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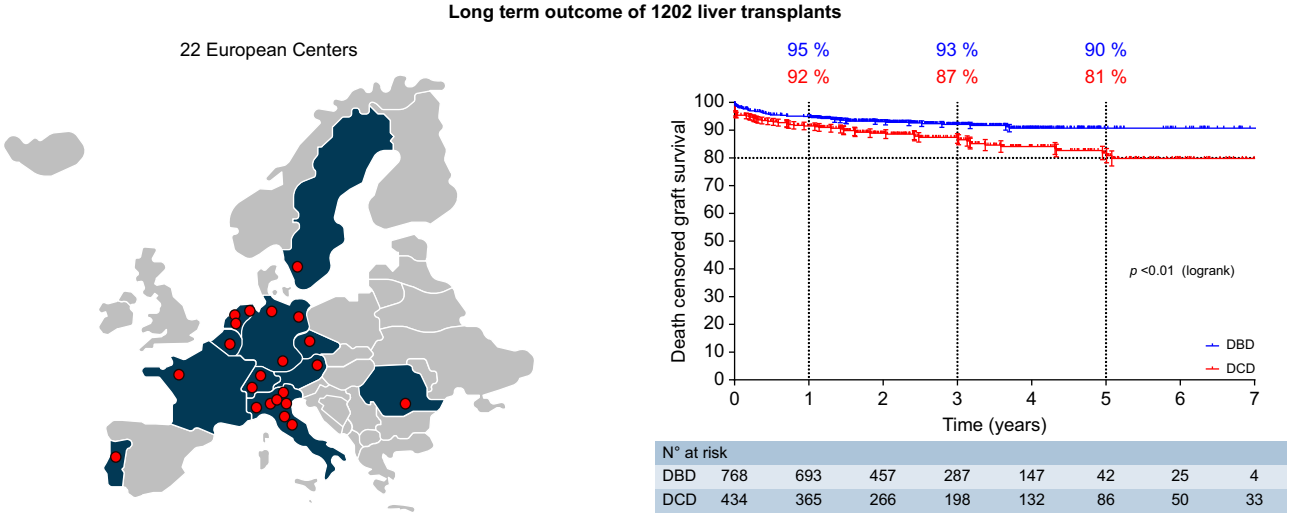
Authors

Janina Eden, Isabel M.A. Brüggenwirth, Gabriela Berlakovich, …, Robert J. Porte, Philipp Dutkowski, Vincent E. de Meijer

Correspondence

v.e.de.meijer@umcg.nl (V.E. de Meijer).

Graphical abstract



Highlights

- We report excellent 5-year survival after transplantation of 1,202 HOPE-treated DBD and DCD livers in 22 European centres.
- HOPE-treatment has now reached IDEAL-D stage 4.
- These findings support the implementation of HOPE in routine clinical practice.

Impact and implications

This study demonstrates the excellent long-term performance of hypothermic oxygenated machine perfusion (HOPE) treatment of donation after circulatory and donation after brain death liver grafts irrespective of their individual risk profile in a real-world setting, outside the evaluation of randomised-controlled trials. While previous studies have established safety, feasibility, and efficacy against the current standard, according to the IDEAL-D evaluation framework, HOPE treatment has now reached the final IDEAL-D stage 4, which further supports its implementation in routine clinical practice.

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Long-term outcomes after hypothermic oxygenated machine perfusion and transplantation of 1,202 donor livers in a real-world setting (HOPE-REAL study)

Janina Eden[1,2], Isabel M.A. Brüggenwirth[1,2], Gabriela Berlakovich[3], Bettina M. Buchholz[4], Florin Botea[5], Stefania Camagni[6], Matteo Cescon[7], Umberto Cillo[8], Fabio Colli[9], Philippe Compagnon[10], Luciano G. De Carlis[11], Riccardo De Carlis[11], Fabrizio Di Benedetto[12], Jule Dingfelder[3], Dulce Diogo[13], Daniele Dondossola[14], Moritz Drefs[15], Jiri Fronek[16], Giuliana Germinario[7], Enrico Gringeri[8], Georg Györi[3], Matej Kocik[16], Efrayim H. Küçükerbil[17], Dionysios Koliogiannis[15], Hwai-Ding Lam[18], Georg Lurje[19], Paolo Magistri[12], Diethard Monbaliu[20], Mostafa el Moumni[21], Damiano Patrono[9], Wojciech G. Polak[17], Matteo Ravaioli[7], Michel Rayar[22], Renato Romagnoli[9], Gustaf Sörensen[23], Deniz Uluk[19], Andrea Schlegel[14,24,#], Robert J. Porte[1,17,#], Philipp Dutkowski[25,#], Vincent E. de Meijer[1,2,*,#]

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Background & Aims: Despite strong evidence for improved preservation of donor livers by machine perfusion, longer post-transplant follow-up data are urgently needed in an unselected patient population. We aimed to assess long-term outcomes after transplantation of hypothermic oxygenated machine perfusion (HOPE)-treated donor livers based on real-world data (*i.e.*, IDEAL-D stage 4).

Methods: In this international, multicentre, observational cohort study, we collected data from adult recipients of HOPE-treated livers transplanted between January 2012 and December 2021. Analyses were stratified by donation after brain death (DBD) and donation after circulatory death (DCD), sub-divided by their respective risk categories. The primary outcome was death-censored graft survival. Secondary outcomes included the incidence of primary non-function (PNF) and ischaemic cholangiopathy (IC).

Results: We report on 1,202 liver transplantations (64% DBD) performed at 22 European centres. For DBD, a total number of 99 benchmark (8%), 176 standard (15%), and 493 extended-criteria (41%) cases were included. For DCD, 117 transplants were classified as low risk (10%), 186 as high risk (16%), and 131 as futile (11%), with significant risk profile variations among centres. Actuarial 1-, 3-, and 5-year death-censored graft survival rates for DBD and DCD livers were 95%, 92%, and 91%, *vs.* 92%, 87%, and 81%, respectively (log-rank $p = 0.003$). Within DBD and DCD strata, death-censored graft survival was similar among risk groups (log-rank $p = 0.26$, $p = 0.99$). Graft loss due to PNF or IC was 2.3% and 0.4% (DBD), and 5% and 4.1% (DCD).

Conclusions: This study shows excellent 5-year survival after transplantation of HOPE-treated DBD and DCD livers with low rates of graft loss due to PNF or IC, irrespective of their individual risk profile. HOPE treatment has now reached IDEAL-D stage 4, which further supports its implementation in routine clinical practice.

Trial registration: ClinicalTrials.gov Identifier: NCT05520320.

Introduction

While the field of liver transplantation has witnessed remarkable advances over the past 60 years, preservation methods have only recently experienced significant improvement.[1] Given the escalating risks associated with an increasing proportion of lower quality donor livers, traditional static cold storage preservation may not be sufficient. The recent introduction of dynamic preservation by oxygenated machine perfusion has revolutionised the field, marking a substantial breakthrough in liver transplantation. Accordingly, machine perfusion of donor livers evolved in the last 10 years from a pure experimental approach to clinical implementation in an increasing number of transplant centres worldwide. This evolution has been driven by extensive mechanistic research,[2–4] followed by initial case series, cohort studies, and several randomised-controlled trials (RCT) in the last 5 years, reporting safety and efficacy of liver machine perfusion compared to cold storage.[5] While most of these RCTs show benefits for machine liver perfusion, their primary endpoints were mainly comprised of first week lab

* Corresponding author. Address: Department of Surgery, Section of HPB Surgery and Liver Transplantation, University of Groningen and University Medical Center Groningen, Groningen, the Netherlands.
E-mail address: v.e.de.meijer@umcg.nl (V.E. de Meijer).
Shared senior authorship
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values, and the longest reported outcome is limited to 1 year.[5] Despite level one evidence (from RCTs) for improved preservation of donor livers by machine perfusion, outcome data with longer post-transplant follow-up are urgently needed in an unselected patient population.

Introduction of liver machine perfusion technology into clinical practice should follow the IDEAL-D concept, *i.e.* Idea, Development, Exploration, Assessment, Long-term (IDEAL) study framework for devices.[6,7] According to this framework, IDEAL-D stages 1 and 2 focus on safety and feasibility to allow for the design of a potential RCT. In stage 3, assessment takes place during large-scale, multicentre RCTs to compare the efficacy of a new device or technique against the current standard. Currently, in the field of liver transplantation, machine perfusion has reached IDEAL-D stage 3. The logical next step refers to monitoring long-term outcomes to establish effectiveness of liver machine perfusion in an unbiased, real-world setting outside strictly controlled RCTs, *i.e.* IDEAL-D stage 4. Such data is highly anticipated because liver machine perfusion is performed with many different parameters among centres worldwide, including different perfusion temperatures, perfusate compositions, perfusion routes, combined protocols, or the use of different devices.[8]

Herein, we aimed, for the first time, to focus on long-term outcomes after transplantation of livers preserved by hypothermic oxygenated machine perfusion (HOPE) one of the two dominantly performed *ex situ* liver machine perfusion procedures, alongside normothermic machine perfusion (NMP). Our aim was to assess 5-year survival rates after transplantation of donor livers preserved by HOPE based on real-world data, in various patient populations, with different device types, and with inherent variations in perioperative and post-operative transplant care. For this purpose, we conducted a large, multicentre, observational cohort study including expert European liver transplant centres with a well-recorded experience in HOPE. Our results demonstrate excellent 5-year survival after transplantation of HOPE-treated livers, irrespective of their individual risk profile. HOPE treatment has now reached IDEAL-D stage 4, which further supports its implementation in routine clinical practice.

Patients and methods

In this multicentre, international, observational cohort study we collected data from adult recipients (age >18 years) of HOPE-preserved livers transplanted at 22 European liver transplant centres between January 1, 2012 and December 31, 2021 (HOPE-REAL study). HOPE can be performed as single HOPE (*i.e.* via portal vein only) or as dual HOPE (*i.e.* via portal vein and hepatic artery [DHOPE]). Centres were eligible for inclusion according to their experience in the HOPE technique, defined as performance of at least 20 HOPE procedures. All consecutive cases of HOPE performed at the participating centres were included in this study. Data were collected until December 31, 2022 to guarantee a minimum follow-up time of 12 months for each patient. For validation of data and to minimise missing values we conducted three rounds of corrections addressing each centre. We excluded simultaneous multiorgan transplantations, sequential protocols such as HOPE followed by NMP, and living partial liver donation. Donor livers which underwent HOPE after *in situ* normothermic regional perfusion (NRP) were included in the analysis.

The HOPE-REAL study was registered prior to initiation and data collection (ClinicalTrials.gov Identifier: NCT05520320). The Medical Research Ethics Committee of the University Medical Center Groningen reviewed the study and waived the need for informed consent. The study was conducted according to the declaration of Helsinki and according to STROBE guidelines.[9]

Donor variables included age, biological sex (female, male), donor height, donor BMI, donor cause of death, graft type (DBD or DCD), total warm ischaemia time (time interval between donor withdrawal of life support and cold flush), functional warm ischaemia time (time interval between blood pressure <50 mmHg and start of cold *in situ* flush), and asystolic warm ischaemia time (time interval between cardiac arrest and cold *in situ* flush). Based on these parameters, we calculated the donor risk index (DRI)[10] and the UK DCD risk score, consisting of seven donor, graft and recipient parameters, such as donor age, donor BMI, donor functional warm ischaemia time, cold ischaemia time (CIT), recipient age, recipient model for end-stage liver disease (MELD) score and previous liver transplantation.[11]

Preservation parameters consisted of CIT (time interval between cold *in situ* aortic flush and start of cold perfusion treatment), perfusion time, perfusion duration, perfusion flow, perfusion type (HOPE, DHOPE, or NRP-HOPE), and the device type (VitaSmart, Bridge2Life, Wandsworth, England; LiverAssist, XVIVO, Groningen, the Netherlands; other devices).

Recipient parameters included age and biological sex (female, male), laboratory MELD score, the balance of risk (BAR) score,[12] primary transplants or retransplants, the presence of hepatocellular carcinoma (HCC), tumour size, tumour number, and alpha fetoprotein.

The primary study outcome was defined as death-censored graft survival. Secondary outcomes included overall graft survival, overall patient survival, incidence of primary graft non-function (PNF), incidence of biliary complications, incidence of vascular complications, incidence of acute cellular rejection, incidence of chronic rejection, incidence of re-transplantation, incidence of recurrence of the primary disease (including recurrence of malignancies), and incidence of new-onset chronic kidney disease. Biliary complications included non-anastomotic strictures (NAS; defined as any irregularity or narrowing of the lumen of the intrahepatic or extrahepatic donor bile ducts, excluding the biliary anastomosis, diagnosed with the use of cholangiography in combination with clinical symptoms such as jaundice or cholangitis or an elevation of cholestatic laboratory variables, in the presence of a patent hepatic artery), anastomotic strictures (defined as strictures occurring at the anastomosis of donor choledochal duct and recipient choledochal duct or jejunal Roux-limb), and biliary leakage (defined as fluid with an elevated (>3x serum) bilirubin level in the abdominal drain or intra-abdominal fluid on or after post-operative day 3 or the need for radiological intervention owing to biliary collections or re-laparotomy due to biliary peritonitis). Further outcome parameters included laboratory values for the first 10 days (aminotransferases, bilirubin, international normalised ratio) as well as the duration of intensive care unit stay, and length of hospital stay.

Analyses were stratified for DBD and DCD liver grafts. DBD grafts were classified as either benchmark (defined as primary transplant with laboratory MELD score <20 and BAR score <9)[12], standard, or extended criteria (defined as a minimum of one of the following parameters: donor BMI >30 kg/m^2, donor age >65 years, CIT >12 h).[13] DCD livers were classified

according to the UK DCD risk score as low risk (0-5 points), high risk (6-10 points), or futile (>10 points).[11]

The mean ± SD follow-up was 2.9 ± 1.8 years for all patients. Time-to-event data analysis was performed by Kaplan-Meier survival analysis with log-rank testing, and by Cox proportional hazards regression. The proportional hazards assumption was verified. Missing values were not subjected to imputation. Univariate and multivariate regression analysis for death-censored graft survival and patient survival was performed. Continuous data are presented as mean ± SD when normally distributed, or as median (IQR). Statistical analysis was performed using IBM SPSS Statistics version 27 and GraphPad Prism 2022, Version 9.4.1.

Results

We included a total of 1,202 liver transplant recipients from 22 European centres (Fig. 1A). Of these, 768 received a DBD liver graft, while 434 received a DCD liver (Fig. 1B). HOPE treatment for DCD livers commenced in 2012, showing a gradual increase over 10 years, whereas HOPE treatment for DBD livers began in 2016, demonstrating a faster pace of introduction into clinical practice (Fig. 1B). Baseline characteristics are presented in Table 1. For DBD donors, median age was 67 years (IQR 53-76 years), with a DRI of 1.96 (IQR 1.69-2.19) (Table 1). Among DBD donors, 99 were classified as benchmark (13%), 176 as standard (23%), and 493 as extended criteria (64%) (Fig. 1C). For DCD donors, median age was 57 years (IQR 47-64 years), with a DRI of 2.37 (IQR 2.01-2.74) (Table 1). According to the UK DCD risk score, 117 DCD donors were classified as low risk (27%), 186 as high risk (43%), and 131 as futile (30%) (Fig. 1C). Notably, the risk profile varied widely among centres. Some centres predominantly included more DBD and low-risk DCD livers, while others included a substantial proportion of high-risk or even futile DCD livers (Fig. 1D). Differences in the baseline characteristics between the different risk subgroups are presented in Table 1.

For DBD livers, after a median CIT of 384 min (IQR 267-480 min), 381 underwent HOPE (50%) and 387 underwent DHOPE (50%) for a median perfusion time of 150 min (IQR 110-210 min) (Table 1). For DCD livers, after a median CIT of 330 min (IQR 251-408 min), 183 underwent HOPE (42%), 115 underwent DHOPE (26%), and 136 underwent NRP-HOPE (31%) for a median perfusion time of 134 min (IQR 104-195 min) (Table 1). The median recipient age was 59 years (IQR 53-65 years), with a median laboratory MELD score of 13 (IQR 9-19) and a median BAR score of 5 (IQR 3-8). The main indication for liver transplantation was HCC (50%). Tumour criteria generally fell within Milan criteria, with a median tumour size of 2 cm (IQR 1-3), a median tumour number of two, and a median alpha fetoprotein level of 6 ng/ml (Table 1).

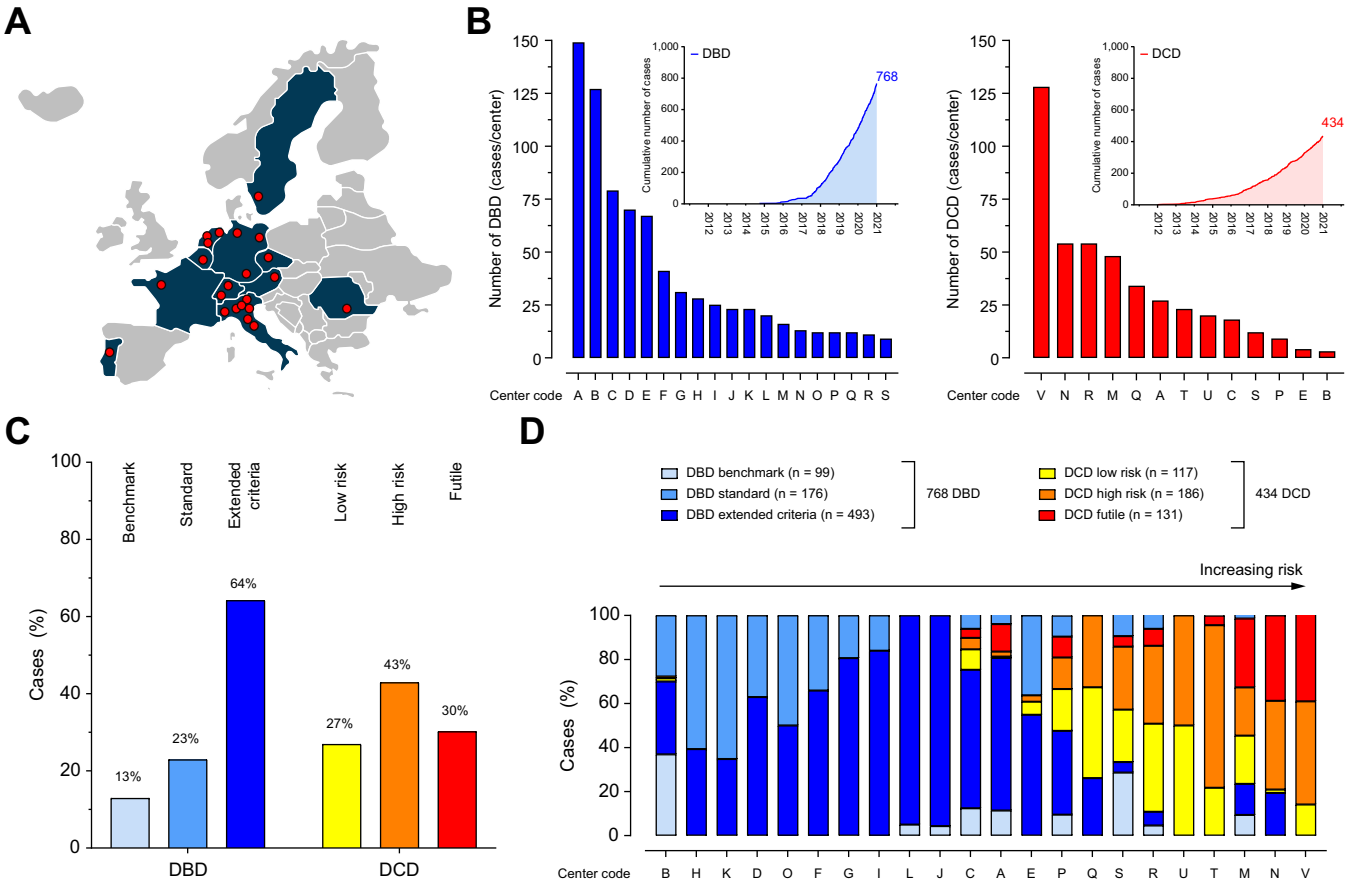


Fig. 1. Participating centres, recruitment rates, and graft risks. The participating centres are visualised on a map (A), with the recruitment of DBD and DCD livers over time, per centre (B). Liver transplants were stratified according to the graft risk (DBD), or according to the UK DCD risk score (C). Centres showed large differences in terms of the graft risk (D). DBD, donation after brain death; DCD, donation after circulatory death.

Table 1. Donor, preservation and recipient parameters.

	Total, N = 1,202	DBD, n = 768	Benchmark DBD, n = 99	Standard DBD, n = 176	Extended-criteria DBD, n = 493	DCD, n = 434	Low-risk DCD, n = 117	High-risk DCD, n = 186	Futile DCD, n = 131	Missing, n (%)
Donor parameters										
Age, years	61 (50-73)	67 (53-76)	53 (41-58)	52 (40-59)	74 (68-80)	57(47-64)	49 (38-55)	57 (48-63)	63 (56-71)	0
Sex male/female	709/493	429/339	62/36	115/61	251/242	280/154	78/39	122/64	80/51	0
Height, cm	172 (165-180)	170 (163-180)	175 (168-180)	175 (168-180)	170 (162-176)	175 (165-180)	175 (168-184)	174 (166-180)	175 (165-180)	19 (1.6)
BMI, kg/m^2	26 (24-29)	26 (24-28)	25 (23-26)	25 (23-26)	27 (24-31)	25 (24-28)	24 (22-26)	25 (23-28)	27 (25-30)	20 (1.7)
dWIT, min	32 (26-41)	—	—	—	—	32 (26-41)	30 (23-36)	31 (26-37)	38 (31-46)	38 (8.7)
fWIT, min	29 (22-38)	—	—	—	—	29 (22-38)	18 (14-22)	27 (22-34)	38 (33-48)	60 (13.8)
aWIT, min	18 (14-24)	—	—	—	—	18(14-24)	16 (13-20)	16 (13-21)	23 (19-27)	86 (19.8)
DRI (points)	2.12 (1,81-2.44)	1.96 (1.69-2.19)	1.67 (1.44-1.89)	1.64 (1.36-1.94)	2.12 (1.91-2.26)	2.37 (2.01-2.74)	2.13 (1.82-2.39)	2.35 (2.0-2.69)	2.65 (2.28-2.95)	232 (19.3)
UK DCD risk score (points)	7 (5-11)	—	—	—	—	7(5-11)	3 (2-5)	8 (6-9)	11 (10-12)	4 (0.9)
Preservation parameters										
Cold storage, min	360 (260-461)	384 (267-480)	360 (282-480)	330 (227-434)	408 (289-497)	330 (251-408)	334 (258-410)	313 (249-375)	345 (245-439)	39 (3.2)
HOPE type (n/%):										
HOPE	564 (46.9)	381 (49)	42 (42.4)	85 (48.3)	254 (51.5)	183 (42)	40 (34.2)	88 (47.3)	55 (42.0)	0 (0)
DHOPE	502 (41.8)	387 (51)	57 (57.6)	91 (51.7)	239 (48.5)	115 (26)	58 (49.6)	51 (27.4)	6 (4.6)	0 (0)
NRP-HOPE	136 (11.3)	—	0 (0)	0 (0)	0 (0)	136 (31)	19 (16.2)	47 (25.3)	70 (53.4)	0 (0)
HOPE device (n/%):										
Liver assist	994 (82.7)	620 (80)	84 (84.8)	157 (89.2)	379 (76.9)	374 (86)	104 (88.9)	157 (84.4)	113 (86.3)	0 (0)
Vitasmart	190 (15.8)	139 (18)	13 (13.1)	17 (9.7)	109 (22.1)	51 (12)	9 (7.7)	26 (14)	16 (12.2)	0 (0)
Other	18 (1.5)	9 (1)	2 (2.0)	2 (1.1)	5 (1.0)	9 (2)	4 (3.4)	3 (1.6)	2 (1.5)	0 (0)
HOPE duration, min	142 (106-202)	150 (110-210)	146 (113-213)	155 (106-235)	146 (110-200)	134 (104-195)	133 (97-196)	130 (107-188)	142 (98-201)	177 (14.7)
Perfusion flow, ml/min	235 (150-250)	235 (150-262)	235 (115-250)	228 (150-338)	240 (150-250)	230 (180-250)	235 (150-240)	223 (180-250)	218 (180-250)	
Recipient parameters										
Age, years	59 (53-65)	59 (52-65)	56 (47-62)	58 (49-63)	61 (55-66)	59 (54-65)	56 (50-60)	59 (54-66)	62 (57-67)	3 (0.2)
Sex, male/female	846/283		70/28	104/45	330/118		87/30	147/39	108/23	0
Lab MELD, points	13 (9-19)	14 (10-21)	13 (10-16)	18 (11-25)	14 (10-20)	12 (9-17)	13 (10-19)	11 (8-16)	12 (9-16)	16 (1.3)
Primary transplant	1,157/1,202	736/768				421/434				
BAR score, points	5 (3-8)	5 (3-9)	4 (2-7)	9 (5-12)	5 (3-9)	4 (3-7)	4 (2-8)	4 (3-7)	5 (4-6)	120 (9.9)
HCC, n (%)	599 (49.8)	350 (45)	45 (45.5)	56 (31.8)	249 (50.5)	249 (57)	53 (45.3)	111 (59.7)	85 (64.9)	0
Tumour size, cm	2.0 (1-3)	1.8 (1-2.7)	2.3 (1.6-3.6)	1.0 (1.0-2.0)	1.7 (1.0-2.6)	2.0 (1.2-3)	2.0 (1.4-3.0)	2.0 (1.3-3.0)	2.0 (1.0-3)	72 (12.0)*
Tumour number	2 (1-3)	2 (1-3)	2 (1-2)	2 (1-10)	1 (1-3)	2 (1-3)	2 (1-3)	2 (1-3)	2 (1-3)	66 (11.0)*
AFP, ng/m	6 (3-15)	5 (3-15)	7 (3-32)	7 (3-25)	5 (3-13)	6 (4-16)	7 (4-15)	6 (3-31)	6 (4-11)	108 (18.0)*

AFP, alpha fetoprotein; BAR, balance of risk; DBD, donation after brain death; DCD, donation after circulatory death; DRI, donor risk index; HCC, hepatocellular carcinoma; (D)HOPE, (dual) hypothermic oxygenated machine perfusion; MELD, model for end-stage liver disease; NRP, normothermic regional perfusion; (a,d,f)WIT, (asystolic, donor, functional) warm ischaemia time.

*Percentage missing refers to HCC population only.

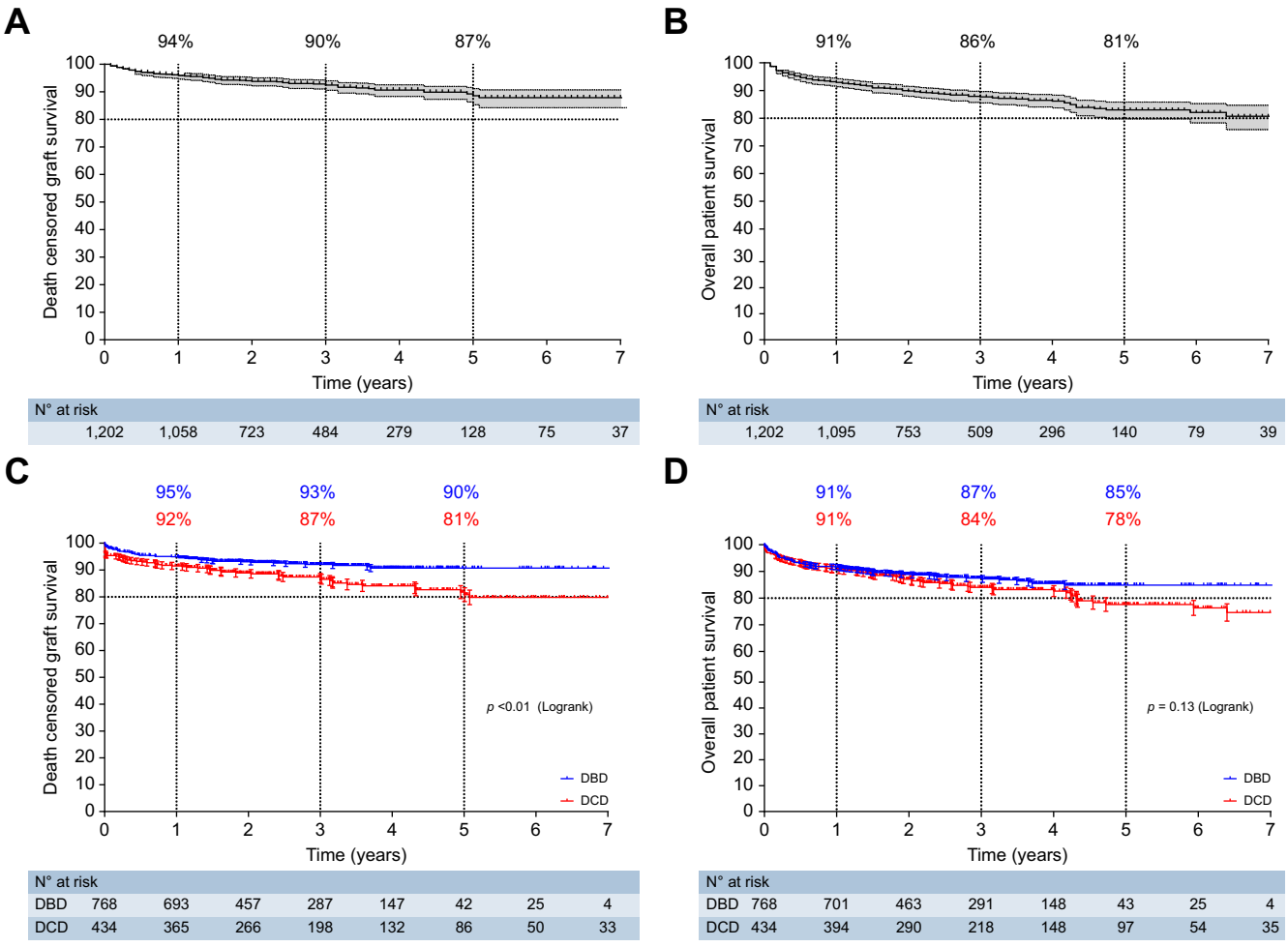


Fig. 2. Death-censored graft survival and overall patient survival for the entire cohort. Death-censored graft survival (A) and overall patient survival (B) are shown with corresponding 95% confidence interval (in grey) for the entire cohort, as well as stratified by DBD and DCD livers (C, D). Level of significance: p <0.05 (log-rank test). DBD, donation after brain death; DCD, donation after circulatory death.

Primary outcome death-censored graft survival and patient survival

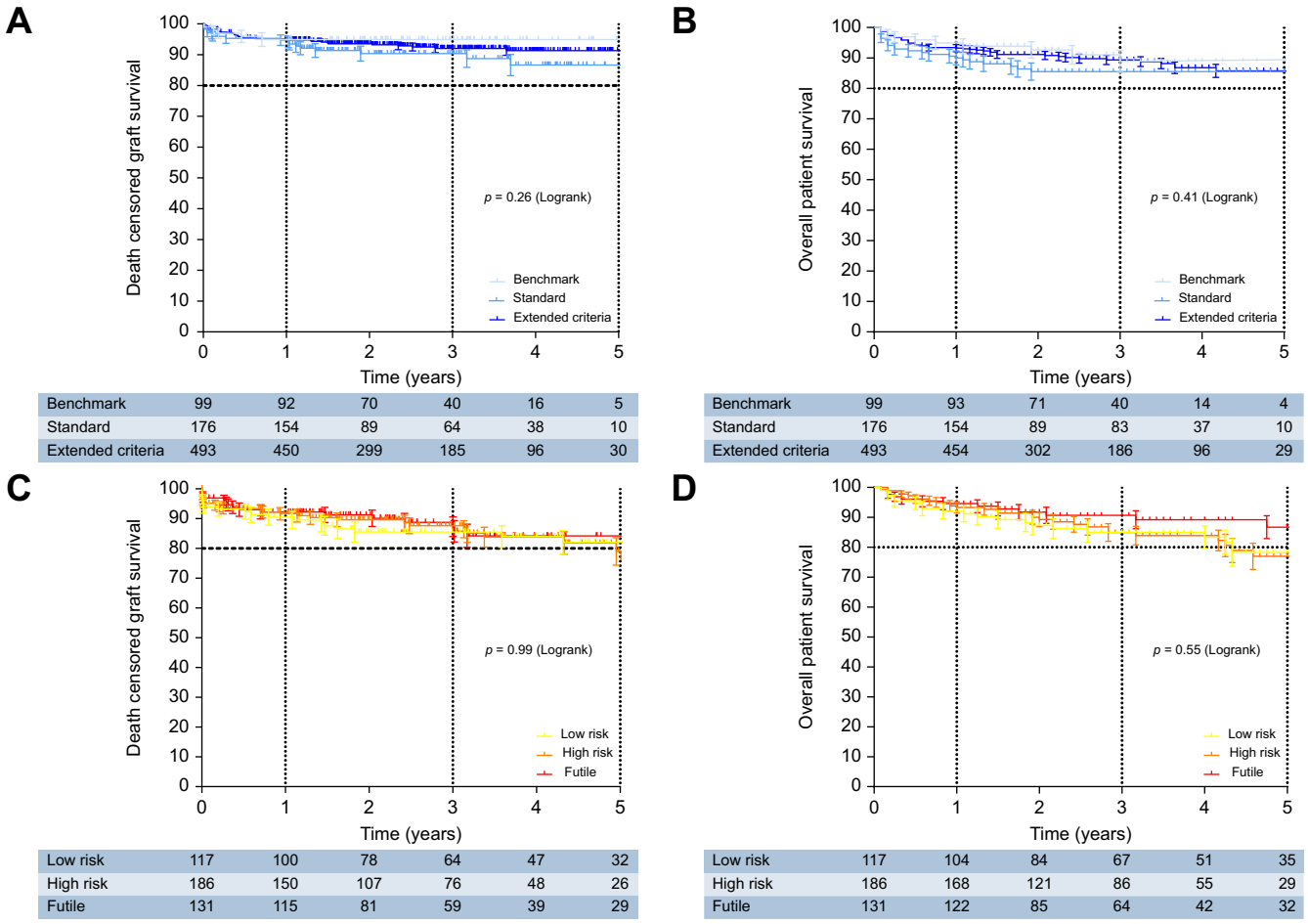
We observed excellent 1-, 3-, and 5-year death-censored graft survival rates of 94%, 90%, and 87% (Fig. 2A), respectively, with overall patient survival rates of 91%, 86%, and 81%, respectively (Fig. 2B). Death-censored graft survival was slightly but significantly better in DBD compared to DCD liver transplants (Fig. 2C; log-rank p = 0.003). Overall graft survival was also significantly better in DBD *vs.* DCD transplants (Fig. S1; log-rank p <0.01). DCD graft type was independently associated with a relative risk of 1.85 (95% CI 1.24-2.76, p = 0.003) compared to DBD graft type for death-censored graft survival on multivariate analysis (adjusted for donor age, donor BMI, CIT and recipient laboratory MELD) (Table S1). However, overall patient survival for DBD and DCD liver transplants was similar (Fig. 2D; log-rank p = 0.131). Of note, 5-year patient survival in our DCD liver transplant cohort (78%) was equal to current benchmark values achieved in ideal DBD liver transplant recipients (78.2%).[14] Furthermore, death-censored graft survival as well as patient survival did not decrease with increasing graft risk, in contrast to data on non-perfused livers.[11] This effect was observed throughout all risk categories within DBD (Fig. 3A; log-rank p = 0.26, and Fig. 3B; log-rank p = 0.41) and DCD strata (Fig. 3C; log-rank p = 0.99, and Fig. 3D; log-rank p = 0.55). No survival differences were observed between HOPE or DHOPE perfusion (Fig. S2) or between different devices (Fig. S3).

Secondary outcomes

Despite higher risk, DCD livers were not inferior to DBD livers in terms of immediate post-transplant liver injury, *i.e.* release of aminotransferases (Fig. 4A,B), and in terms of graft function, which was slightly better in DCD livers (international normalised ratio and bilirubin) during the first week after transplantation (Fig. 4C,D). Accordingly, graft loss due to PNF was not significantly different between DBD and DCD liver transplants. Median intensive care unit and hospital stays were somewhat shorter for DCD liver recipients, *i.e.* 72 h (IQR 48-120 h) *vs.* 120 h (IQR 72-192 h; p = 0.001), and 15 days (IQR 11-25 days) *vs.* 17 days (IQR 13-27 days; p = 0.004), respectively.

The cumulative incidence of NAS after 6, 12, and 24 months was 2.2%, 2.3%, and 2.5% for DBD grafts, and 8.0%, 10.0%, and 11.5% for DCD grafts, respectively (log-rank p <0.001%; Fig. 4E). The overall incidence of NAS was 2.5% in all DBD

Long-term outcomes after HOPE



Benchmark	99	92	70	40	16	5
Standard	176	154	89	64	38	10
Extended criteria	493	450	299	185	96	30

Benchmark	99	93	71	40	14	4
Standard	176	154	89	83	37	10
Extended criteria	493	454	302	186	96	29

Low risk	117	100	78	64	47	32
High risk	186	150	107	76	48	26
Futile	131	115	81	59	39	29

Low risk	117	104	84	67	51	35
High risk	186	168	121	86	55	29
Futile	131	122	85	64	42	32

Fig. 3. Death-censored graft survival and overall patient survival for different risk categories. Death-censored graft survival (primary endpoint) and overall patient survival are shown for different graft risk categories in DBD liver transplants (A, B), and in DCD liver transplants (C, D). Level of significance: p <0.05 (log-rank test). DBD, donation after brain death; DCD, donation after circulatory death.

transplants and 12.4% in all DCD transplants (p <0.001). Overall incidence of graft loss due to NAS was 0.4% for DBD liver recipients, and 4.1% for DCD liver recipients (p <0.0001; Fig. 4F). Also, anastomotic biliary strictures occurred more frequently in DCD liver transplants (26%) than DBD liver transplants (13%; p <0.001; Fig. 4F). No significant differences were observed in the incidence of renal replacement therapy, acute rejection, HCC recurrence, biliary leakage, and hepatic artery thrombosis among DBD and DCD liver recipients (Fig. 4F). In a subgroup analysis of DBD and DCD risk groups, the incidence of secondary outcomes was largely similar among the subgroups (Fig. S4).

Finally, we performed a subgroup analysis to investigate the effect of preservation strategy on outcome after DCD liver transplantation, specifically. A total of nine centres performed HOPE/DHOPE for 298 DCD livers, whereas five centres performed NRP-HOPE for 136 DCD livers (Table S2). For the centres that performed HOPE/DHOPE, 67% of DCD livers were either in the high-risk or futile category, whereas for NRP-HOPE this was 86%, indicating very high-risk livers in either group (Fig. 5A). Actuarial 1-, 3-, and 5-year death-censored graft survival rates for HOPE/DHOPE and NRP-HOPE preservation were 91.3%, 89.9%, and 87.5% *vs.* 92.6%, 90.6% and 79.5%, respectively (log-rank p = 0.18; Fig. 5B). For DCD grafts only, the cumulative incidence of NAS after 6, 12, and 24 months was 9.9%, 12.6%, and 14.7% for HOPE/DHOPE-perfused grafts, and 3.8%, 4.6%, and 4.6% for NRP-HOPE-perfused grafts, respectively (log-rank p <0.001 Fig. 5C). Post-transplant biliary complications, including incidence of NAS, graft loss due to NAS, as well as incidence of anastomotic biliary strictures were all significantly lower after NRP-HOPE preservation compared to HOPE/DHOPE preservation (Fig. 5D).

Discussion

Herein, we present the largest, multicentre, observational cohort study on HOPE-treated liver transplants worldwide. We found that, despite large differences in donor graft risk, long-term outcomes after transplantation of HOPE-treated livers were excellent for all risk strata for both DBD and DCD cohorts. Accordingly, even high-risk and futile DCD liver transplants reached an excellent 5-year death-censored graft survival rate of more than 80%, which is similar to the reported benchmark for DBD liver transplants.[14] We found no differences between HOPE types (*i.e.* HOPE or DHOPE), and no differences between perfusion devices, supporting the view of a highly reproducible, simple, and robust procedure. With this study, HOPE treatment

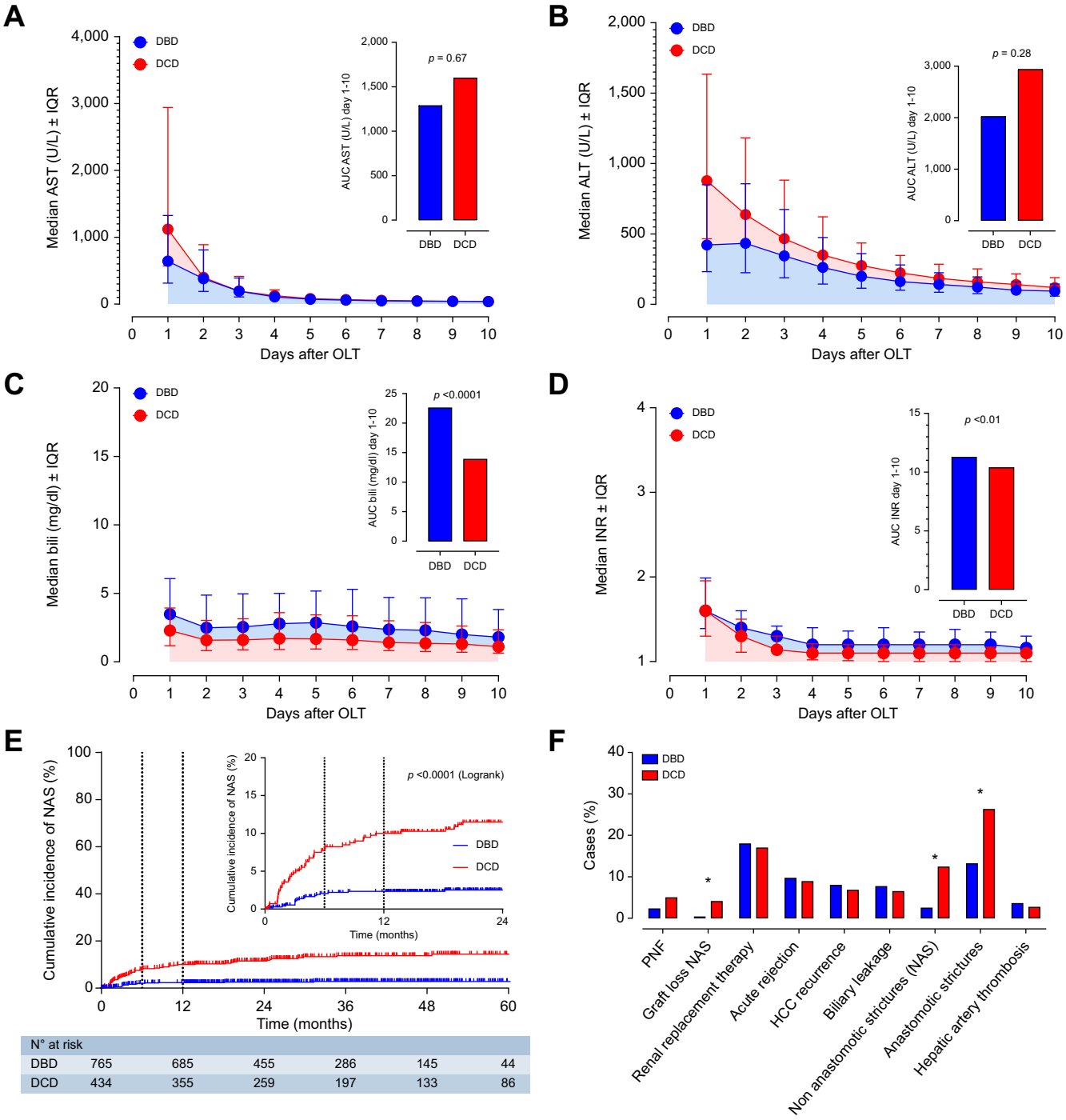


Fig. 4. The post-operative course of AST, ALT, bilirubin, and INR within the first 10 post-operative days is shown for DBD and DCD livers. The figure inserts depict the median corresponding AUC. Cumulative incidence of NAS over time, stratified by DBD and DCD livers (E). Secondary endpoints stratified by DBD and DCD livers (F). Level of significance: p <0.05 (Mann-Whitney U test; panels A-D), p <0.05 (log-rank test; panel E), *p <0.01 (Chi square test; panel F). ALT, alanine aminotransferase; AST, aspartate aminotransferase; DBD, donation after brain death; DCD, donation after circulatory death; INR, international normalised ratio; NAS, non-anastomotic strictures.

of donor livers before transplantation has reached IDEAL-D stage 4.

Dynamic preservation by liver machine perfusion is currently of emerging interest because of the increasing number of suboptimal, high-risk livers that need optimisation and assessment before use to increase utilisation rates given the worldwide organ shortage.[15] However, despite the relatively old concept of oxygenated perfusion instead of anaerobic cold storage, *ex situ* liver perfusion has not been widely adopted until recently. Accordingly, in the past 3 years six RCTs have been published showing short-term benefits of HOPE over static cold storage, with primary endpoints ranging from 1 week to up to 1 year. All six published RCTs were performed with well-selected, homogeneous patient populations and perfusion

Long-term outcomes after HOPE

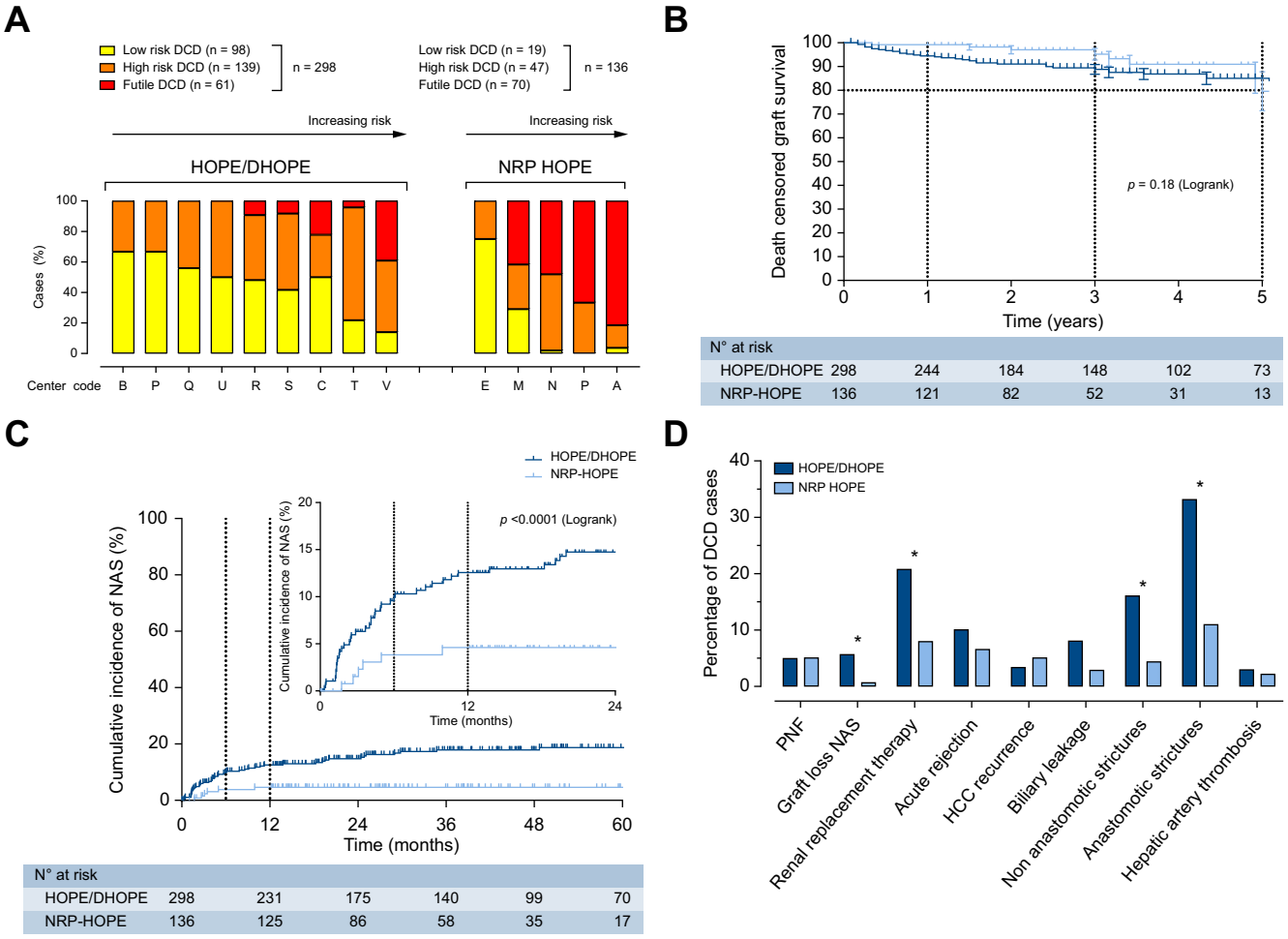


Fig. 5. In a subgroup analysis of DCD livers only, HOPE/DHOPE and NRP-HOPE strategies are compared. Centres showed substantial differences in terms of the graft risk (A). Death-censored graft survival between HOPE/DHOPE and NRP-HOPE was similar (B). Cumulative incidence of NAS over time, stratified by HOPE/DHOPE and NRP-HOPE strategy (C). Secondary endpoints stratified by HOPE/DHOPE and NRP-HOPE strategy (D). Level of significance: p <0.05 (log-rank test; panels B, C), *p <0.01 (Chi square test; panel D). DCD, donation after circulatory death; (D) HOPE, (dual) hypothermic oxygenated machine perfusion; HCC, hepatocellular carcinoma; NAS, non-anastomotic strictures; NRP, normothermic regional perfusion; PNF, primary non-function.

procedures.[5] In five RCTs, a total of 252 HOPE-treated DBD livers were included,[16–20] whereas only one RCT recruited 78 DHOPE-treated DCD livers.[21] Thus, a randomised investigation of all these confounders, *i.e.* variations in study population, procedures, or device types, would require a very high case-load, which may be very difficult to accomplish.

Alternatively, and in line with the IDEAL-D framework, we conducted a large, multicentre observational cohort study of prospectively collected data in 22 European transplant centres representing a real-world setting, and compared the results with internationally established benchmark values for DBD and DCD liver transplants.[5,14] The choice of comparator is important because interpretation of results depends on clinical acceptance and reliability. The benchmark data chosen here refer to the best possible outcomes after liver transplantation of so called “ideal” low-risk DBD and DCD grafts, and were established in livers that have not undergone machine perfusion. These data consisted of 2,024 low-risk DBD liver transplants, published in 2018, and of 1,012 low-risk DCD liver transplants, published in 2022.[14,22] Of note, the concept of using an external control from a source of secondary data has recently been advocated in terms of real-world data acquisition.[23] Real-world data is of emerging interest and has garnered the highest level of clinical evidence in this regard. Collected patient healthcare data is unfiltered, rendering its value potentially superior to that of RCT data. This is because it may present an untarnished depiction of reality. Consequently, our data consisted of a high heterogeneity in graft type, graft risk, surgical approach, HOPE perfusion technique, and post-transplant treatment. Despite these differences and many confounders, we identified by multivariate analysis only graft type, *i.e.* DCD *vs.* DBD, as a significant prognostic factor for death-censored graft survival. Notably, traditional donor or recipient key risk factors, including commonly employed risk scores such as DRI, BAR score, or UK DCD risk score, were not associated with either graft or patient survival. This lack of significance can be attributed to the fact that these prediction models were developed in the pre-machine perfusion era and are lacking validation with machine perfusion cohorts.

Another important observation was the cumulative incidence of NAS in HOPE/DHOPE-treated DCD livers. Results from the DHOPE-DCD trial showed a 6-month cumulative NAS

incidence of 6% for DHOPE-treated DCD livers.[21] In the current study, the 6-month cumulative NAS incidence for DHOPE/HOPE-treated DCD livers was 9.9%, and slowly continued to increase to 12.6% after 12 months, and 14.7% after 24 months but without impacting graft loss. In both studies, a similar definition for clinically relevant NAS was used, but the longer follow-up in the current study suggests that the onset of NAS following HOPE/DHOPE treatment may not be entirely prevented but instead delayed. The long-term follow-up results from the DHOPE-DCD trial are eagerly anticipated to corroborate these findings.

At the same time, NRP-HOPE treatment demonstrated superiority in preventing post-transplant biliary complications after DCD liver transplantation, when compared to HOPE/DHOPE treatment alone. *In situ* NRP followed by HOPE in our study was predominantly performed in Italy in the setting of very long donor warm ischaemia times. NRP allows viability assessment of the donor liver, which in Italy results in a utilisation rate of approximately 60%.[24] While this method of viability assessment proves highly effective in identifying donor livers with a subsequent low risk of post-transplant biliary complications, it is noteworthy that 40% of these DCD livers are still discarded during NRP.[15] While it is plausible that HOPE after NRP further enhanced the reconditioning potential of NRP alone, a subset within this group might have been suitable for transplantation.[25] In contrast, the HOPE/DHOPE cohort included many cases from the earliest era of the machine perfusion without the possibility of viability testing during HOPE. Only recently, in Zurich, a method of *ex situ* viability assessment during HOPE was developed, which was associated with low rates of graft loss due to cholangiopathy.[26] Alternatively, HOPE/DHOPE-treated livers can undergo sequential rewarming and NMP for *ex situ* viability assessment.[27,28] When applied to high-risk DCD livers, this approach resulted in utilisation rates of 66% (transplanted livers/HOPE-NMP procedures) and excellent long-term outcomes, without signs of clinically relevant NAS, despite long functional donor warm ischaemia times.[29] These observations highlight the importance of viability assessment of DCD livers during perfusion in decreasing the incidence and burden of post-transplant biliary complications. The mechanisms underlying the potential beneficial effect of NRP in combination with HOPE are unclear. They may be related to ischaemia-reperfusion injury-triggered defence mechanisms during NRP followed by restoration of mitochondrial energy reserves during subsequent HOPE. Future studies should focus on the validation of *in situ* (*i.e.* NRP) and *ex situ* viability assessment protocols (*i.e.* during hypothermia or normothermia) in unselected cohorts of DCD livers, and on designing RCTs for comparison of machine liver perfusion strategies.

This study has a number of limitations. The presented data were collected retrospectively with a varying proportion of missing values. Yet, the study was registered, and a study protocol was drafted prior to initiation and data collection (ClinicalTrials.gov Identifier: NCT05520320). Nevertheless, independent data monitoring and validation was not performed, as is typically done during mandatory monitoring in RCTs. We aimed to include all cases in each centre that fulfilled the inclusion criteria, however, inherent to the observational study design, any residual bias cannot be ruled out. Additionally, because we captured the evolution of the introduction of this new technology into clinical practice, follow-up times for different centres varied; yet the minimal follow-up for each included patient was set at 1 year. Importantly, most patients in this study were not included in the six previously published RCTs, but rather represented an intentionally, unselected and inherently heterogeneous patient population, which accurately reflects the complexities of real-world scenarios.

In summary, we demonstrate excellent 5-year death-censored graft and patient survival after transplantation of HOPE-treated DBD and DCD livers with low rates of graft loss due to PNF or cholangiopathy, despite a considerably proportion of high-risk grafts. HOPE treatment has now reached IDEAL-D stage 4, which further supports its implementation in routine clinical practice.

Affiliations

[1]*Department of Surgery, Section of HPB Surgery and Liver Transplantation, University of Groningen and University Medical Center Groningen, Groningen, the Netherlands;* [2]*UMCG Comprehensive Transplant Center, Groningen, the Netherlands;* [3]*Division of Transplantation, Medical University of Vienna, Vienna, Austria;* [4]*Department of Visceral Transplantation, University Medical Center Hamburg-Eppendorf, Hamburg, Germany;* [5]*Fundeni Clinical Institute, Center of General Surgery and Liver Transplantation; "Titu Maiorescu" University, Bucharest, Romania;* [6]*Department of Organ Failure and Transplantation, ASST Papa Giovanni XXIII, Bergamo, Italy;* [7]*Department of General Surgery and Transplantation, IRCCS, Azienda Ospedaliero-Universitaria di Bologna, University of Bologna, Bologna, Italy;* [8]*Chirurgia Generale 2, Hepato-Biliary-Pancreatic Unit and Liver Transplant Center, Padova University Hospital, Padova, Italy;* [9]*General Surgery 2U, Liver Transplant Centre, Azienda Ospedaliero Universitaria Città della Salute e della Scienza di Torino, Turin, Italy;* [10]*Department of Transplant Surgery, University of Geneva, Geneva, Switzerland;* [11]*Department of General Surgery and Transplantation, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy;* [12]*Hepato-Pancreato-Biliary Surgery and Liver Transplantation Unit, University of Modena and Reggio Emilia, Modena, Italy;* [13]*Adult Liver Transplantation Unit, Department of Surgery and Gastroenterology, Coimbra Hospital and University Center, Coimbra, Portugal;* [14]*General and Liver Transplant Surgery Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, and Department of Pathophysiology and Transplantation Università degli Studi di Milano, Milan, Italy;* [15]*Department of Transplant Surgery, University of Munich Grosshaderm, Germany;* [16]*Transplant Surgery Department, Institute for Clinical and Experimental Medicine (IKEM), Prague, Czech Republic;* [17]*Erasmus MC Transplant Institute, University Medical Center Rotterdam, Division of HPB and Transplant Surgery, Rotterdam, the Netherlands;* [18]*Department of Surgery, Leiden University Medical Center, Leiden, the Netherlands;* [19]*Department of Surgery, Campus Charité Mitte, Campus Virchow-Klinikum, Charité-Universitätsmedizin Berlin, Berlin, Germany;* [20]*Department of Abdominal Transplantation, Leuven Transplant Center, University Hospitals Leuven, Leuven, Belgium;* [21]*Department of Surgery, Section of Epidemiology and Statistics, University of Groningen and University Medical Center Groningen, Groningen, the Netherlands;* [22]*CHU Rennes, Service de Chirurgie Hépatobiliaire et Digestive, Rennes, France;* [23]*Transplant Institute, Sahlgrenska University Hospital, Gothenburg, Sweden;* [24]*Transplantation Center and Lerner Research Institute, Cleveland Clinic Ohio, USA;* [25]*Swiss HPB and Transplant Center, Department of Visceral Surgery and Transplantation, University Hospital Zurich, Zurich, Switzerland*

Abbreviations

BAR, balance of risk; CIT, cold ischaemia time; DBD, donation after brain death; DCD, donation after circulatory death; DRI, donor risk index; HCC, hepatocellular carcinoma; HOPE, hypothermic oxygenated machine perfusion; MELD, model for end-stage liver disease; NAS, non-anastomotic strictures; NMP, normothermic machine perfusion; NRP, normothermic regional perfusion; PNF, primary non-function; RCT, randomised-controlled trial.

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Conflict of interest

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Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

J.E.: study design, data collection, data analysis, data interpretation, writing the manuscript, verification of data. I.M.A.B: study design, data collection. G.B., B.M.B., F.B., S.C., M.C., U.C., F.C., P.C., L.G.C., R.C., F.B., J.D., D.D., D.D., M.D., J.F., G.G., E.G., G.G., M.K., E.H.K., D.K., H.D.L., G.L., P.M., D.M., D.P., W.G.P., M.R., M.R., R.R., G.S., D.U., A.S., R.P.: data collection. M.M.: statistical analysis, data interpretation, verification of data. P.D.: data analysis, data interpretation, writing the manuscript, verification of data. V.M.: study design, data interpretation, data analysis, writing the manuscript, verification of data. All authors: critical appraisal and review of the manuscript.

Data availability statement

All data supporting the findings of this study are available within the paper and its supplementary Information. The individual patient data that support the findings of this study are not openly available due to legal and ethical restrictions associated with patient confidentiality. However, anonymised data can be made available from the corresponding author upon reasonable request.

Ethics committee approval

The Medical Research Ethics Committee of the University Medical Center Groningen reviewed the study and waived the need for informed consent.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2024.06.035.

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Author names in bold designate shared co-first authorship

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