP observed in respective dose groups over the 120 h from start of infusion and shaded areas represent the normal range for healthy subjects for the respective parameter. Panels B, D, and F plots show HR, SBP, and DBP observed in control and remodulin groups. Data are expressed as median and range

groups at the beginning and recovered to normal range within the first week of transplantation. The area under plasma concentrations vs time curve for ALT, AST, total bilirubin, and SCr calculated using linear trapezoidal method was similar between control and treprostinil groups (Figure 7).

Hepatobiliary secretory function (Figure 8) was assessed by indocyanine green plasma disappearance (ICG-PDR) test. Control subjects were not subjected to any of the study-related procedures including ICG-PDR test. The median values for ICG-PDR were 21 and 19.9%/min on post-LTx days 2 and 5, respectively. Most of the patients in treprostinil group showed ICG-PDR values similar to healthy subjects on both test days. No primary graft non-functional events were seen in either group. All adverse events encountered during the study were evaluated by physician investigators and were found to be unrelated to the study drug.

Patients were followed for up to 180 days post-LTx. All the grafts and patients in treprostinil group survived, but there was one death in the control group. Subjects in treprostinil group needed fewer days of mechanical ventilation support when compared to control group (1 vs. 2.5 days, $P < .05$). The lengths of ICU and hospital stays were lower in treprostinil group, but the difference was not statistically significant (Table 4).

4 | DISCUSSION

Cold static organ storage has been the standard for allograft preservation but is associated with I/R injury. Many precious organs are often not used, because of their increased susceptibility to I/R injury.[23] Our experience at UPMC and the literature indicates that more than 26% of the livers retrieved from donors after circulatory death (DCD) are not transplanted because of their propensity for PNF.[24] A newer approach such as organ preservation with machine perfusion at cold and normothermic conditions is under investigation to overcome I/R injury-related complications. Ravikumar et al[25] utilized normothermic machine perfusion in human liver transplantation and

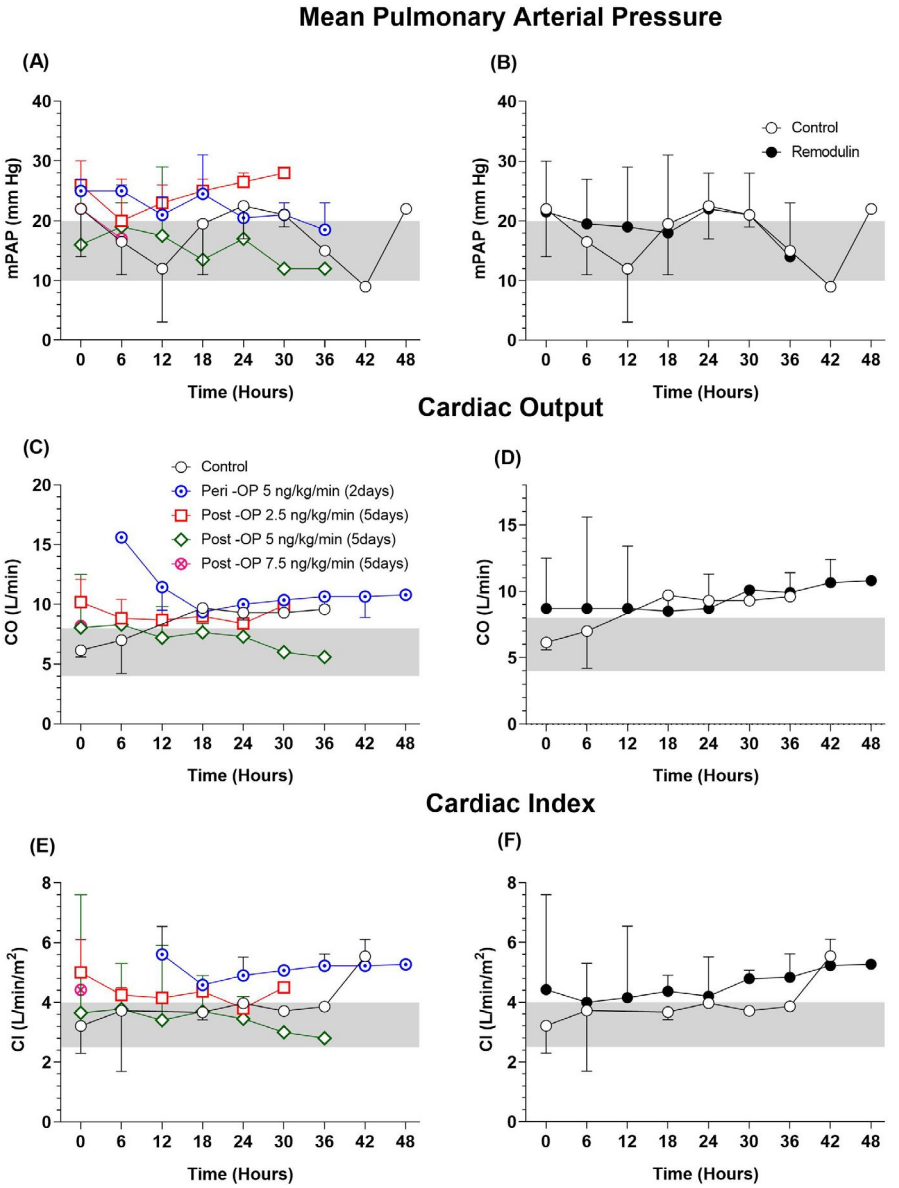

FIGURE 4 Hemodynamic parameters observed in patients receiving treprostinil infusion. Panels A, C, and E depict mean pulmonary arterial pressure, cardiac output, and cardiac index observed in LTx patients in respective treprostinil dose groups over two days and shaded areas represent the normal range for the respective parameter in healthy subjects. Panels B, D, and F plots show mPAP, CO, and observed in control and remodulin groups. Data are expressed as median and range

reported that peak AST in the first week was significantly reduced but the other parameters were similar to cold static preservation. Using a cell-free oxygen carrier solution that complements the use of liver perfusion machine under hypothermic conditions has also been investigated.[26] However, one of the more suitable and easier ways to prevent allograft damage is the addition of a pharmacological agent to improve LTx outcomes. In the current study, we explored the safety and feasibility of infusing treprostinil, a drug already FDA approved for pulmonary arterial hypertension (PAH) and marketed, IV during and immediately after liver transplantation.

This is the first clinical study to systematically investigate the safety and feasibility of a continuous IV infusion of treprostinil (a stable synthetic PGI_2), in liver transplant patients, in order to attenuate the hepatic I/R injury. This study was initiated based on the proof of concept study in rats, where treprostinil significantly prevented I/R injury.[20] The hypothesis that treprostinil can be administered safely in liver transplant patients was based on our observations in two liver transplant recipients who were diagnosed with PAH and have been using treprostinil infusion for 6 and 11 months prior to LTx. These patients received 36 and 45 ng/kg/min, as part of PAH therapy, during the liver transplantation procedure and afterward in the ICU without any treprostinil-related adverse events.[21]

All the study participants were accepted as liver transplant candidates at our center and were treated in accordance with the standard of care protocols including immunosuppression and other elements of pre- and post-operative care. In the current study, all the subjects received whole liver transplant with end-to-end anastomosis of portal vein, hepatic artery, hepatic vein, and infra-hepatic vena cava, respectively, of donor organ and recipient. Biliary anastomosis was performed duct to duct.

PGE_1 has been administered intraoperatively during liver transplant surgery at a maximum dose of 1 μg/kg/min and was found to be safe.[27] Clinically, switching from IV epoprostenol to SC treprostinil infusion can be done at 1:1 ratio.[28] However, a twofold increase

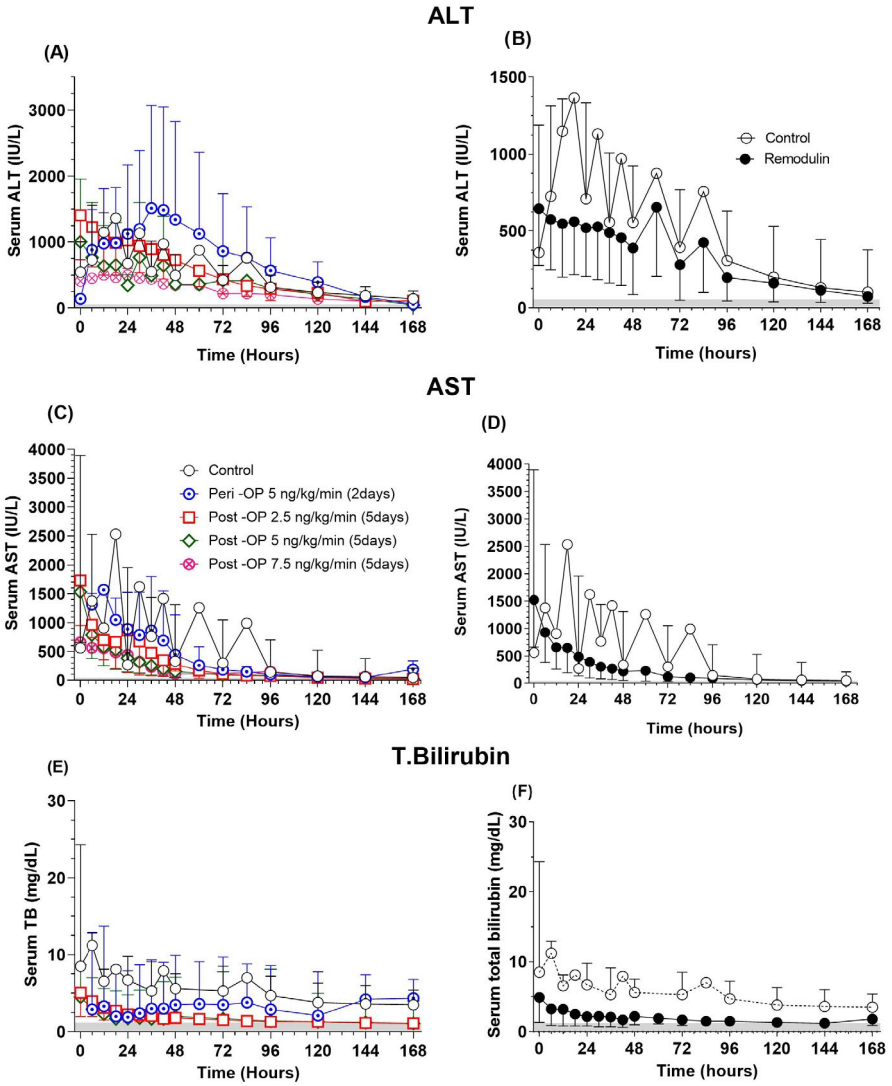

FIGURE 5 Time course of hepatic injury markers during first week after LTx. Panels A, C, and E show ALT, AST, and total bilirubin levels observed in LTx patients in respective treprostinil dose groups during the first-week post-LTx. Shaded areas represent the normal range for the respective parameter in healthy subjects. Panels B, D, and F plots show ALT, AST, and Total bilirubin levels observed in control (open circles) and remodulin groups (closed circles). Data are expressed as median and range

in treprostinil dose will typically be required to obtain similar clinical response as IV PGI_2.[29] Based on the pharmacokinetic data of treprostinil in patients with liver disease and typical doses of treprostinil used in PAH, we identified a starting dose of 5 ng/kg/min for the initial study.

Out of a total 166 subjects screened, thirty-five subjects signed informed consent form and fourteen patients completed the planned course of treprostinil infusion. These patients were distributed into four dose levels chronologically escalated in terms of treprostinil dose and duration, 5 ng/kg/min for 2 days, 2.5, 5, and 7.5 ng/kg/min for 5 days. Our results reveal that there are no safety concerns in terms of cardiovascular measurements, LFTs, or kidney function directly or indirectly related to treprostinil up to the 5 ng/kg/min dose for 120 h. The consented patients who did not receive treprostinil were considered as the control group and no study-related procedures were performed. All the data from control group were obtained from the medical records.

In the first three patients, treprostinil was initiated at 5 ng/kg/min at the time of induction of anesthesia during liver transplantation and continued for nearly 48 h. Hemodynamic instability in these patients presumably related to liver transplant procedure itself led to interruption of the drug infusion and reinitiation at various time points. Therefore, the protocol was modified to avoid interruption of treprostinil infusion. Treprostinil infusion was started after the patient was hemodynamically stable and taken off inotropes post-completion of the liver transplant surgery. None of the patients (in control and treprostinil groups) needed inotropes during the study. Three patients received 2.5 ng/min/kg for 120 h. Subsequently, three patients received 5 ng/min/kg for 120 h. Further, Three patients were started with 7.5 ng/min/kg for 120 h, of which one subject completed the study and two subjects experienced hemodynamic instability in 3–4 h after starting the infusion. Infusion was reinitiated after blood pressure returned to normal (1–2 h after stopping infusion), but the subjects experienced hemodynamic changes and the study was discontinued. As per the study design, treprostinil dose was deescalated to 5 ng/kg/min and three more subjects were studied. Our results show that starting treprostinil infusions in liver transplant recipients after they were hemodynamically stabilized was generally safe and hemodynamic parameters remained stable over the study period. Systemic and pulmonary arterial pressure

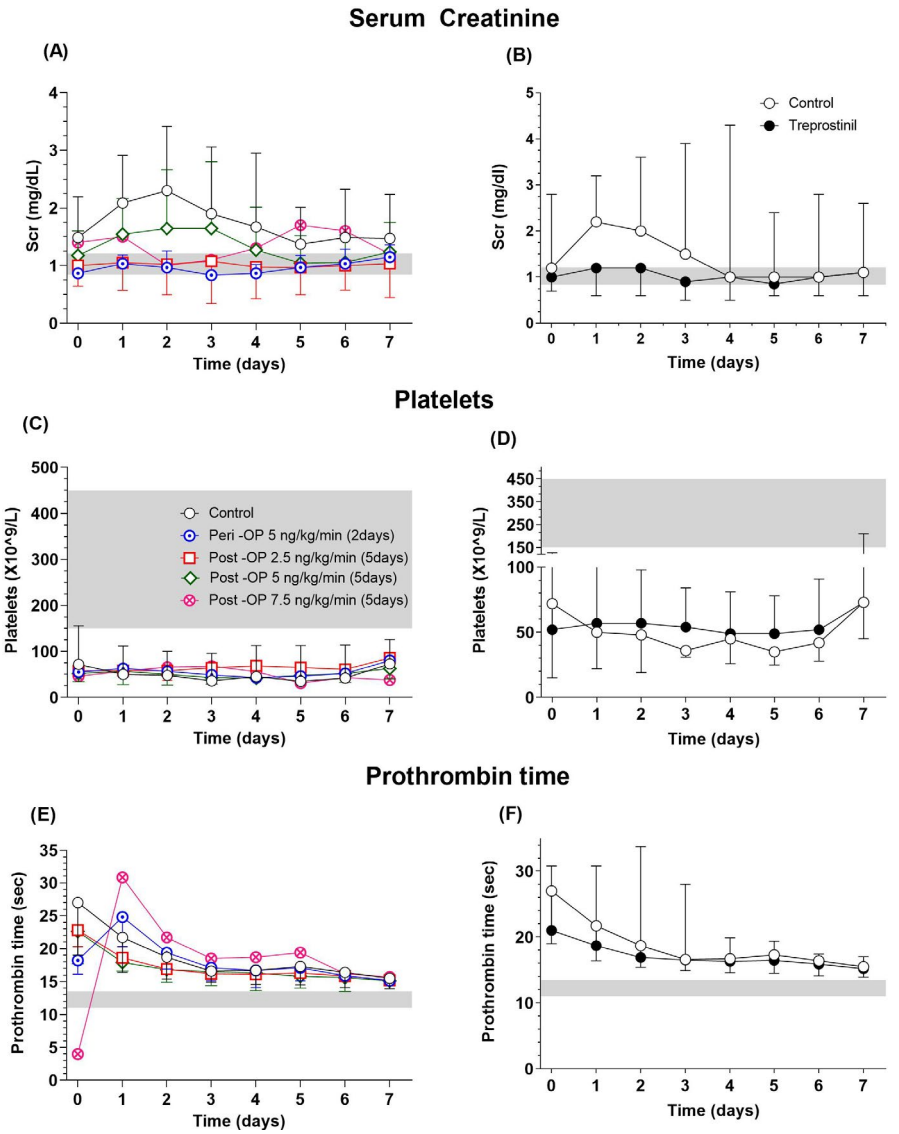

FIGURE 6 Time course of blood coagulation and kidney function markers for 7 days post-LTx. Panels A, C, and E show time course of serum creatinine levels, platelet counts, and prothrombin time observed in LTx patients in respective treprostinil dose groups during the first-week post-LTx. The shaded areas represent normal ranges of each parameter in healthy subjects. Panels B, D, and F show serum creatinine, platelet counts, and prothrombin time observed in control (open circles) and remodulin groups (closed circles). Data are expressed as median and range

parameters were closely monitored and were not affected by treprostinil administration. No participants experienced any significant complications or needed inotropes during study. In the present study, our primary endpoints for safety were achieved, since mean pulmonary arterial pressure, systemic blood pressure, and cardiac index values stayed within or close to the normal range. A reduction in the mPAP is essential for PAH patients and is considered the only predictor of patient's survival,[30] but our results show that mPAP was not altered in LTx patients at the dosing regimens used in this study asserting its safety in the study subjects.

Assessment of plasma concentrations of transaminases[31] showed that the liver injury status and enzymes levels dropped rapidly over time and reached to normal levels within the first-week post-transplantation. We have previously reported in a rodent OLT that treprostinil not only reduced the peak levels of both ALT and AST significantly but also reduced AUCs. Less necrosis was observed in the treprostinil-treated rat livers.[20] Similar results were replicated in an ex vivo isolated perfused rat liver model (unpublished data). Excretory function assessed by serum total bilirubin levels where 10 mg/dl of total bilirubin level is considered as a predictor of initial poor function,[32] bilirubin levels recovered to normal levels (<1.5 mg/dl) within 3 days for all the patients in our study. The synthetic function of the allograft was assessed by estimating prothrombin time, a coagulation parameter. Prothrombin time was similar in both control and treprostinil-treated groups. The renal function evaluated by serum creatinine levels was similar in both control and treprostinil groups. Our results are in agreement with the recent publication by Cochrane et al that PGs significantly minimized the risk of acute kidney failure.[17]

Disappearance of Indocyanine green (ICG) (an inert and water-soluble dye) from plasma was used to measure the dynamic function of livers. Hepatic uptake of ICG is mediated via organic anionic transporters such as SLCO1B3 (OATP1B3) and SLC10A1 (NTCP), and efflux is mediated via ABCC2 (MRP2), ABCB4 (MDR3), and ABCB1 (MDR1; P-gp). Almost all of the ICG dose is excreted unchanged into the bile, with no enterohepatic recirculation.[31,33] Reported values for ICG-PDR ranged between <8 and >20%/min, where 8%/min or less is considered organ failure with a

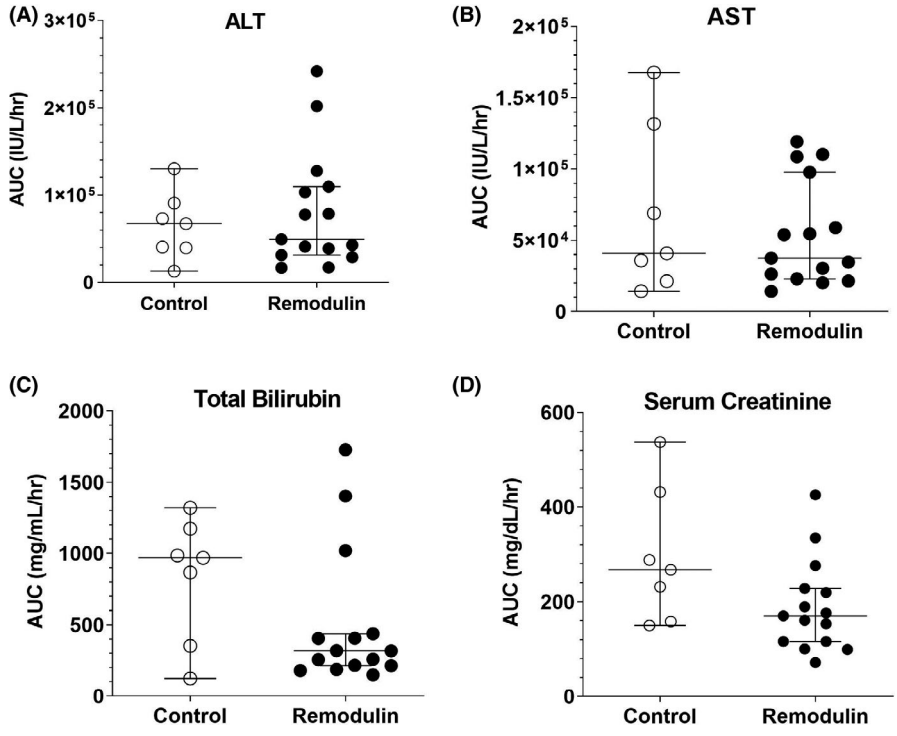

FIGURE 7 Area under the concentration time curves (AUC) for liver and kidney biomarkers over 7 days post-LTx. AUCs for ALT (A), AST (B), total bilirubin (C), and SCr (D) observed in control (open circles) and remodulin groups (closed circles) in first week after LTx. Data are expressed as median and range

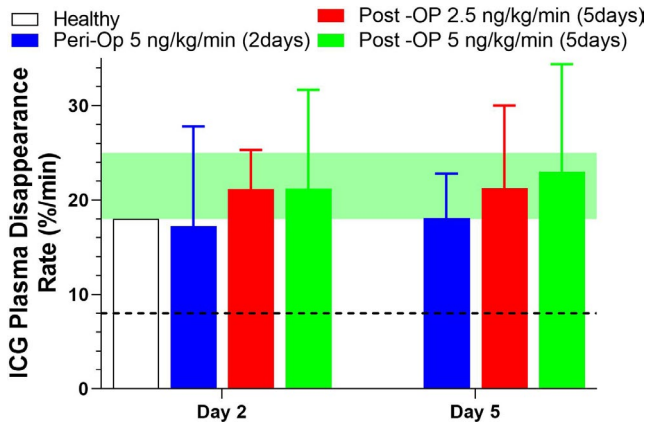

FIGURE 8 ICG-PDR observed on Day2 and Day5 post-LTx. Left panel illustrates ICG-PDR values observed in respective treprostinil dose groups on day 2 post-LTx and right panel illustrates ICG-PDR values observed in respective treprostinil dose groups on day 5, respectively. The shaded areas represent the normal range observed in healthy subjects, and dotted line is the lowest threshold below which the liver is deemed non-functional or compromised. Data are expressed as median and range. *ICG-PDR values for healthy and control subjects were obtained from previous study

TABLE 4 Summary of outcomes

	Treprostinil	Control
Safety outcomes		
Survival rates		
Liver graft (180 days)	100%	86%
Subject (180 days)	100%	86%
Primary graft non-functional (PNF)	0%	14%
Preliminary efficacy outcomes		
On ventilator (days)	1 (1–2)*	2.5 (1–3)
ICU (days)	3 (2–4)	4 (2–12)
Hospital (total days)	8 (6–26)	15 (7–58)
Exploratory outcomes		
Indocyanine green plasma disappearance rate (ICG-PDR) (%/min)		
At day 2	21.1 (13.6–31.7)	ND
At day 5	21.25 (12.2–34.4)	ND

Note: Data are presented as median (range).
Abbreviation: ND: Not measured.
*$P < .05$ compared to control group

sensitivity and specificity of 81 and 70%, respectively.[32,34,35] The PDR test results in our patients on days 2 and 5 after transplantation showed that almost all the grafts cleared ICG as efficiently as normal healthy subjects.[36,37]

Primary graft non-function (PNF), with an incidence up to 23% in ECD livers,[7] was also examined. All grafts functioned well throughout the study period (180 days). The patient survival was 100% for treprostinil group, and 1 subject in control group died 3 days after liver transplant. National liver transplantation data show ~7% graft failure in deceased donor liver transplant recipients at 6 months.[38] A retrospective analysis of a study reported that continuous subcutaneous infusion of treprostinil (19–53 ng/kg/min) in five liver transplant recipients diagnosed with moderate to severe portopulmonary hypertension survived an average of 30 months.[30] Barthel et al[19] reported that primary graft dysfunction and six-month mortality were not different between control and iloprost, a PGI_2 analog, groups.

The impact of treprostinil on the need for supportive care, ICU, and total hospital stays was also evaluated. Treprostinil-treated patients needed ventilation support for a shorter time with a median of 1 day; however, control subjects needed a significantly longer duration of ventilation, a median of 2.5 days. The length of stay in both ICU and hospital was reduced from 4 to 3 days and 15 to 8 days for treprostinil and control groups, respectively. This was in line with a study reported by Henley et al that showed PGE_1 significantly reduced the hospital and ICU stays by 20% and 40%, respectively.[18] However, longer ICU (6–12 days) and hospital (27–32 days) stays have been reported in iloprost-treated patients.[19,39] The current study was not powered to show statistically significant outcomes. But the observed beneficial effects are encouraging for future studies with more patients.

In conclusion, this clinical study shows the safety and feasibility of using treprostinil in liver transplant patients, during the post-surgical phase. A maximum tolerable dose of 5 ng/kg/min of treprostinil was identified in patients who had undergone liver transplantation. Preliminary observation indicated some signs of efficacy in terms of improvement in hepatobiliary excretory function and trend toward improvement in hospitalization, survival parameters, and PNF. However, a larger, randomized, double-blind, placebo-controlled, multicenter, phase III clinical study is warranted to demonstrate the safety and efficacy of treprostinil in protecting the livers from I/R injury following transplantation.

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CONFLICT OF INTREST

The authors of this manuscript have conflicts of interest to disclose as described by the Clinical Transplantation. The study was partially funded by a grant from United Therapeutics Corporation and United Therapeutics Corporation provided treprostinil (intervention drug). Pillai VC currently works at Office of Clinical Pharmacology, US Food and Drug Administration; Miah MK currently works at Department of Clinical Pharmacology; Astra Zeneca and Almazroo OA currently work at Saudi Food and Drug Authority, Riyadh, Saudi Arabia.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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