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ORIGINAL ARTICLE

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Improved outcomes after hypothermic oxygenated machine perfusion in liver transplantation – Long-term follow-up of a multicenter randomized controlled trial

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Abstract

Background: While 4 randomized controlled clinical trials confirmed the early benefits of hypothermic oxygenated machine perfusion (HOPE), high-level evidence regarding long-term clinical outcomes is lacking. The aim of this follow-up study from the HOPE-ECD-DBD trial was to compare long-term outcomes in patients who underwent liver transplantation using extended criteria donor allografts from donation after brain death (ECD-DBD), randomized to either

Abbreviations: CCI, Comprehensive Complication Index; CD, Clavien-Dindo; DBD, donation after brain death; DCD, donation after circulatory death; D-HOPE, dual-hypothermic oxygenated machine perfusion; ECD, extended criteria donor; HOPE, hypothermic oxygenated machine perfusion; LT, liver transplantation; MP, machine perfusion; RCT, randomized controlled trial.

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HOPE or static cold storage (SCS).

Methods: Between September 2017 and September 2020, recipients of liver transplantation from 4 European centers receiving extended criteria donor-donation after brain death allografts were randomly assigned to HOPE or SCS (1:1). Follow-up data were available for all patients. Analyzed endpoints included the incidence of late-onset complications (occurring later than 6 months and graded according to the Clavien-Dindo Classification and the Comprehensive Complication Index) and long-term graft survival and patient survival.

Results: A total of 46 patients were randomized, 23 in both arms. The median follow-up was 48 months (95% CI: 41–55). After excluding early perioperative morbidity, a significant reduction in late-onset morbidity was observed in the HOPE group (median reduction of 23 Comprehensive Complication Index-points [$p = 0.003$] and lower incidence of major complications [Clavien-Dindo ≥ 3, 43% vs. 85%, $p = 0.009$]). Primary graft loss occurred in 13 patients (HOPE n = 3 vs. SCS n = 10), resulting in a significantly lower overall graft survival ($p = 0.029$) and adverse 1-, 3-, and 5-year survival probabilities in the SCS group, which did not reach the level of significance (HOPE 0.913, 0.869, 0.869 vs. SCS 0.783, 0.606, 0.519, respectively).

Conclusions: Our exploratory findings indicate that HOPE reduces late-onset morbidity and improves long-term graft survival providing clinical evidence to further support the broad implementation of HOPE in human liver transplantation.

INTRODUCTION

Liver transplantation (LT) has evolved as the standard treatment for end-stage liver disease. The circumstance of donor scarcity and the increase of patients with end-stage liver disease forces clinicians and transplant programs to accept extended criteria donation (ECD) liver allografts that have 1 or multiple risk factors for adverse outcomes after transplantation.[1,2] While the transplantation of ECD organs saves patients from waiting list dropout, these predamaged organs also exhibit an increased susceptibility to ischemia-reperfusion injury, translating into impaired clinical outcomes after ECD organ transplantation.[3–5]

Machine perfusion (MP) is increasingly recognized as a powerful strategy to protect allografts from ischemia-reperfusion injury, increase preservation time and improve short-term and long-term outcomes after LT.[2,6–10] Hypothermic oxygenated machine perfusion (HOPE) is a dynamic preservation method of the liver with cooled, oxygenized perfusate that replenishes tissue energy reserves before normothermic reperfusion *in vivo* and reduces allograft injury.[11] The potent clinical effects of HOPE, especially when using high-risk livers, have been described consistently in 5 randomized controlled clinical trials (RCT).[8–10,12–14] These trials could demonstrate a reduction of early allograft injury,[10,13,14] cumulative morbidity,[13,14] liver-related complications,[8] length of stay,[13] treatment costs,[15,16] and a mitigating effect on nonanastomotic biliary complications in donation after circulatory death (DCD) when using HOPE-treated allografts.[9] The most recent trial published very recently by Grąt et al[14] from Warsaw could also confirm the short-term benefits of HOPE on early allograft dysfunction and morbidity in high-risk livers with donor risk index over 1.7, but not for standard criteria allografts. Since these trials were focused on short-term perioperative outcomes (maximum follow-up of 1 year), long-term outcome data with over 1-year follow-up on MP in human LT are still lacking.

We therefore analyzed long-term clinical outcomes in patients who were randomly assigned to HOPE versus static cold storage (SCS) from our HOPE-ECD-donation after brain death (DBD) multicenter RCT and report late-onset morbidity and long-term graft survival and patient survival.

METHODS

Trial design

The HOPE-ECD-DBD trial (clinicaltrials.gov: NCT03124641) was conducted between September 2017 and

September 2020 as an investigator-initiated open-label multicenter RCT to assess the effects of HOPE versus SCS in patients receiving ECD allografts following DBD.[13] The trial was approved by the leading ethics committee (University Hospital RWTH Aachen; EK 049/17). The full study protocol was published before the initiation of enrollment.[12]

The trial was designed and conducted based on principles of good clinical practice guidelines (International Conference on Harmonization-Good Clinical Practice), Declaration of Helsinki, and Declaration of Istanbul. Reporting is in line with recommendations of the Consolidated Standards of Reporting Trials (CONSORT) statement,[17] http://links.lww.com/HC9/A787. Written informed consent was obtained from all participants before enrollment to the study.

Participants, definitions, trial interventions, and methods

Details of the trial design, inclusion/exclusion criteria, and definition of ECD criteria are listed below. Further information on the trial design was described in our initial trial report and *ex ante* study protocol.[12,13]

Eligibility of an ECD allograft was defined based on the fulfillment of at least one of the following criteria:

1. Donors 65 years of age and older
2. Intensive care therapy of the donor was required before donation for at least 7 days
3. Obesity of the donor with a Body Mass Index >30 kg/m²
4. Fatty liver (with histology) $>40\%$ macrosteatosis or mixed steatosis
5. Serum-sodium >165 mmol/L
6. Serum transaminases >3x upper limits of normal
7. Serum-bilirubin >2 mg/dL

Trial inclusion criteria:

1. Signed informed consent
2. Patients 18 years or older
3. Patients suffering from end-stage liver disease and/or malignant liver tumors
4. Listed for LT
5. Receiving ECD allografts

Trial exclusion criteria:

1. Recipients of split or living donor LT
2. Previous LT
3. Combined transplantations (liver-kidney, liver-lung, etc.)
4. Participation in other liver-related trials
5. The subject received an investigational drug within 30 days prior to inclusion
6. The subject is unwilling or unable to follow the procedures outlined in the protocol
7. The subject is mentally or legally incapacitated
8. The patient is not able to understand the procedures due to language barriers
9. Family members of the investigators or employees of the participating department

Patients were randomized based on a stratified randomization model (ratio 1:1) into a “control” group undergoing SCS according to local center-specific clinical standards versus an “intervention” group in which all allografts were treated using end-ischemic HOPE with a median dynamic preservation time of 145 minutes (101–203 minutes) (LiverAssist; XVIVO Perfusion AB, Göteborg, Sweden) as described.[13]

Further details concerning sample size calculation, randomization, organ preservation, MP protocols, surgical procedures, and standards of perioperative care were described in detail in the original trial report and study protocol.[12,13]

Follow-up and outcome measures

Follow-up data until the time point of death or last follow-up of all patients randomized for the HOPE-ECD-DBD trial were included in this analysis. Follow-up data were available for all randomized patients alive, and none of the participants withdrew from the study or were lost to follow-up.

Analyzed data included laboratory parameters, the incidence of late-onset morbidity, defined as complications registered later than 6 months and quantified according to the Clavien-Dindo (CD) Classification and Comprehensive Complication Index (CCI), readmissions, long-term graft survival, and patient survival.

Data collection and statistical methods

Data integrity standards of the original study report were adopted for follow-up data collection and analysis.[13] Briefly, data were collected prospectively by trained investigators on paper case report forms and subsequently transferred into the trial database according to International Conference on Harmonization-Good Clinical Practice standards. Following the final follow-up data collection in January 2023, the database was locked. The pseudonymized data were analyzed according to an intention-to-treat concept.

Statistical analysis was carried out using SPSS Statistics v24 (IBM Corp., Armonk, NY). The alpha level was set to 0.05 or indicated otherwise. It should be emphasized that the secondary analyses reported here are considered explorative, and *p*-values need to be interpreted in a descriptive fashion. Values for metric

parameters are displayed as medians with IQR and absolute plus relative frequencies for nominal data. Continuous variables were compared with the Mann-Whitney *U* test, while for the analysis of categorical data, the χ^2 and Fisher exact tests were used. Spearman correlation coefficient was used to express associations between early-onset and late-onset morbidity. Median follow-up time, graft survival and patient survival data were analyzed using the reverse Kaplan-Meier and Kaplan-Meier methods, respectively. Log-rank test was used for statistical comparisons. Univariable and multivariable analysis using binary logistic regression was performed to identify factors associated with late-onset major morbidity. Variable with *p*-values ≤ 0.1 in the univariable setting were included in the multivariable binary logistic regression analysis.

RESULTS

Baseline characteristics and follow-up

Out of 59 patients who were screened for eligibility, a total of 46 patients (23:23; SCS:HOPE) were randomized into the HOPE-ECD-DBD trial. Figure 1 depicts the patient flow modified for the present long-term follow-up setting in accordance with the CONSORT guidelines. Baseline donor, recipient, intraoperative, and perfusion characteristics were described in the initial trial report and displayed in Supplemental Table S1,[13] http://links.lww.com/HC9/A788. Briefly, all baseline characteristics were well balanced between trial arms.

The median Eurotransplant Donor Risk Index score of 2.050 (1.878–2.218) indicated an elevated allograft-related risk in our ECD-DBD allograft population with comparable distribution between groups (Supplemental Table S1, http://links.lww.com/HC9/A788). Further parameters indicating the overall donor-recipient risk, including the recipient Model for End-stage Liver Disease and Balance of Risk scores, were similar in both groups.

The median follow-up was 48 months (95% CI: 41–55) and was not significantly different between the SCS (48 months) and the HOPE group (47 months, Table 1).

Impact of HOPE on late-onset complications, morbidity, and hospital admission rates

Late-onset major complications (CD $\geq$ 3) occurring $>$ 6 months after LT was registered in 26 (63%) patients. Recipients receiving HOPE-treated allografts experienced significantly fewer late-onset major complications compared to the SCS group (9 [43%] HOPE vs. 17 [85%] SCS, CD $\geq$ 3, $p = 0.009$, Table 1). In addition, minor complications (CD1-2) were observed in 1 patient (5%) versus 6 patients (29%) in the SCS versus HOPE groups, respectively (Table 1). In total, 8 recipients (n = 2 in the SCS and n = 6 in the HOPE group, not significant) did not develop any late-onset complications during their clinical course following LT (Table 1).

The difference in late-onset complications (CD $\geq$ 3) was associated with a marked gap between the trial arms in terms of cumulative morbidity. The median CCI for late-onset complications was reduced by 50% in the intervention group in which patients received HOPE-treated livers compared to SCS (23 [0–37] HOPE vs. 46 [34–95] SCS, CCI points, $p = 0.003$, Table 1 and Figure 2A).

Further analysis was carried out to explore baseline characteristics and factors associated with new-onset morbidity (Figure 2 and Table 2). A moderate level of correlation was observed between 90-day and new-onset CCI with a correlation coefficient of 0.500 (Figure 2B). In addition, the partial dissociation between cumulative complications in the early phase after LT and later (90-day vs. late onset) was further visualized for the whole cohort using slope charts, where no clear patterns could be observed (Supplemental Figure S1, http://links.lww.com/HC9/A789).

The comparison of baseline characteristics and perioperative data for patients who developed new-onset major morbidity, independently of their randomization status, shows that they initially underwent a tendentially longer LT procedure (345 [166–460] minutes without vs. 420 [350–478] minutes with CD $\geq$ 3, 1.005 OR [0.999–1.011 95% CI], $p = 0.088$, Table 2) and spent slightly longer periods in hospital (20 [15–27] days without vs. 25 [18–44] days with CD $\geq$ 3, OR 1.050 [0.989–1.116 95% CI], $p = 0.111$, Table 2). In the univariable and multivariable regression model, only HOPE as the treatment group showed a significant ($p = 0.030$) and independent association with a markedly reduced risk of developing late-onset major complications with an OR of 0.153 (0.029–0.848 95% CI, $p = 0.030$, Table 2).

Further, a detailed breakdown of complications showed trends toward a higher incidence of infectious complications (7 [33%] HOPE vs. 11 [55%] SCS, $p = 0.215$, Table 1) or recirrhosis/graft failure (1 [5%] HOPE vs. 5 [25%] SCS, $p = 0.093$, Table 1) after SCS.

In the complete cohort, 36 (78%) patients were readmitted at least once during the follow-up period without any significant difference between groups (16 [70%] HOPE vs. 20 [87%] SCS, $p = 0.491$, Table 1).

The rate of biliary complications registered during the whole follow-up period, incidence of biliary stenosis ($p = 0.751$), and requirement of biliary interventions ($p = 0.538$) did not differ in patients receiving HOPE-treated and HOPE-untreated allografts (Table 2). Of note, biochemical parameters, including surrogate markers of hepatocellular and biliary injury as well as retention parameters did not show any differences between the trial arms (Table 1).

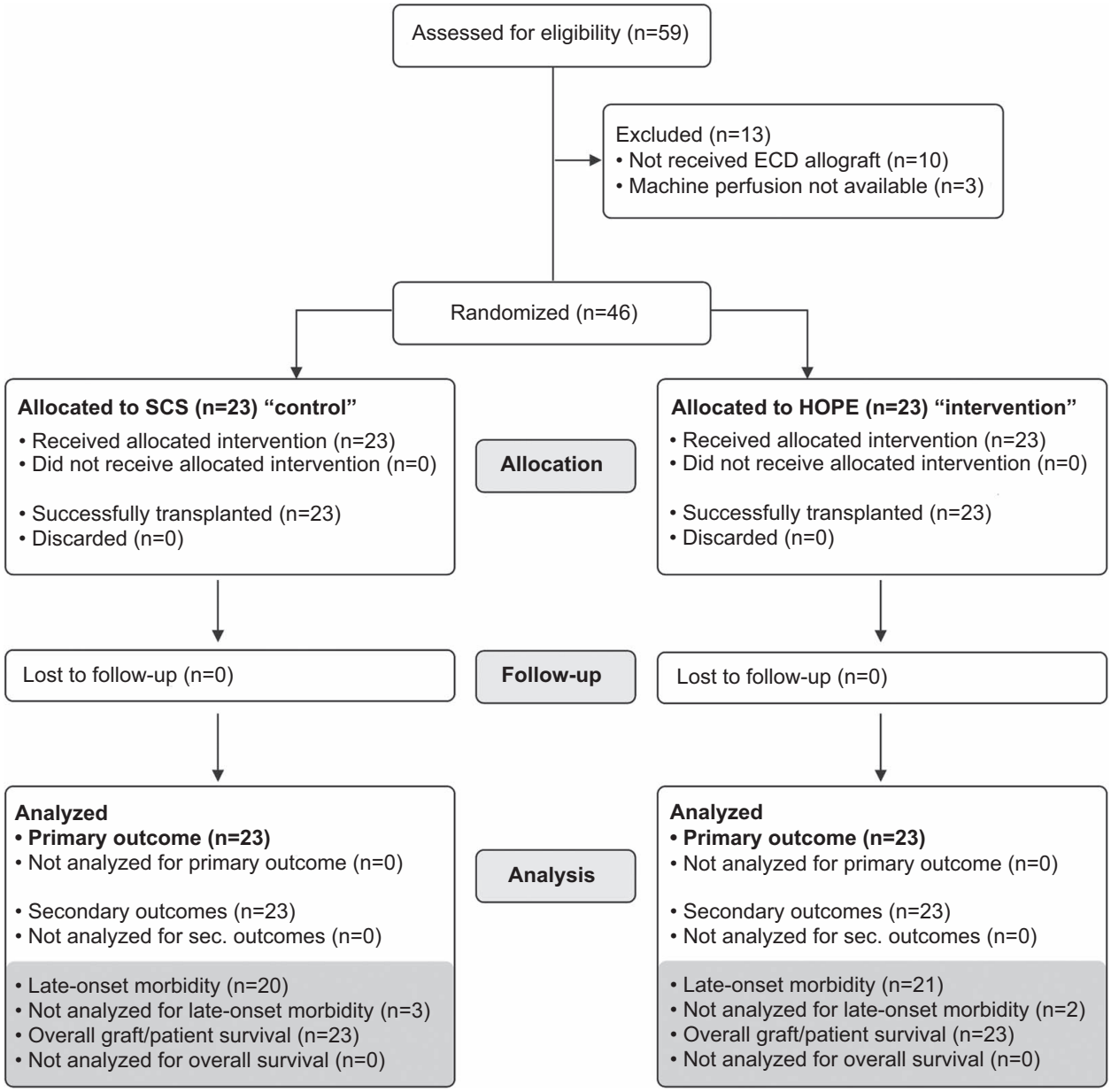

FIGURE 1 Consolidated Standards of Reporting trial flowchart, including the analysis of long-term outcomes. Abbreviations: ECD, extended criteria donation; HOPE, hypothermic oxygenated machine perfusion; SCS, static cold storage.

Impact of HOPE on overall graft survival and patient survival

A total of 13 patients had primary graft loss during the whole follow-up. Causes of primary graft loss included death (HOPE n = 1 vs. SCS n = 7), primary nonfunction (HOPE n = 1 vs. SCS n = 1), and chronic graft failure (HOPE n = 1 vs. SCS n = 2).

Eleven patients died within the whole follow-up period. Causes of death included HCC recurrence (HOPE n = 0 vs. SCS n = 3), graft failure (HOPE n = 2 vs. SCS n = 2), cardiac or septic complications (HOPE n = 1 vs. SCS n = 1), and domestic death events (HOPE n = 0 vs. SCS n = 2). Table 1 details further characteristics of late graft loss and death, while earlier events (< 6 months) have been described in the initial study report.[13]

Accordingly, the Kaplan-Meier estimate of overall patient survival for the whole follow-up period did not differ relevantly between the HOPE and SCS groups (log-rank $p = 0.107$, Figure 3A). The Kaplan-Meier estimate of overall graft survival for the complete cohort was lower with worse 1-, 3-, and 5-year graft survival probabilities in the SCS group (HOPE 0.913, 0.869, 0.869 vs. SCS 0.783, 0.606, 0.519, overall graft survival, respectively; log-rank $p = 0.029$, Figure 3B).

After excluding recipients who died during the initial hospitalization following LT (n = 4) and censoring for death

TABLE 1 Long-term biochemical outcomes and late-onset morbidity

Outcome	All patients (n = 41)	SCS (n = 20)	HOPE (n = 21)	p[a]
Biochemical findings and surrogate parameters at 24 mo				
ALT/median IU/L [IQR]	21 [15–32]	15 [12–31]	22 [17–38]	0.197
AST/median IU/L [IQR]	25 [19–34]	23 [16–42]	30 [20–35]	0.327
Total bilirubin/median mg/dL [IQR]	0.52 [0.41–1.05]	0.90 [0.41–1.88]	0.50 [0.41–0.71]	0.423
Creatinine/median mg/dL [IQR]	1.23 [1.04–1.75]	1.23 [1.13–2.08]	1.23 [0.99–1.30]	0.379
eGFR/median mL/min/1.73 m^2 [IQR][b]	62 [38–71]	59 [36–65]	63 [53–78]	0.178
Late-onset morbidity and other outcomes[c]				
Late-onset complications/n (%)[d]				
No complications	8 (20)	2 (10)	6 (29)	0.238
Minor complications (CD 1–2)	7 (17)	1 (5)	6 (29)	*0.093*
CD 3–4	20 (49)	12 (60)	8 (38)	0.217
CD 5	6 (15)	5 (25)	1 (5)	0.093
Major complications (CD ≥ 3)	26 (63)	17 (85)	9 (43)	0.009
Late-onset cumulative CCI/median [IQR][e]	34 [21–52]	46 [34–95]	23 [0–37]	0.003
Late-onset complication type, n (%)				
Biliary stenosis	6 (15)	3 (15)	3 (14)	> 0.999
Biliary other	4 (10)	3 (15)	1 (5)	0.343
Hepatic vascular	3 (7)	1 (5)	2 (10)	> 0.999
Rejection	2 (5)	0 (0)	2 (10)	0.488
Infectious	18 (44)	11 (55)	7 (33)	0.215
Recirrhosis/graft failure[f]	6 (15)	5 (25)	1 (5)	0.093
Recurrence of original disease	5 (12)	4 (20)	1 (5)	0.184
Retransplantation	2 (5)	2 (10)	0 (0)	0.232
Pulmonary	5 (12)	3 (15)	2 (10)	0.663
Cardiovascular	5 (12)	2 (10)	3 (14)	> 0.999
Gastrointestinal	8 (20)	5 (25)	3 (14)	0.454
Malignancy	4 (10)	2 (10)	2 (10)	> 0.999
Other	15 (37)	9 (45)	6 (29)	0.341
Overall biliary complications/n (%)[g]				
Overall biliary stenosis	15 (33)	8 (35)	7 (30)	0.751
Overall biliary other	10 (22)	5 (22)	5 (22)	> 0.999
Overall biliary interventions	20 (43)	11 (48)	9 (39)	0.538
Readmissions/n ()[g]	36 (78)	20 (87)	16 (70)	0.491
Follow-up time /median months (95% CI)	48 (41–55)	48 (35–61)	47 (38–56)	0.674
Death later than 6 mo due to graft failure[h]	2 (5)	1 (5)	1 (5)	NA
Death later than 6 mo with functional grafts[i]	4 (10)	4 (20)	0 (0)	NA
Graft loss later than 6 mo without death[j]	1 (2)	1 (5)	0 (0)	NA

Note: Data presented as median and interquartile range [IQR], absolute and relative frequencies/n (%), median and 95% CI.
Earlier death and graft loss events have been already reported in our initial study report (Czigany et al[13]).
[a]Statistical analyses are exploratory and *p*-values are descriptive (see also "Data collection and statistical methods").
[b]Based on CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation.[18]
[c]Late-onset morbidity refers to complications registered later than 6 months following liver transplantation as a new diagnosis requiring a particular treatment. Recurrent complications, which have been treated earlier during the clinical course but reoccurred or required long-term treatment, were not counted here.
[d]Based on Clavien et al.[19]
[e]Based on Slankamenac et al.[20]
[f]Refers to biopsy-proven significant refibrosis/cirrhosis of the allograft or clinically manifest graft failure (please also see Supplemental Table S2, http://links.lww.com/HC9/A791 for more information).
[g]Reported for the whole study cohort (n = 46/23/23) and for the complete follow-up period.
[h]Group SCS: n = 1 complex and severe biliary complications presenting as cholangiosepsis, stenosis at multiple levels, and biliary casts building leading to graft failure and retransplantation, no recovery and progressing critical illness, failure of the second graft with multiorgan failure and death after 13 months. Group HOPE: n = 1 young recipient with unclear acute on chronic graft failure, suspected association with recurrent alcohol abuse with multiorgan failure, and death 18 months after LT.
[i]Group SCS: n = 3 HCC recurrence; n = 1 domestic death; Group HOPE: n = 0.
[j]Group SCS: n = 1 retransplantation, due to therapy-resistant nonanastomotic biliary stenosis with cholangiosepsis and progressing graft failure.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CCI, comprehensive complication index; CD, Clavien-Dindo; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; HOPE, hypothermic oxygenated machine perfusion; ICU, intensive care unit; NA, not applicable; SCS, static cold storage.

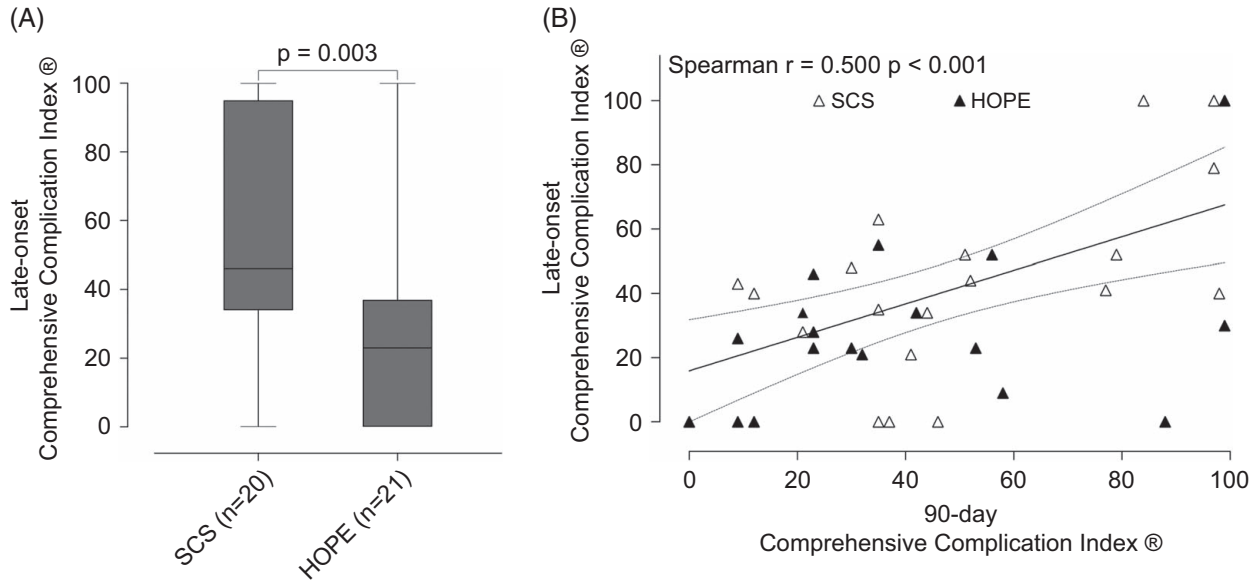

FIGURE 2 Cumulative Comprehensive Complication Index for late-onset complications observed later than 6 months after liver transplantation (A), Data demonstrating a moderate correlation between early and late-onset complications (B). Data are presented as median and IQR for the box plots, min-max for the whiskers (A) or single values (B). Mann-Whitney *U* *p*-values for group comparisons (A). Spearman correlation coefficient (B). Abbreviations: HOPE, hypothermic oxygenated machine perfusion; SCS, static cold storage.

related to tumor recurrence (n = 3) in a supplementary analysis, the difference in graft survival yielded a marginally nonsignificant result (log-rank $p = 0.060$); however, with a numerically worse outcome for patients who received SCS-treated allografts (HOPE 1.000, 0.952, 0.952 vs. SCS 0.855, 0.801, 0.687, 1-, 3-, and 5-year overall graft survival probabilities, respectively, Supplemental Figure S2, http://links.lww.com/HC9/A790).

DISCUSSION

This is the first long-term follow-up study from a randomized controlled trial using *ex vivo* MP technology in human LT. The present analysis demonstrates that the beneficial effects of HOPE are not limited to its early mitigating effects on allograft injury and perioperative morbidity but are also associated with a sustained reduction of late-onset postoperative complications and improved graft survival.[13]

We demonstrated in our initial report of the HOPE-ECD-DBD trial that HOPE significantly reduces early allograft injury and improves posttransplant outcomes.[13] This is in line with 3 other clinical trials and cohort studies in DCD and DBD LT, showing the favorable early effects of HOPE.[1,8–10,21,23–28] The importance of *ex vivo* MP is further highlighted by the recent International Liver Transplantation Society enhanced recovery for liver transplantation guidelines, recommending HOPE in the setting of DCD and ECD LT.[22]

The only long-term study reporting outcomes after HOPE in human LT is a retrospective analysis by Schlegel et al,[1] comparing HOPE-treated DCD-allografts with a matched cohort of untreated DCD-allografts from the Birmingham group. This retrospective cohort study could not demonstrate a difference in 1-year CCI ($p = 0.898$) in DCD, and a recent multicenter HOPE trial by the same group also failed to show an effect on 1-year CCI and on major morbidity (CD ≥ 3).[8] A *post hoc* analysis, however, within the same trial revealed that liver-related CD ≥ 3b complications (risk ratio 0.26, $p = 0.027$), as well as subsequent associated graft failure, occurred less frequently after HOPE when compared to SCS (7% vs. 0%). It must be noted that the eligibility criteria in the abovementioned Zurich trial were not restricted to a specific allograft risk profile or ECD criteria but were open to the randomization of any non-ECD DBD donor allograft.[8]

To assess the long-term effects of HOPE, the present analysis was focusing solely on late-onset complications occurring later than 6 months after LT, demonstrating a significant reduction of late-onset major morbidity (CD ≥ 3) as well as cumulative postoperative complications (CCI) in HOPE-treated ECD allografts. Notably, we could not show a significant correlation on a cohort level between patients who developed severe complications in the early phase and late phase. As such, the development of late-onset complications in our HOPE-ECD-DBD trial population cannot be explained solely as a direct and associated consequence of early postoperative morbidity.[13] This is further supported by a logistic regression analysis of baseline characteristics and perioperative factors, in which HOPE was the only parameter showing a significant association with a reduction in late-onset major morbidity.

TABLE 2 Comparison of baseline characteristics and perioperative data for those individuals who developed late-onset major morbidity (Clavien-Dindo ≥ 3 later than 6 mo) versus those who did not

	Late-onset complications		Univariable analysis		Multivariable analysis	
Characteristics	**Major (CD ≥ 3)[a] n = 26**	**No/minor (CD1-2)[a] n = 15**	**OR (95% CI)/*p*[b]**			
Donor age /median years [IQR]/	72 [65–79]	69 [59–78]	1.015 (0.965–1.068)	0.566	—	—
Donor BMI /median kg/m^2 [IQR]/	28 [24–30]	29 [27–33]	0.928 (0.825–1.044)	0.213	—	—
Donor sex female /n (%)/	11 (42)	6 (40)	1.179 (0.321–4.326)	0.804	—	—
ET-DRI Score[c]	2.060 [1.935–2.195]	1.960 [1.870–2.370]	1.218 (0.130–11.377)	0.863	—	—
Recipient age /median years [IQR]/	60 [55–66]	62 [55–64]	0.994 (0.922–1.072)	0.880	—	—
Recipient BMI /median kg/m^2 [IQR]	28 [25–31]	28 [27–31]	0.945 (0.815–1.097)	0.459	—	—
Recipient sex female/n (%)	5 (19)	2 (13)	1.625 (0.273–9.658)	0.593	—	—
labMELD /median [IQR]	17 [9–25]	12 [8–17]	1.056 (0.970–1.149)	0.207	—	—
BAR Score /median [IQR][d]	7 [4–11]	5 [3–8]	1.116 (0.944–1.319)	0.198	—	—
Total cold preservation time/median min [IQR]	495 [471–575]	480 [408–523]	1.005 (0.998–1.011)	0.182	—	—
Warm ischemic time/median min [IQR]	45 [38–52]	39 [32–51]	1.033 (0.972–1.099)	0.291	—	—
Duration of surgery/median min [IQR]	420 [350–478]	345 [166–460]	1.005 (0.999–1.011)	0.088	1.006 (0.999–1.013)	0.097
Intraoperative RBC/median units [IQR]	4 [2–7]	4 [2–7]	1.041 (0.891–1.215)	0.615	—	—
Intraoperative FFP/median units [IQR]	16 [10–25]	18 [0–25]	1.015 (0.954–1.079)	0.643	—	—
Early (90-d) major complications yes /n (%)	15 (58)	7 (47)	1.558 (0.434–5.596)	0.496	—	—
Length of initial ICU stay /median days [IQR]	7 [4–14]	5 [3–11]	1.057 (0.958–1.167)	0.270	—	—
Length of initial hospital stay /median days [IQR]	25 [18–44]	20 [15–27]	1.050 (0.989–1.116)	0.111	1.030 (0.963–1.101)	0.388
Treatment group HOPE /n (%)	9 (35)	12 (80)	0.141 (0.031–0.634)	0.011	0.153 (0.029–0.848)	0.030

Note: Data presented as median and interquartile range [IQR] or absolute and relative frequencies/n (%).

[a]Based on Clavien et al.

[b]*p*-values ≤ 0.1 in the univariable setting were included in the multivariable binary logistic regression analysis. Statistical analyses are exploratory, and *p*-values are descriptive (see also "Data collection and statistical methods").

[c]Based on Braat et al.[21]

[d]Based on Dutkowski et al.[22]

Abbreviations: BAR, balance of risk; BMI, body mass index; CD, Clavien-Dindo; ET-DRI, Eurotransplant donor risk index; FFP, fresh frozen plasma; HOPE, hypothermic oxygenated machine perfusion; MELD, Model for End-stage Liver Disease; RBC, red blood cells.

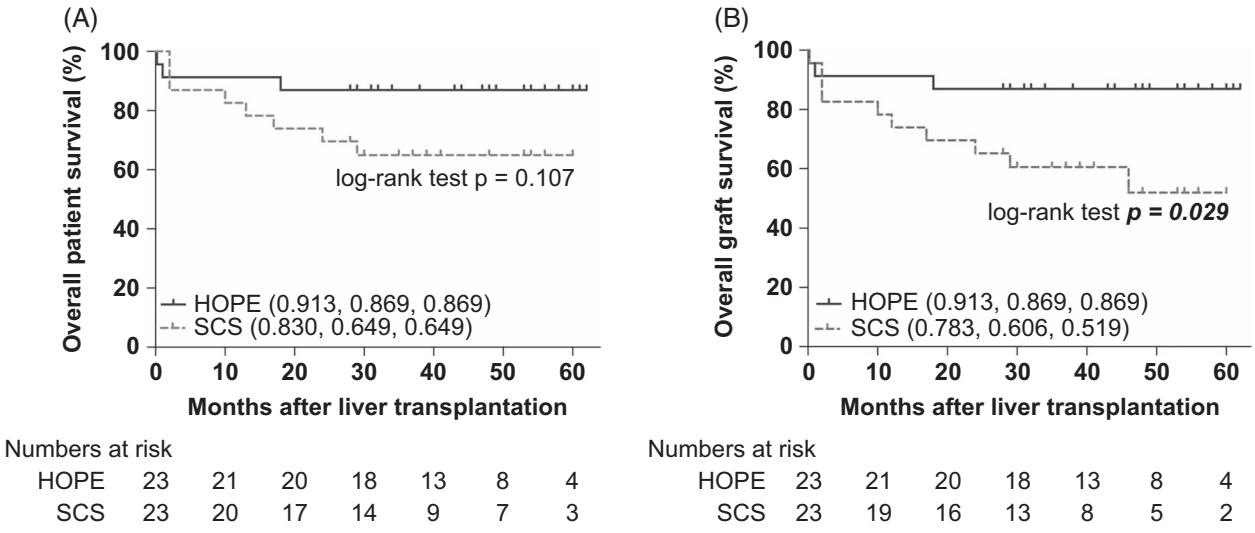

FIGURE 3 Kaplan-Meier curves of overall graft survival and patient survival including all trial patients (A, B). One-, 3-, and 5-year survival probabilities are reported in brackets, respectively. Abbreviations: HOPE, hypothermic oxygenated machine perfusion; SCS, static cold storage.

While the pivotal D-HOPE (dual-HOPE) trial by the Groningen group showed a significant reduction of non-anastomotic biliary structures after 6 months in DCD (6% D-HOPE vs. 18% SCS, $p = 0.03$),[9,29] and a matched cohort of DCD donor allografts by Schlegel et al[1] revealed similar findings to the D-HOPE trial, a detailed breakdown of complications in our HOPE-ECD-DBD trial could not show significant differences in any of the complication subcategories, including biliary complications. This is in line with other prospective ECD-DBD cohorts, such as the Bologna and Zurich trials,[8,10] but also with a retrospective cohort study by the Turin group that did not find a significant reduction of biliary complications using HOPE and D-HOPE, respectively.[23]

Even though improved short-term outcomes of DCD and ECD-DBD allografts have been reported previously in 4 RCTs,[8,9,13] long-term outcome data from an RCT supporting the use of HOPE in human LT are still lacking. With a median follow-up of 48 months, another important finding of the present analysis is superior long-term graft survival for ECD allografts undergoing HOPE treatment.

Certain limitations to this secondary analysis from our multicenter RCT need to be considered. First, due to a relatively small cohort of only 46 recipients who were randomly assigned to each group, some analyses were carried out with a limited number of patients and events. Second, this study reports late-onset morbidity and long-term graft survival and patient survival, even though neither of these was the primary endpoint of the initial HOPE-ECD-DBD trial.[12,13] Therefore, these analyses are potentially underpowered, thus they need to be interpreted in an explorative fashion, and the reported *p*-values need to be considered descriptive. Third, the follow-up for the study population was not fully mature and some patients are yet to reach the 60-month follow-up. Therefore, we could not and also did not aim to report absolute 5-year survival rates. Survival data were reported as survival probabilities according to the Kaplan-Meier method.

Notwithstanding these limitations, this is the first study to report favorable effects of HOPE on long-term morbidity and graft survival in an RCT cohort of patients who underwent LT using ECD-DBD allografts. Furthermore, the lack of any lost to follow-up cases and the homogeneous prospective RCT cohort suggest high data integrity and support the strength of our findings.

While HOPE is a simple, practical, and cost-efficient back-to-base dynamic *ex vivo* preservation technology, this trial provides first-time evidence that HOPE, in comparison to SCS, significantly improves long-term post-transplant outcomes in ECD LT. Further well-designed, multimodal, high-volume MP trials with clinically more relevant nonsurrogate primary endpoints, such as our HOPE-normothermic machine perfusion trial (NCT04644744), will provide further high-level evidence for the broad implementation of MP on a global scale.

DATA AVAILABILITY STATEMENT

All data are available in the manuscript and the supplementary files. Further data would be made available upon reasonable request and addressed to the corresponding author.

AUTHOR CONTRIBUTIONS

The study was designed by the initiating study team of Zoltan Czigany, Georg Lurje, and Ulf P. Neumann. Zoltan Czigany, Deniz Uluk, Sandra Pavicevic, Isabella Lurje, Theresa Keller, Jiří Froněk, Pavel Strnad, Rene H. Tolba, Decan Jiang, Tom Gevers, Dionysios

Koliogiannis, Markus Guba, Franziska A. Meister, Ulf P. Neumann, Matej Kocik, Marek Kysela, Frank Tacke, Johann Pratschke, and Georg Lurje: data collection, interpretation, and analysis. Zoltan Czigany and Georg Lurje: drafted the manuscript. Deniz Uluk, Sandra Pavicevic, Isabella Lurje, Theresa Keller, Jiří Froněk, Pavel Strnad, Rene H. Tolba, Decan Jiang, Tom Gevers, Dionysios Koliogiannis, Markus Guba, Franziska A. Meister, Ulf P. Neumann, Matej Kocik, Marek Kysela, Igor M. Sauer, Nathanael Raschzok, Wenzel Schöning, Irinel Popescu, Frank Tacke, Johann Pratschke: contributed to the final version of the manuscript. All authors have read and approved the final version of the manuscript.

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CONFLICTS OF INTEREST

Zoltan Czigany is on the speakers' bureau and received grants from Xvivo and received grants from Astellas. Tom Gevers received grants from Gilead and AbbVie. Rene Tolba is employed and owns stock in Vivalyx and owns stock in Protembis. Ulf Neumann is on the speakers' bureau for Dr. Falk, Merck, and AstraZeneca and received grants from Chiesi. Frank Tacke consults, advises, is on the speakers' bureau, and received grants from Gilead. He consults, advises, and is on the speakers' bureau for AbbVie. He consults, advises, and received grants from Allergan, Bristol-Myers Squibb, and Inventiva. He consults and advises AstraZeneca, Alnylam, Intercept, Pfizer, Novartis, Novo Nordisk, and Sanofi. He is on the speakers' bureau for Falk, Merz, and Orphalan. Georg Lurje reports receiving travel support and speakers' fees from Astellas Pharma, XVIVO, Bridge to Life, and Aferetica S.R.L outside the submitted work. Zoltan Czigany reports receiving travel support from Astellas Pharma, outside the submitted work. The remaining authors have no conflicts to report.

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