

Donor Simvastatin Treatment Is Safe and Might Improve Outcomes After Liver Transplantation: A Randomized Clinical Trial

Duilio Pagano, MD, PhD,¹ Jaime Bosch, MD, PhD,² Fabio Tuzzolino, MS,³ Elisabetta Oliva, MS,⁴ Burcin Ekser, MD, PhD,⁵ Giovanni Zito, MS, PhD,³ Davide Cintonino, MD, PhD,¹ Fabrizio di Francesco, MD, PhD,¹ Sergio Li Petri, MD,¹ Calogero Ricotta, MD, PhD,¹ Pasquale Bonsignore, MD, PhD,¹ Sergio Calamia, MD,¹ Bianca Magro, MD,¹ Gianluca Trifirò, MD, PhD,⁶ Rossella Alduino, MS,³ Marco Barbara, MS,³ Pier Giulio Conaldi, MD, PhD,³ Alessia Gallo, MS, PhD,³ Francesca Venuti, MS,³ Angelo Luca, MD,⁷ and Salvatore Gruttadauria, MD, PhD^{1,8}

Background. The current curative approaches for ischemia/reperfusion injury on liver transplantation are still under debate for their safety and efficacy in patients with end-stage liver disease. We present the SIMVA statin donor treatment before Liver Transplants study. **Methods.** SIMVA statin donor treatment before Liver Transplants is a monocentric, double-blind, randomized, prospective trial aiming to compare the safety and efficacy of preoperative brain-dead donors' treatment with the intragastric administration of 80mg of simvastatin on liver transplant recipient outcomes in a real-life setting. Primary aim was incidence of patient and graft survival at 90 and 180 d post-transplant; secondary end-points were severe complications. **Results.** The trial enrolled 58 adult patients (18–65 y old). The minimum follow-up was 6 mo. No patient or graft was lost at 90 or 180 d in the experimental group (n = 28), whereas patient/graft survival were 93.1% ($P = 0.016$) and 89.66% ($P = 0.080$) at 90 d and 86.21% ($P = 0.041$) and 86.2% ($P = 0.041$) at 180 d in the control group (n = 29). The percentage of patients with severe complications (Clavien-Dindo \geq IIIb) was higher in the control group, 55.2% versus 25.0% in the experimental group ($P = 0.0307$). The only significant difference in liver tests was a significantly higher gamma-glutamyl transferase and alkaline phosphatase at 15 d ($P = 0.017$), ($P = 0.015$) in the simvastatin group. **Conclusions.** Donor simvastatin treatment is safe, and may significantly improve early graft and patient survival after liver transplantation, although further research is mandatory.

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INTRODUCTION

The number of liver transplantations (LTs) is continuously increasing worldwide¹ since LT is currently the only curative treatment for patients with end-stage liver disease

(ESLD), with or without liver tumors.² However, this increasing clinical application is limited by a chronic shortage of donor organs. Therefore, multiple strategies and

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¹ Department for the Treatment and Study of Abdominal Diseases and Abdominal Transplantation, Istituto di Ricovero e Cura a Carattere Scientifico - Istituto Mediterraneo per i Trapianti e Terapie ad alta specializzazione (IRCCS-ISMETT), University of Pittsburgh Medical Center (UPMC) Italy, Palermo, Italy.

² Department of Visceral Surgery and Medicine, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland.

³ Department of Research, IRCCS-ISMETT, UPMC Italy, Palermo, Italy.

⁴ Fondazione Ri.MED, Palermo, Italy.

⁵ Division of Transplant Surgery, Department of Surgery, Indiana University School of Medicine, Indianapolis, IN.

⁶ Department of Diagnostics and Public Health - University of Verona, Verona, Italy.

⁷ Department of Diagnostic and Therapeutic Services, IRCCS-ISMETT, UPMC Italy, Palermo, Italy.

⁸ Department of Surgery and Surgical and Medical Specialties, University of Catania, Catania, Italy.

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that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or nonfinancial interest (such as personal or professional relationships, affiliations, knowledge, or beliefs) in the subject matter or materials discussed in this manuscript.

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Limited data have been collected and analyzed because patient enrollment is in the initial phase, which is not available to the public at this point. The data that support the findings of this study are available from Ri.MED Foundation and ISMETT, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are, however, available from the authors on reasonable request and with permission of corresponding (S.G.) and first authors (D.P.).

All patients involved in the study provided written informed consent.

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surgical technical improvements have been implemented over time to reduce the rate and severity of complications after LT and optimize graft and recipient survival.³⁻⁵ Introducing simple measures such as pharmacologically ameliorating the metabolic status of the liver graft may thus represent a new way of improving LT outcomes, especially with an increasing use of extended criteria donors (ECD).

In Italy, and in particular in regions such as Sicily, the use of ECDs remains crucial for treating ESLD and related oncologic diseases, obtaining patient and graft survival at 3 and 6 mo after LT of 96% and 93%, and of 91% and 89%, respectively.^{6,7}

Nevertheless, in this last decade, there has been an increased mortality on the waiting list due to lack of transplantable livers.^{8,9} In many occasions, liver allografts judged unsuitable by other transplant centers have to be used for transplant. The considerable distances, and the need to keep short ischemic times related to the transport of organs, represents a challenge, particularly during the severe acute respiratory syndrome coronavirus 2 pandemic.¹⁰⁻¹³ Additionally, the management of donors after brain death (DBDs) can be difficult, and the time required for initial judgment of nonsuitability by the reference transplant center where the DBD is certified can lead to an extension of intensive care.^{6,14,15} This can increase the risk of severe ischemia-reperfusion injury for available liver grafts and ultimately lead to severe clinical outcomes following LT, such as primary nonfunction.⁵ Preclinical studies suggested that simvastatin administration pretransplant or added to the preservation solution enhances preservation time while preventing postreperfusion liver injury by preventing liver endothelial dysfunction.¹⁶⁻²⁰

It has been demonstrated that statins, including simvastatin, show antithrombotic and anti-inflammatory properties.^{21,22} Additionally, they have vasoprotective capacities

via the activation of Krüppel-like Factor 2 (KLF2), an effector recently identified with a protective role in liver and kidney ischemia/reperfusion injury (IRI).^{23,24}

This effect is similar to that of pulsatile ex vivo machine liver perfusion, a sophisticated approach that has been shown to successfully improve outcomes of LT.²⁵ Recently, a randomized clinical trial (RCT) in the ideal setting for the hepatic metabolism, such as heart transplant from DBDs, indicated that simvastatin can represent a viable option to prevent the risk of IRI.¹²

This article reports the SIMVastatin donor treatment before Liver Transplants (SIMVALT) study, a double-blinded, placebo-controlled, randomized phase 2 trial designed to compare 3- and 6-mo graft and recipient survival in 2 parallel groups of eligible adult LT recipients. Simvastatin or placebo was administered as a single intragastric administration DBD donor before organ procurement for LT.

MATERIALS AND METHODS

Trial Design, Participants, Randomization, and Interventions

The study was conducted in accordance with the principles outlined in the Declaration of Helsinki of 1996, ISMETT's Institutional Research Review Board approved the protocol (protocol number IRRB/72/14). Before the outbreak of severe acute respiratory syndrome coronavirus 2 pandemic, the enrollment of 71 patients was calculated for providing 75% power to detect a difference of 15% between the 2 groups, at 0.05 2-sided alpha level. Based on the maximum recommended dose in an adult patient, the therapeutic intervention object of RCT consisted in the administration via a nasogastric tube of a single dose of 80 mg of simvastatin (experimental group), or placebo (control group) in a hydro-alcoholic solution. Simvastatin is absorbed rapidly following oral administration, reaching peak plasma concentration (T_{max}) within 1.8–1.0 h. In this trial, the administration to DBD was done 2 h before donor cross-clamping for liver graft procurement. Informed consent for study participation was obtained from the patient legal representative before any study-related procedure. Randomization was coordinated by the independent statistical service, using sealed opaque envelopes containing the allocated treatment code according to a randomization list generated in a random and unpredictable method using (one for each study strata) an “on-off” program in SAS Version 9.4 using the PROC PLAN with a fixed seed. The randomization codes were reported on the drug/placebo containers and on the clinical report forms. The randomization system was stratified according to donor age. A specific website is dedicated for electronic clinical report form depository (<https://simvalt.fullcro.org/>). The randomization was in blocks of 4 and with a 1:1 equal allocation ratio. Randomization was done after declaration of donor brain death and on acceptance of liver donation for the recipient with written informed consent for the study. The investigators' request for supply of study drug or identical placebo took place through an automated system, which generated an e-mail communication addressed to the institutional pharmacy service, the principal investigator, and the donor surgical team that was the keeper of the drug/placebo, according to the drug

Standard Randomized Controlled Trial Registry. URL of registry: <http://www.isrctn.com>. Date of registration: October 10, 2015. Date of enrollment of the first participant to the trial: June 29, 2018. Date of enrollment of the last participant to the trial: April 30, 2020.

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D.P. and E.O. had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: D.P., J.B., P.G.C., A.L., and S.G.. Acquisition, analysis, or interpretation of data: D.P., M.B., R.A., D.C., F.D.F., S.L.P., P.B., G.Z., A.G., S.C., and B.M. Drafting of the manuscript: D.P. Critical revision of the manuscript for important intellectual content: D.P., J.B., and P.G.C. Statistical analysis: F.T. and R.A. Obtained funding: D.P. and E.O. Administrative, technical, or material support: E.O., P.G.C., F.V., C.R., B.E., and G.T. Supervision: J.B., P.G.C., A.L., and S.G.

Correspondence: Salvatore Gruttadauria, MD, Department for the Treatment and Study of Abdominal Diseases and Abdominal Transplantation, IRCCS-ISMETT, UPMC Italy, Via E. Tricomi 5, Palermo 90127, Italy. (sgruttadauria@ismett.edu).

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storage procedures. All donor procedures were performed in operating theaters of other hospitals where the DBDs were located at the time of the declaration of donor brain death. According to Eurotransplant, we defined ECD as a graft meeting one or more of the extended donation criteria published elsewhere.²⁶

The surgeon responsible for the organ recovering phases administered the randomized treatment on arrival of the DBD in the operating room, before proceeding with the surgical maneuvers to ensure proper removal of the entire organ according to usual institutional clinical practice and under strict double-blind conditions.

Study Population

Donor allocation, liver transplant recipient care, and follow-up were performed, according to the national allocation policy and institutional written clinical protocol. Eligibility criteria included adult recipients over 18 y of age, DBDs over 18 y of age, patients with ESLD or primary hepatic tumors. Donor exclusion criteria were as follows: (1) Pregnant or breast-feeding donors. (2) Donor with autoimmune disease or allergies to statins. (3) Donors requiring ongoing dosing with a systemic immunosuppressive drug at time of recovering procedures. (4) Donors known to be positive for HIV and/or coronavirus disease 2019 (COVID-19). (5) Donors with present or past malignancy, except for nonmetastatic basal or squamous cell skin cancer treated successfully. Recipient exclusion criteria were as follows: (1) Patients with acute liver disease. (2) Patients undergoing liver retransplantation, patients undergoing split or living donor LT, patients undergoing combined liver or other solid (abdominal or thoracic) organ(s) transplantation. (3) Patient who is participating or have been participated to another clinical trial/study in the last 30 d. (4) Patients with history of allergy or intolerance to statins. (5) Patients greater than 65 y old.

Immunosuppression in LT adult recipients was conducted as suggested by the national scientific board, in accordance with the consensus recommendations from the Italian Society for Organ and Tissue Transplantation and the International Liver Transplantation Society consensus guidelines.²⁷

Both groups received tacrolimus that was administered at 0.15 mg/kg/d by mouth, or through a nasogastric tube, within 24 h after the LT, and adjusted to achieve trough levels in the range of 8–10 ng/mL. At 30 d post-transplantation, the target trough level was reduced to 5–7 ng/mL for the first post-transplant year. An induction of the immunosuppressive regimen was performed in the entire study population with 20 mg basiliximab, which was administered by IV bolus in both groups, during anhepatic phase and on postoperative day (POD) 4. The mammalian target of rapamycin inhibitor (eg, everolimus), and/or a cell cycle inhibitor (mycophenolic acid), were used as combined therapy with reduced-exposure of calcineurin inhibitors.

Aims

The main aims of the study were the safety and efficacy of intragastric administration of simvastatin to DBD liver donors increasing the viability of DBD liver grafts, and improving early graft and recipient survival.

Outcome Measures

The primary end-point of the study were early LT outcomes in terms of graft and patient survival rates at 90 and 180 d, comparing patients receiving grafts from donors treated with simvastatin versus those receiving placebo, as well as safety. Postoperative complications were graded according to the Clavien-Dindo classification.²⁸ All participants underwent LTs with standard technique, and as part of the routine management had an intraoperative liver biopsy, intensive care monitoring, daily laboratory, and ultrasound examinations for the first week after the operation and when required during their hospital stay. The early allograft failure simplified estimation (EASE) scores were calculated with Version 2.0 (available at <https://transplant-tools.com/product/ease-calc-version-2-0>), which allows for entering data for multiple patients.²⁹ The patients were followed-up by expert medical and nursing staff at 15 and 30 d, 3 and 6 mo (primary end-point assessments). At the follow-up visits, the patients underwent clinical examination and liver function tests. The last blinded follow-up date for this 6-mo report was November 30, 2020. Liver biopsy was performed during the follow-up only when clinically indicated. Clinically relevant rejection, characterized by histological evidence of rejection, biochemical signs of liver damage and/or clinical manifestations was recorded and analyzed.

Sample Size Calculation

Given the mobility restrictions and severe donor shortage associated with the COVID-19 pandemic, and the occurrence of four 6-mo post-LT graft losses in the recruited population at the scheduled midterm interim analysis, 58 patients had been included and the last included patient had a follow-up of 6 mo, to detect a 15% difference in a bilateral test with α of 0.05 and β of 0.20. The study was interrupted because of data pointing to a difference in the main end-point, accordingly with the data safety monitoring board. The Italian national competent authority and the institutional ethics committee reviewed and authorized the related amendment.

Gene mRNA Expression by Real-time Reverse Transcription Polymerase Chain Reaction

Gene expression analysis on hepatic tissue specimens from the simvastatin and placebo arms before and after reperfusion was performed to assess whether simvastatin caused a significant upregulation of endothelium protective genes. Tissue specimens from simvastatin/placebo-treated livers were collected and stored at -80°C until analysis. Total RNA was isolated with the Pure Link RNA Mini Kit (Life Technologies), according to the manufacturer's protocol. A total of 100 ng of RNA was reverse-transcribed with the high-capacity RNA-to-complimentary DNA kit protocol (Thermo Fisher Scientific, Waltham, MA) to produce single-stranded complimentary DNA. Expression of mRNA was quantified by real-time polymerase chain reaction using StepOnePlus Real-Time PCR System (Thermo Fisher Scientific). TaqMan gene assay (Life Technologies) was used for the analysis of specific genes, including flow-induced transcription factor KLF2 (Thermo Fisher Scientific, reference gene Hs00360439_g1), intercellular adhesion molecule (ICAM) (Thermo Fisher Scientific,

reference gene Hs00609563_m1), endothelial nitric oxide synthase (eNOS; Thermo Fisher Scientific, reference gene Hs00167223_m1), and hepatocyte growth factor (HGF) (Thermo Fisher Scientific, reference gene Hs00300159_m1). Glyceraldehyde 3-phosphate dehydrogenase (Thermo Fisher Scientific, reference gene Hs02786624_g1) was used as a reference gene for the relative quantification, assessed by $2^{-\Delta\Delta CT}$ calculation for each mRNA.

Statistical Analysis

Analyses were conducted on an intention-to-treat strategy. The main variables, namely the incidence of graft failure, patient and graft survival at 3 and 6 mo in the 2 arms was analyzed using the Z test for 2 proportions.

All the variables collected in the clinical report forms were included in the descriptive tables. The continuous variables are presented as mean and SD (or medians and interquartile ranges), whereas the discrete variables are summarized through absolute values, percentages and cumulative percentages. To evaluate the homogeneity between the groups, the continuous variables were evaluated using the Student's *t* test for independent data or by the Wilcoxon test if the assumptions of the Student's *t* test were not met, whereas the discrete or nominal variables were evaluated by χ^2 test or Fisher exact test when appropriate.

Death-censored analyses were performed in terms of EASE score for evaluating any survival advantage using study drug between the 2 groups. The statistical evaluation of gene expression analysis was made on differences between post-reperfusion liver biopsy versus preoperative liver biopsy for each sample. Statistical tests were considered significant at $P < 0.05$. Probability of graft

loss and survival were estimated with Kaplan-Meier estimators and tested for difference by means of the log-rank test. Data handling and analyses were performed with SAS Version 9.4.

RESULTS

Trial Patients

The SIMVALT study was conducted between June 30, 2018, and April 30, 2020, at the IRCCS-ISMETT in Italy (Palermo, Sicily). At this moment, the trial recruitment was closed because of the restrictions imposed by the COVID pandemic. Of the 118 LTs performed with whole liver grafts procured from consecutive adult DBD donors, 103 adult recipients were subjected to the informed consent administration prior to formal inclusion in the LT-waiting list, and defined as eligible to be included in this study. Fifteen DBD donors were excluded as they underwent split surgery (9 for pediatric recipients and 6 adults). Seventy-one DBDs were enrolled for the study. Only 58 liver allograft DBDs were randomly assigned at a 1:1 ratio to receive either 80 mg of simvastatin via nasogastric tube, or placebo, on day 0 (before donor surgery), as showed into the flow chart of the study (Figure 1). Specifically, 13 organs were not used because of intraoperative evidence of exclusion factors related to DBD characteristics. In detail, in 4 cases, a steatohepatitis proved by intraoperative liver biopsy was found; in 3 cases, a compensated macroscopic liver cirrhosis was detected; in 2 cases, an extrahepatic malignancy was found; in 2 cases, there was an advanced liver disease (Ishack grade ≥ 3), and in 1 case, it was not possible to perform the intragastric administration of the

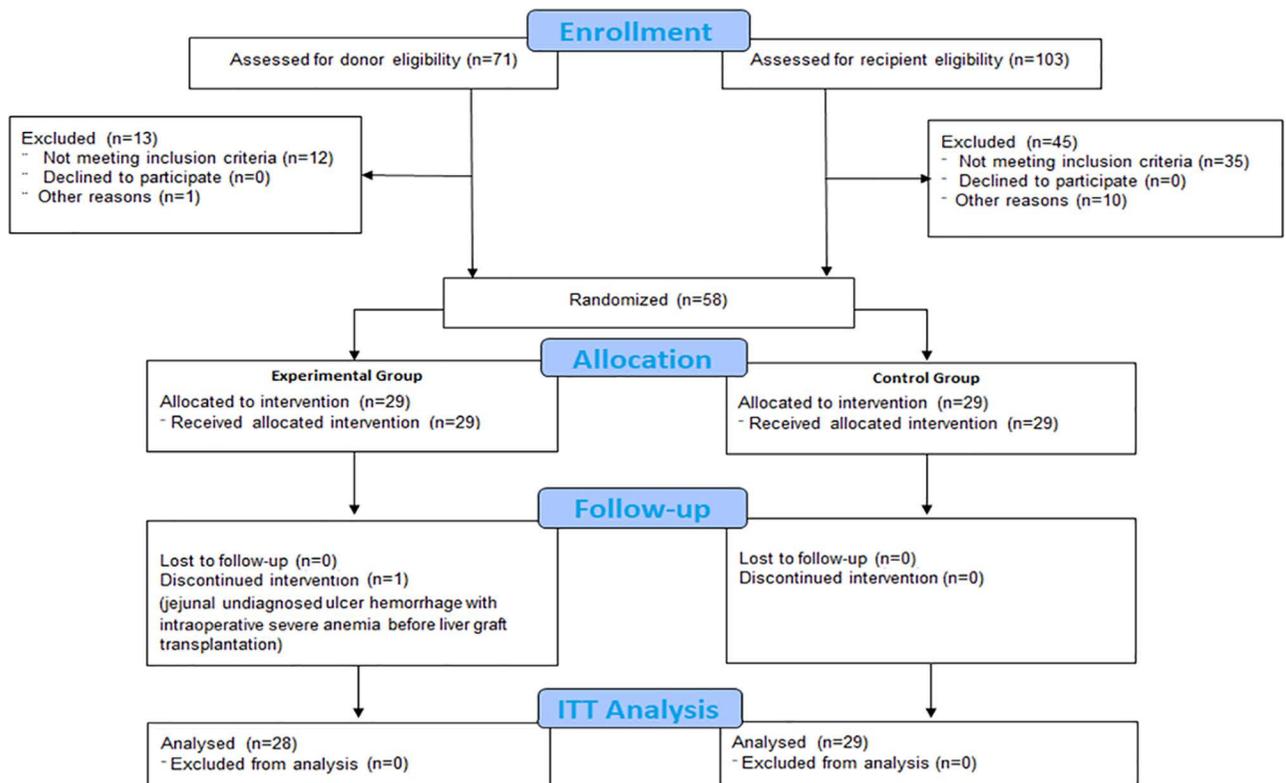


FIGURE 1. Study flow diagram. Study population, enrollment, and randomization of the donors to either the simvastatin or placebo arms, or their respective recipients. ITT, intention-to-treat analysis.

drug because of deteriorating hemodynamic conditions of the DBD. Simvastatin or placebo administration before the cross-clamp surgical procedure did not alter the timing of the donor's surgical procedure for organ procurement.

One patient randomized in the simvastatin arm was excluded as he/she was nonevaluable. The patient had hypovolemic shock due to massive intraoperative digestive bleeding before completing the organ implantation and died at the end of the operation on transferal to the intensive care unit (ICU). Therefore, the final number of patients

was 29 in the placebo arm and 28 in the simvastatin arm, configuring the conduction of a modified intention-to-treat analysis (Figure 1). Among the grafts, 52 (91.2%) were ECD; these were not differently assigned and transplanted in both groups (26 in the experimental group and 26 in the control arm, $P = 0.33$). Median donor age (interquartile range) was 61 (54–68) y (with 31.3% women), which was not statistically significantly different from the control arm. There were also no differences in previous exposure to statin therapy (Table 1).

TABLE 1.
Donor demographics

	Study population (N = 57)				
	Control group (N = 29)		Experimental group (N = 28)		P
	Mean	SD	Mean	SD	
Age, y	58.4	15.9	60.7	11.0	0.530
Body mass index, kg/m ²	27.3	4.4	28.1	4.2	0.487
Intensive care unit stay, d	4.6	3.1	6.1	5.1	0.160
Sodium serum level, mEq/L	153.7	14.7	150.7	10.1	0.378
	Study population (N = 57)				
	Control group (N = 29)		Experimental group (N = 28)		P
	No.	%	No.	%	
Gender					
Female	12	41.4	9	32.1	0.585
Male	17	58.6	19	67.9	
Donor procurement hospital					
Abroad	1	3.4	0	0.0	1.000
National	11	37.9	12	42.9	
Regional	17	58.6	16	57.1	
Donor medical history					
Hypertension	15	51.7	14	50.0	1.000
Diabetes mellitus type II	1	3.4	5	17.9	0.102
Cardiopathy	3	10.3	7	25.0	0.179
Dyslipidemia	3	10.3	5	17.9	0.470
Previous statin assumption	3	10.3	6	21.4	0.297
Donor cause of death					
Traumatic brain injury	2	6.9	3	10.7	0.915
Cerebral hypoxia	6	20.7	6	21.4	
Cerebrovascular accident	21	72.4	19	67.9	
Hemodynamic instability					
Amine IV continue administration ≥ 1	22	75.9	24	85.7	0.505
Amine IV continue administration > 1	11	37.9	11	39.3	1.000
Donor risk (National Transplant Center)					
Nonstandard	15	51.7	17	60.7	0.596
Standard	14	48.3	11	39.3	
Ultrasonographic examination					
Steatosis	4	13.8	5	17.9	0.730
Liver biopsy histologic findings					
No fibrosis	25	86.2	23	82.1	
Ishack grade I	3	10.3	5	17.9	0.586
Ishack grade II	1	3.4	0	0.0	
Microvesicular ($\geq 30\%$)	2	6.9	3	10.7	0.670
Macrovesicular ($\geq 30\%$)	3	10.3	4	14.3	0.706
Perfusion solution					
Celsior	14	48.3	13	46.4	1.000
Servator C	15	51.7	15	53.6	

The most common transplant diagnoses were liver cirrhosis related to hepatitis C virus infection, cirrhosis secondary to nonalcoholic steatohepatitis, and alcohol-related liver cirrhosis. Recipients in the donor's simvastatin treatment group had a similar Model of End-stage Liver Disease score to the control group (16 [11–17] versus 15 [11–22]), but a greater presence of portal thrombosis (4 versus 0) than to those of the control group. There were no differences in immunosuppressive treatment between the groups. All recipients received basiliximab induction therapy and initial calcineurin inhibitor (tacrolimus) immunosuppressive treatment. Recipients were also treated with tacrolimus maintenance immunosuppression in 90%, combined with a cell cycle inhibitor (mycophenolic acid) in 95% of recipients. Reduced doses of corticosteroids were used as part of immunosuppressive treatment only in 1 patient transplanted for autoimmune liver disease belonging to the control group, and in 3 patients with sclerosing cholangitis (2 in the experimental group and 1 in the control group; Table 2).

Graft and Patients Survivals

During the 6 mo of clinical follow-up to which the transplanted patients were subjected according to the institutional standard clinical practice, no loss to follow-up was recorded during the observation period. In the simvastatin arm, the overall graft and patient survival was 100% at 180 d. In contrast, in the control group, patient and graft survival were 89.7% ($P = 0.0804$) and 93.1% ($P = 0.1572$), respectively, at 90 d, and 86.2% ($P = 0.0415$) and 86.2% ($P = 0.0415$) at 180 d (Table 3). Four grafts were lost in the control group for the following causes: (1) primary nonfunction in a case requiring retransplantation in the POD 4 with death of the patient in POD 38 from the primary transplant; (2) graft malfunction in a case requiring retransplantation in the 63rd POD with death of the patient in POD 100 from the primary transplant; (3) Infectious complications associated with delayed allograft dysfunction with severe sarcopenia, septic shock, and prolonged stay in ICU and death of the patient in POD 166; and (4) Death at home from cardiocirculatory arrest in a patient on POD 61, after being discharged on POD 51 for

TABLE 2.
Recipient demographics

	Study population (N = 57)				
	Control group (N = 29)		Experimental group (N = 28)		P
	Mean	SD	Mean	SD	
Age, y	55.2	8.3	51.7	11.5	0.192
Body mass index, kg/m ²	27.3	4.2	27.2	4.6	0.925
	Study population (N = 57)				
	Control group (N = 29)		Experimental group (N = 28)		P
	No.	%	No.	%	
Gender					
Female	10	34.5	6	21.4	0.379
Male	19	65.5	22	78.6	
Etiology					0.914
HCC-HBV	1	3.7	2	7.1	
HCC-HCV	2	7.4	2	7.1	
HCV	4	14.8	5	17.9	
HBV	2	7.4	1	3.6	
NASH	6	22.2	6	21.4	
Alcohol	9	33.3	7	25.0	
Other	5	18.5	5	17.9	
Recipient medical history					
Hypertension	9	31.0	9	32.1	1.000
Diabetes mellitus type II	20	69.0	17	60.7	0.585
Dyslipidemia	1	3.45	3	10.7	0.111
Portal vein thrombosis	0	0.0	4	14.8	0.040
	Study population (N = 57)				
	Control group (N = 29)		Experimental group (N = 28)		P
	Median	25th–75th percentile	Median	25th–75th percentile	
MELD score	15.0	11–21	16.0	11–17	0.779
Cold ischemia time, min	360.0	330–380	400.0	360–450	0.066
Warm ischemia time, min	41.0	37–57.50	45.0	40–60	0.746
Total ischemia time, min	407.5	397.5–439	440.0	407.5–502.5	0.196

HBV, hepatitis B virus infection; HCC, hepatocellular carcinoma; HCV, hepatitis C virus infection; MELD, Model of End-stage Liver Disease; NASH, non-alcoholic steatohepatitis.

TABLE 3.**Transplant outcomes**

	Study population (N = 57)				P
	Control group (N = 29)		Experimental group (N = 28)		
	No.	%	No.	%	
Graft survival					
3 mo	26	89.66	28	100.00	0.080
6 mo	25	86.21	28	100.00	0.041 ^a
Patient survival					
3 mo	27	93.10	28	100.00	0.157
6 mo	25	86.21	28	100.00	0.041 ^a
Primary nonfunction	1	3.45	0	0.00	0.312
Clavien-Dindo grading system (multitier analysis)					
I	2	6.90	3	10.71	0.080
II	3	10.34	3	10.71	
IIIa	1	3.45	2	7.14	
IIIb	9	31.03	2	7.14	
IV	3	10.34	5	17.86	
V	4	13.79	0	0.00	
Clavien grading system (dichotomous analysis)					
≤IIIa	13	44.83	21	75.0	0.031 ^a
≥IIIb	16	55.17	7	25.0	

	Study population (N = 57)				P
	Control group (N = 29)		Experimental group (N = 28)		
	Median	25th–75th percentile	Median	25th–75th percentile	
Length of hospital stay, d	18.00	13–32	19.50	14.5–38	0.551
Length of intensive care unit stay, d	6.00	3–10	4.50	3–6.5	0.586

a cardiologic complication that required ICU admission (severe cardiac arrhythmia). Kaplan-Meier survival probability curves for graft survival at 6 mo was statistically

significant higher in the patients randomized to receive a graft pretreated with simvastatin than in those receiving a graft pretreated with placebo ($P = 0.0435$; Figure 2).

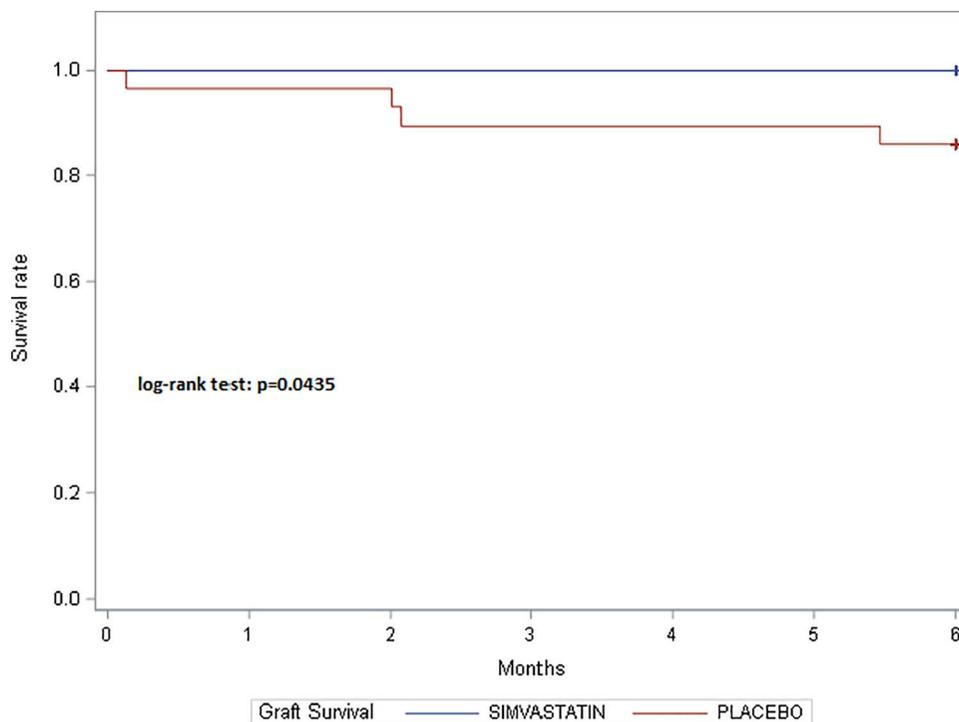


FIGURE 2. Kaplan-Meier survival probability curve. Kaplan-Meier curve graft survivals, with related log-rank test.

Gene Expression Analysis of Liver Biopsies

To determine the candidate genes that might suggest the protective role of simvastatin pretreatment on IRI on transplanted patients, we decided to investigate the expression of KLF2, eNOS, ICAM-1, and HGF in the post-transplant specimens of patients pretreated with simvastatin in comparison with patients under placebo conditions. KLF2 ($P = 0.003$) and HGF ($P = 0.003$) genes were upregulated in the postreperfusion liver biopsies of patients in the experimental group in comparison with those receiving placebo. Accordingly, we found that eNOS, whose transcription is positively regulated by KLF2, was upregulated in post-reperfusion liver biopsies of patients in the experimental group, although the difference did not reach statistical significance ($P = 0.06$). Furthermore, no significant changes were experienced in simvastatin-treated patients in terms of downregulation of ICAM-1 expression ($P = 0.56$; Figure 3).

Postoperative Clinical Course

Overall and ICU hospitalization times were not significantly different between the 2 groups. However, the

number of severe Clavien-Dindo complications (\geq IIIb) was significantly lower in patients receiving simvastatin (25.0% experimental group versus 55.2% control group, $P = 0.0307$; Table 3). No significant changes were detected in trends of liver function tests in the postoperative course (Table 4). The only noticeable difference was a significant increase of gamma-glutamyl transferase (GGT) and alkaline phosphatase levels in POD 15 (respectively, $P = 0.0174$ and $P = 0.0152$), and in GGT at POD 30 ($P = 0.0375$). No difference was detected between the experimental and control groups in the EASE score ($P = 0.89$; Figure 4). There were 4 episodes of acute cell rejection in the control group versus 4 similar episodes in the experimental group treated with short course of corticosteroids.

DISCUSSION

LT results can provide effective and sustainable overall survival benefit, remission of ESLD-related comorbidities, and improvement of quality of life in the long term.^{3,4} In 2019, the annual number of donors for organ transplantation and the related percentage of donor suitable for LT performed in Italy were 1743 and 79%, respectively.^{5-7,10}

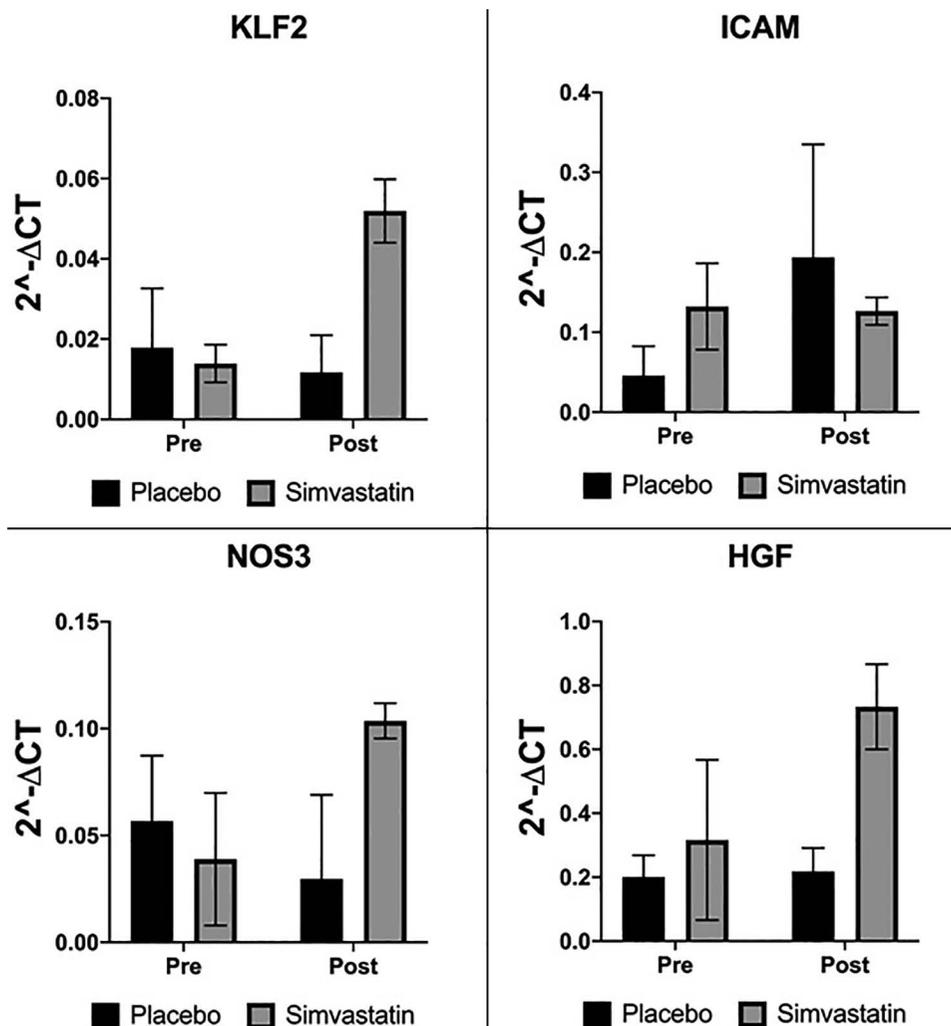


FIGURE 3. Gene expression analyses. The statistical evaluation of gene expression analysis was made on differences (Δ) obtained from postreperfusion liver biopsy minus preoperative liver biopsy for KLF2, NOS3, ICAM1, and HGF in the preoperative and postreperfusion liver biopsies of grafts pretreated with simvastatin in comparison with grafts under placebo conditions. Histograms were obtained from raw gene expression data. HGF, hepatocyte growth factor; ICAM1, intercellular adhesion molecule 1; KLF2, Kruppel-like factor 2; NOS3, endothelial nitric oxide synthase.

TABLE 4.
Liver function tests

	Study population (N = 57)				P
	Control group (N = 29)		Experimental group (N = 28)		
	Median	25th–75th percentile	Median	25th–75th percentile	
Aspartate transaminase, U/L					
POD 2	572.0	339.0–1173.0	539.5	306.0–2123.5	0.793
POD 7	50.0	35.0–64.0	53.5	45.0–71.0	0.346
POD 15	24.5	18.5–44.0	40.5	29.0–62.0	0.072
POD 30	24.5	18.5–34.0	32.5	20.5–68.0	0.089
POD 90	24.0	16.0–39.0	28.0	18.0–39.0	0.478
POD 180	20.0	17.0–34.0	24.0	17.0–32.0	0.764
Alanine transaminase, U/L					
POD 2	792.0	526.0–1502.0	653.0	334.5–2031.5	0.849
POD 7	173.0	77.0–229.0	147.0	80.5–263.5	0.861
POD 15	48.0	31.5–66.5	53.5	36.5–88.0	0.273
POD 30	32.5	23.5–42.0	42.0	24.5–92.0	0.144
POD 90	30.0	22.0–55.0	42.0	24.0–68.0	0.301
POD 180	32.5	24.0–49.0	29.0	25.0–61.0	0.896
Gamma-glutamyl transferase, U/L					
POD 2	89.0	65.0–116.0	128.0	54.0–203.0	0.198
POD 7	258.0	153.0–352.0	290.0	154.0–468.0	0.332
POD 15	119.0	74.0–265.0	198.0	110.0–385.0	0.017 ^a
POD 30	63.0	38.0–130.0	129.0	72.0–230.0	0.015 ^a
POD 90	63.5	27.5–132.0	95.0	39.0–197.0	0.369
POD 180	52.5	24.0–183.0	46.5	26.0–98.0	0.934
Alkaline phosphatase, U/L					
POD 2	108.0	83.0–136.0	106.0	67.0–156.0	0.930
POD 7	255.0	177.0–456.0	286.0	163.0–431.0	0.640
POD 15	190.0	128.0–257.0	275.0	188.0–360.0	0.037 ^a
POD 30	154.0	121.0–225.0	201.0	145.0–359.0	0.169
POD 90	154.0	92.0–367.0	126.0	89.0–212.0	0.500
POD 180	135.5	81.0–197.0	108.5	87.0–169.0	0.658
International normalized ratio, n					
POD 2	1.5	1.3–2.0	1.5	1.2–2.2	0.836
POD 7	1.3	1.2–1.4	1.3	1.2–1.5	0.432
POD 15	1.2	1.1–1.3	1.2	1.1–1.3	0.512
POD 30	1.2	1.1–1.3	1.2	1.1–1.3	0.711
POD 90	1.1	1.1–1.2	1.1	1.1–1.2	0.409
POD 180	1.1	1.0–1.1	1.1	1.1–1.1	0.161
Total bilirubin, mg/dL					
POD 2	4.5	2.5–7.7	4.4	2.2–10.4	0.832
POD 7	4.1	3.0–8.1	9.3	3.6–13.3	0.087
POD 15	1.7	1.2–2.6	3.1	1.4–6.6	0.110
POD 30	1.1	0.8–1.8	1.6	0.9–2.7	0.110
POD 90	0.6	0.5–0.8	0.7	0.5–1.0	0.269
POD 180	0.5	0.4–0.8	0.8	0.3–1.4	0.230

POD, postoperative day.

Still, demand for donor organs far exceeds its availability, and this dramatic gap is particularly severe in regions with a donation rate of less than 12.6 donors per million per year, as in Sicily.^{6,11,15,30}

This study examined whether pretreating the donor with a single dose of simvastatin at the start of organ recovering could improve the outcomes of deceased donor LT, which is a novel approach that has not been used previously but for which there is biological rationale and preclinical evidence. This study found that donor simvastatin treatment

was safe, and was associated with improved recipient outcomes at 90 and 180 d, the period where improved graft function is more likely to influence clinical outcomes. These findings are of great relevance overall, and even more in regions with low donor rates where the increasing adoption of ECDs remains crucial.³¹

For explaining the potential mechanism for how and why treatment drug could lead to a positive impact on outcome, we have investigated several gene expression. Particularly, HGF was upregulated in the experimental

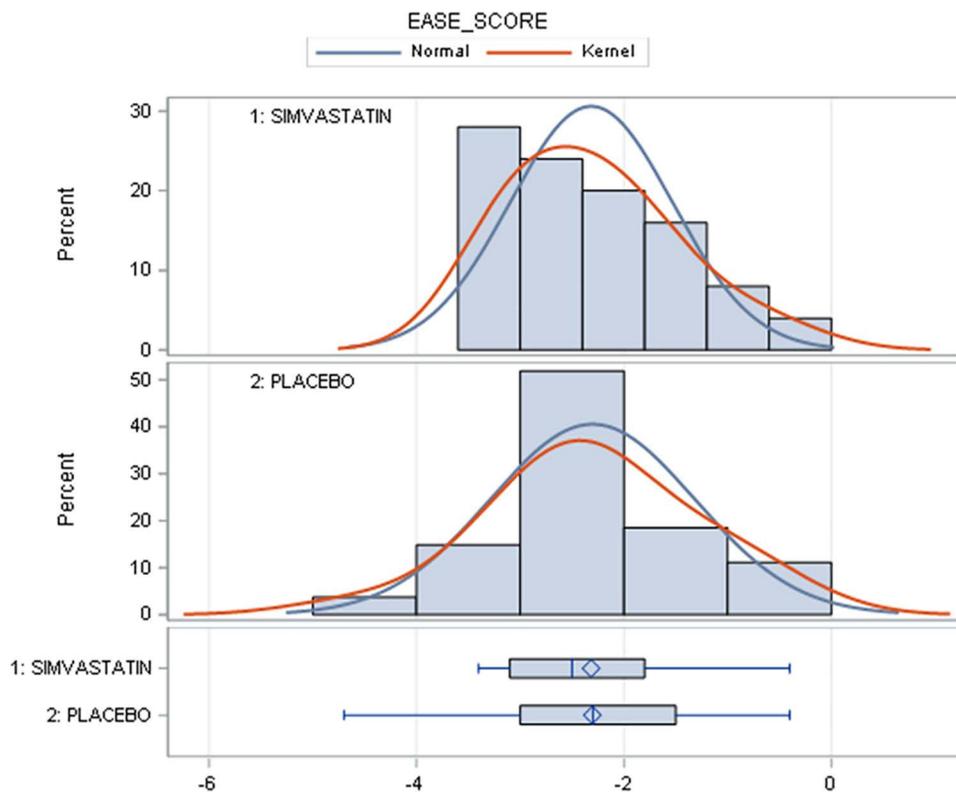


FIGURE 4. Distributions and box-ploting of EASE scores. Normal and kernel density estimation were used to estimate the probability density function of EASE score. EASE, early allograft failure simplified estimation.

group, in comparison with the ones in the placebo arm. This finding is in strong accordance with previous data from literature suggesting that HGF-met signaling activation in reperfused livers attenuate the damage from IRI.³²

Recent preclinical studies have demonstrated previously unknown therapeutic targets for hydroxymethylglutaryl-coenzyme A reductase inhibitor reductase inhibitors, such as KLF2, a nuclear transcription factor regulating eNOS expression in liver endothelial cells, which we have confirmed in this study. It should be noted that upregulation by simvastatin of KLF2 results in increased transcription of several vasoprotective genes besides eNOS, including thrombomodulin, angiopoietin, altogether maintaining a healthy endothelial phenotype, which is antiproliferative, provasodilatory, anti-inflammatory, and anti-thrombotic.³³ KLF2 is rapidly downregulated in liver grafts, which provides a rationale for using statins to prevent it and its associated consequences on reperfusion. Notably, the natural stimulus for KLF2 expression is flow-induced shear stress, which is a major mechanism for improved results in LT using machine perfusion of the liver grafts until the moment of transplantation. Therefore, our data suggest that the very simple, inexpensive, and widely available administration of simvastatin to the donor before organ procurement may allow obtaining results that could be similar or approach those afforded by ex vivo liver graft machine perfusion.^{16,34}

Taken together, the data obtained from the gene expression analysis are a proof of target engagement by demonstrating that simvastatin pretreatment indeed stimulated the transcription of genes associated with protection from liver IRI on transplant. Moreover, the data indicate that giving simvastatin via the nasogastric tube effectively

delivered the drug to the liver despite varying degrees of absorption may occur in ECD with hemodynamic instability.

Over the past 2 decades, several complex therapeutic approaches for facing ischemia-reperfusion injury in the transplanted patient following the phases of transport of the organ (cold ischemia) and those necessary at the time of transplant on the recipient before achieving effective reperfusion of the graft (warm ischemia-reperfusion injury). This led to the recent suggestion to use with caution liver grafts from DBDs with 3 or more ECD features or >33% steatosis and livers from circulatory death donors with any ECD features to reduce short-term graft loss and complication prompted by severe ischemia-reperfusion injury.¹⁴ Different therapeutic strategies have been proposed without any clear outcome difference in terms of graft and patient survivals, nor incidences of early allograft dysfunction.^{11,14,25} Therefore, our current findings are of potential clinical relevance as they point out to a clear benefit in terms of graft survival in the context of organ shortage making mandatory using predominantly donors with extended criteria. On the other hand, simvastatin is a well-known drug with a minimal risk of liver damage,^{13,35-39} which was reinforced by the absence of safety concerns in our patients receiving the drug. All DBDs were exposed to study treatment for the planned dose, and all enrolled recipients attended the visits for assessment of primary end-point. Its use in our study was safe, not associated with signs of liver damage in any treated patient. Our study closed with a total of 57 patients enrolled, and in 52 (91.2%) cases, an ECD grafts was used. After unblinding the data, there was a significant difference in 6-mo graft survival that was of 13.8% ($P = 0.041$; 86.2% in the

control group versus 100% in the simvastatin arm). Thus, the SIMVALT study has provided preliminary evidence on the effectivity and safety of the oral administration of 80 mg of simvastatin via a nasogastric tube to the DBD just before organ recovering. Furthermore, the assessment of early allograft dysfunction in both groups did not show differences in terms of EASE score between the 2 groups. The only significant effect in liver enzymes was an apparent increase in GGT and alkaline phosphatase at POD 15, and of GGT at POD 30. More important, the number of severe complications (>IIIa of the Clavien-Dindo classification) was half in the patients randomized to simvastatin as compared with controls (25% versus 55.17%; $P = 0.031$).

Limitations and Strengths

This study has several limitations. In analogy to what recently emerged with the introduction of surgical devices such as perfusion machines, this RCT focused on a single center clinical practice in an area with low rate of deceased donation.^{25,35,36} Another limitation of the study is that enrollment was severely impacted by the COVID-19 pandemic, which led to a protocol amendment and premature termination of the study. This detracted power and therefore robustness to the results, that should be considered preliminary and need further confirmation. Anyhow, our findings strongly support new investigations confirming the clinical benefit afforded by simvastatin in the context of LT. In this regard, our study provides valuable evidence both for the design of future studies, and to inform clinical practice related to early-term post-LT outcomes. A major advantage of the proposed simvastatin single dose administration to the donor before graft recovering is that it is very simple, easy to perform in any setting, and extremely inexpensive as compared with very sophisticated device-based procedures.³⁷⁻⁴¹

CONCLUSIONS

Donor simvastatin treatment was safe, and further resulted in a reduced frequency of major complications after LT. Donor simvastatin treatment on top of standard static cold storage was associated with significantly improved graft and recipient survival at 6-mo after LT. Our results could encourage future large-scale clinical trials to determine the clinical benefit from this new simple and inexpensive approach to optimize the results of deceased donor LT, and to inform clinical practice related to early-term post-LT outcomes.

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