Hypothermic Oxygenated Liver Perfusion (HOPE) Prevents Tumor Recurrence in Liver Transplantation From Donation After Circulatory Death

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Objective: The aim of this study was to investigate tumor recurrence after liver transplantation for hepatocellular carcinoma (HCC), with and without hypothermic oxygenated liver perfusion (HOPE) before transplantation.

Patients and Methods: We analyzed all liver recipients with HCC, transplanted between January 2012 and September 2019 with donation after circulatory death (DCD) livers after previous end-ischemic HOPE-treatment (n = 70, Center A). Tumor parameters and key confounders were compared to consecutive recipients with HCC, transplanted during the same observation period with an unperfused DBD liver (n = 70). In a next step, we analyzed unperfused DCD (n = 70) and DBD liver recipients (n = 70), transplanted for HCC at an external center (Center B).

Results: Tumor parameters were not significantly different between HOPE-treated DCD and unperfused DBD liver recipients at Center A. One-third of patients were outside established tumor thresholds, for example, Milan criteria, in both groups. Despite no difference in tumor load, we found a 4-fold higher tumor recurrence rate in unperfused DBD livers (25.7%, 18/70), compared to only 5.7% (n = 4/70) recipients with tumor recurrence in the HOPE-treated DCD cohort ($P = 0.002$) in Center A. The tumor recurrence rate was also twice higher in unperfused DCD and DBD recipients at the external Center B, despite significant less cases outside Milan. HOPE-treatment of DCD livers resulted therefore in a 5-year tumor-free survival of 92% in HCC recipients, compared to 73%, 82.7%, and 81.2% in patients receiving unperfused DBD or DCD livers, from both centers.

Conclusion: We suggest that a simple machine liver perfusion approach appears advantageous to protect from HCC recurrence after liver transplantation, despite extended tumor criteria.

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Machine liver perfusion is currently a hot topic in transplantation aiming to optimize graft quality before implantation and to assess liver function.[1,2] Besides an immediate effect on graft function, machine liver perfusion impacts also on the early immune response,[3–5] with therefore potential clinical implications, for example, on tumor recurrence. Yet, the mechanisms for tumor recurrence after hepatectomy and liver transplantation (LT) remain not fully understood. Several factors, including tumor seeding, unrecognized residual micro-lesions in lymph nodes, active hepatitis, increased liver regeneration, and immunosuppressive treatment, may promote cancer growth or recurrence.[6–8] In addition, ischemia reperfusion injury has been recognized as an important initial driver of microvascular dysfunction with subsequent tissue hypoxia and ongoing inflammation, which promotes tumor cell reseeding and growth.[6] This is of clinical relevance because many candidates listed for hepatocellular carcinoma (HCC) currently receive livers from donation after circulatory death (DCD) donors, where medical fitness is required to sustain a potentially occurring reperfusion syndrome.

We have introduced in Zurich a clinical DCD liver transplant program in January 2012, where all human DCD livers undergo standard procurement with cold storage and end-ischemic hypothermic oxygenated perfusion (HOPE) before implantation.[9] Our philosophy was to utilize DCD livers for transplant candidates listed with an HCC with expected long waiting time.

We have now assessed outcomes after transplantation of HCC candidates who receive HOPE-treated DCD livers in comparison with un-perfused DBD livers. In a second step, we compared our cohort with DBD and DCD transplantations, performed at another high-volume European transplant center.

PATIENTS AND METHODS

Patients and Transplant Program University Hospital Zurich (Center A)

We analyzed all liver recipients with HCC, transplanted between January 2012 and September 2019 in Zurich with DCD livers and end-ischemic HOPE-treatment (n = 70), and a minimal follow-up of 6 months. These results were compared to consecutive recipients with HCC, transplanted during the same observation period with an unperfused DBD liver (n = 70).

Tumor parameters, including tumor number, size (diameter of largest lesion), total tumor volume (TTV), and microvascular invasion were determined on pathological specimen.[10,11] Key confounders including alpha-fetoprotein (AFP), bridging treatment (TACE, RFA), waiting time, and tumor recurrence-free survival were documented. Based on these data, we assessed tumor classification systems, including Milan criteria (3 lesions ≤3 cm each or 1 lesion ≤5 cm), UCSF criteria (1 lesion ≤6.5 cm or up to 3 lesions with the largest measuring ≤4.5 cm and a total tumor volume of ≤8 cm), and Metroticket 2.0 [sum of tumor number+size (cm) ≤7 and AFP <200 ng/mL; or sum of tumor number+ size (cm) ≤5 and AFP 200–400 ng/mL; or sum of tumor number + size (cm) ≤4 and AFP 400–1000 ng/mL].[12]

In addition, we collected donor, graft and recipient parameters, such as donor age and body mass index (BMI), functional donor warm ischemia (fDWIT; defined as MAP <50 mm Hg to cold aortic flush), cold storage preservation time (Institute-George-Lopez-1), recipient age, and recipient lab MELD score. To estimate risk profiles, we calculated current risk scores, including the balance of risk score (BAR), donor risk index (DRI), and UK DCD risk score.[13–15]

LT was routinely performed by cava replacement technique with graft reperfusion through the portal vein first. Post-transplantation immunosuppression was performed with tapered steroids and tacrolimus monotherapy. In recipients with impaired kidney function after LT, we started tacrolimus with 24-hour delay and added basiliximab (2 × 20 mg). The median follow-up was 2.6 years in the DCD- and 4.4 years in the DBD cohort.

The primary endpoint of this study was tumor recurrence. Secondary endpoints included operation time, transfusion rates, intensive care and hospital stay, biopsy proven acute rejection rates, graft and patient survival, and de novo tumor growth.

External Cohort Queen Elizabeth Hospital Birmingham (Center B)

In a next step, we analyzed outcomes after transplantation of consecutive un-perfused DCD (n = 70) and DBD liver recipients (n = 70), listed with an HCC at the Queen Elisabeth Hospital Birmingham, United Kingdom. Patients were included when transplanted during the same time frame, starting January 2012 to 2019, securing a minimal follow-up of 6 months (median follow-up: 4.4 years and 3.7 years). Any machine perfused livers, split grafts, combined transplants and domino transplants were excluded. The withdrawal process for DCD donors was the same compared to Switzerland with super rapid cannulation, cold flush, and hepatectomy with subsequent cold storage (UW solution). Liver implantation was performed with piggyback techniques [modified (side-to-side) or classic] and portal vein reperfusion first in the majority of cases. The immunosuppression protocol consisted of a combination of steroids, tacrolimus, and azathioprine or mycophenolate mofetil. Preoperative tumor listing criteria followed strictly the Milan criteria during the study period.

Statistical Analysis and Data Validation

Data were analyzed with Prism 7 and SPSS, version 25 (SPSS Inc, Chicago, IL). All metric parameters are reported by median and interquartile range and compared by Mann-Whitney *U* test. All categorical variables were expressed in quantities and percentages and compared by Chi-square test or the Fisher exact test. *P* values <0.05 were considered statistically significant. Survival rates were calculated by Kaplan-Meier and compared by log-rank tests. The data analysis was approved by local ethics (KEK No. 2019-01000 and CARMS No: 14535).

RESULTS

Tumor Parameters in HOPE-Treated DCD and DBD Cohorts in Center A

Due to a center allocation for the majority of DCD donors in Zurich, DCD recipients had a shorter waiting time compared to DBD recipients (156 vs 263 days). Tumor parameters, however, between HOPE-treated DCD and unperfused DBD liver recipients, were not different. This included overall HCC size (3.2 vs 3.5 cm), the size of the biggest lesion (2.1 vs 2.0 cm), the number of lesions (2 vs 1), TTV (16.4 vs 17.2 cm^3), and microvascular invasion (17 vs 14%) (Table 1, Fig. 1A–E). The same number of DCD and DBD patients, for example, 56 of 70 cases (75.7%), underwent bridging therapy (TACE or RFA) while waiting for a liver offer (Table 1). Median serum AFP levels at transplantation were in the normal range at the time of listing in both groups (median 8 vs 9 μg/L, ns), and minimally elevated at the time of transplantation in DBD compared to DCD liver recipients (16.7 vs 6.8 μg/L, $P = 0.03$, reference value <13.1) (Table 1). Correspondingly, the percentage of patients exceeding Milan, UCSF, or Metroticket 2.0 criteria was not different between DCD and DBD liver transplants. Approximately one-third of patients (35.7 vs 37.1%) in both groups were outside Milan and UCSF criteria, and a minority of cases was transplanted being outside Metroticket 2.0 (18.6 vs 17.1%, ns) (Fig. 1F).

Donor and Recipient Risk Factors in HOPE-Treated DCD and DBD Transplants in Center A

Median donor age, total preservation time, recipient age, and recipient MELD were comparable between HOPE-treated DCD and DBD recipients in Zurich, resulting in similar low BAR scores (4 vs 4) (Table 1). The DRI was significantly higher in the DCD cohort (2.48 vs 1.66 points), and the UK-DCD-score was close to the futile range with a median of 9 points, due to long fDWIT times (Table 1). Despite this, the duration of transplantation surgery (4.7 vs 5 h), intraoperative blood loss (1500 vs 1000 mL), and required transfusions (0 vs 0 RBC, 0 vs 0 FFP) remained comparable between DCD and DBD recipients (Table 2).

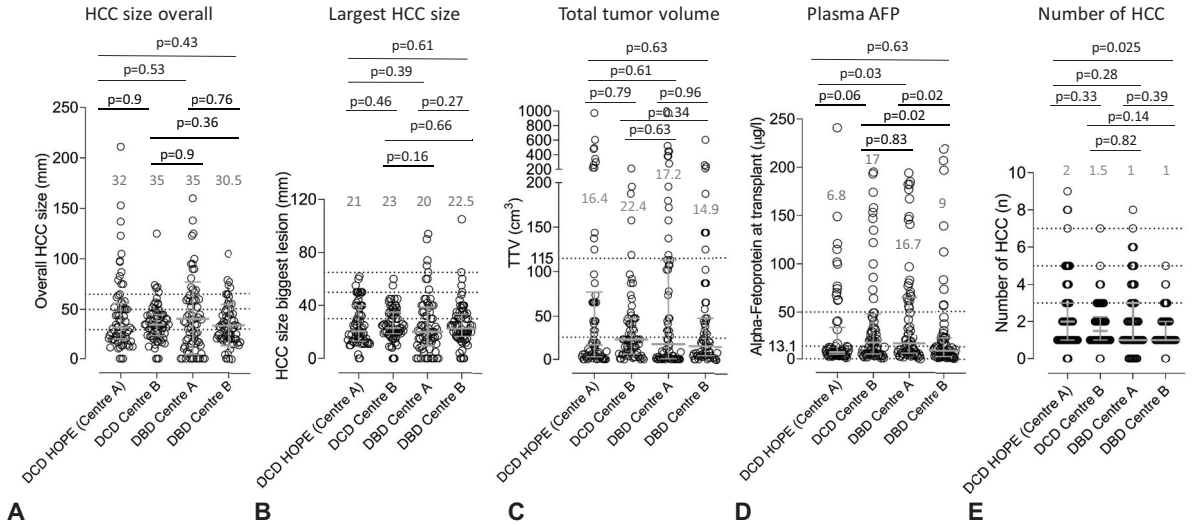
Outcome in HOPE-Treated DCD and DBD Cohorts in Center A

Recipients of DCD livers experienced higher ALT on day 1 after LT (1305 vs 893 U/L, $P = 0.0059$) and higher peak creatinine (212 vs 131 μmol/L) in the first week, with however better early graft function, as shown by INR recovery at day 1 (Table 2, Fig. 2A–C). Consistently, ICU (3 vs 3 days) and hospital stays (17 vs 15 days) were not different in both groups. Of note, the systemic inflammatory response after LT, visualized by cumulative CRP levels in the first week, was significantly lower in HOPE-treated DCD livers compared to untreated DBD grafts (Fig. 2D). The median plasma tacrolimus trough levels were 5.1 and 6.1 on day 7 after LT, and between 5.8 and 7.5 at discharge after 3 months and 1 year comparing both groups (Table 2). The de novo tumor rate was not different between DCD and DBD transplants (8/70 vs 8/70) (Table 2). Despite similar immunosuppressive treatment, however, the biopsy-proven acute rejection rate was significantly lower in HOPE-treated DCD liver recipients, compared to DBD recipients (7.1 vs 20%, $P = 0.0016$). In addition, recipients of HOPE-treated DCD grafts experienced significantly less tumor recurrence during the follow-up (4/70) (5.7%), compared to a 4-times higher recurrence rate found in DBD recipients 25.7% (18/70, $P = 0.002$), mainly found in the new graft [n = 2/4 (50%); n = 12/18, 66.7%]. Recurrence-free recipient survival was consecutively significantly higher with 92% after 5 years in HOPE-treated DCDs compared to untreated DBD liver recipients

TABLE 1. Donor, Recipient, and Tumor Parameter in Liver Transplantation for Candidates With HCC

Risk Factors	DCD HOPE (Center A) (n = 70)	DCD (Center B) (n = 70)	DBD (Center A) (n = 70)	DBD (Center B) (n = 70)	*P* DCD HOPE vs DBD (Center A)	*P* (DCD HOPE Center A vs DCD Center B)
Donor and recipient risk factors						
Donor age, y	59.5 (48.75–72.0)	51 (35–66.25)	57 (45–70.0)	51 (40–67)	0.4697	0.0077
Donor BMI, kg/m^2	24.9 (22.9–27.5)	24.61 (22.6–27.3)	22.20 (20.0–25.1)	26.5 (22.9–31.22)	0.0002	0.5641
fDWIT, min	30.5 (26.0–35.0)	19 (14.25–23)	—	—	—	<0.0001
HOPE duration, h	2 (1.7–2.5)	–	–	–	–	–
Cold ischemia time, h	4.05 (3.2–5)	6.865 (5.87–7.6)	6.8 (5.4–8.475)	7.74 (6.7–9.77)	<0.0001	<0.0001
Total preservation time	6.3 (5.48–7.33)	6.865 (5.87–7.6)	6.8 (5.4–8.475)	7.74 (6.7–9.77)	0.1677	0.1499
Recipient age, y	60.5 (56.75–66.25)	61.0 (57.0–66.0)	62 (56.0 – 65.0)	61 (54–66)	0.5347	0.9499
Recipient MELD (points)	10 (8–14)	7 (5–10)	14 (8–28)	9 (5.8–14)	0.0592	<0.0001
BAR Score (points)	4 (2–4)	3 (2–4)	5 (3–12)	3 (2–5)	0.0003	0.3182
DRI points	2.48 (2.048–2.818)	2.2 (1.9–2.975)	1.66 (1.38–1.910)	1.6 (1.375–2)	<0.0001	0.6497
UK DCD Risk Score (points)	9 (6–11)	5 (4–8)	—	—	—	<0.0001
Duration of follow-up, days	962.5 (474.5–1667)	1620 (944–2063)	1628 (738.0–2634)	1348 (823–2026)	0.0008	0.002
Tumor parameter						
No. of HCCs (n)	2 (1–3)	1.5 (1–2.25)	1 (1–3)	1 (1–2)	0.28	0.33
Size biggest HCC lesion, mm	21 (13.5–39)	23 (18.75–35)	20 (10–38)	22.5 (16.75–33.25)	0.39	0.46
Overall size all HCCs, mm	32 (18.5–53.5)	35 (22.75–45)	35 (12–60)	30.5 (20–45)	0.53	0.9
AFP at transplantation, µg/L	6.8 (4.2–33.9)	17 (5–49)	16.65 (6.05–65.75)	9 (3–23)	0.03	0.06
HCC bridging treatment (yes):	65 (92.86%)	35 (50%)	64 (91.43%)	41 (58.57%)	1.0	0.0001
TACE: n/%	50 (71.4%)	21 (30%)	53 (75.7%)	29 (41.43%)	0.7018	0.0001
RFA: n (%)	6 (8.57%)	13 (18.57%)	3 (4.19%)	11 (15.71%)	0.4932	0.1372
SABR:	1 (1.43%)	0	0	0	1.0	1.0
Combinations or other, n (%)	8 (11.43%)	1 (1.43%)	8 (11.43%)	1 resection	1.0	0.0332
Microvascular invasion, n (%)	12 (17.1%)	33 (47.1%)	10 (14.28%)	33 (47.1%)	0.8169	0.0002
Time on waiting list, days	155.5 (73–276.8)	63 (27–124.3)	263 (134–351)	70 (24.5–238)	0.0051	<0.0001

Median and IQR, *U* test, n and %, n = 70 per group.



Tumour Classification at liver Transplantation	DCD HOPE (Centre A)	DCD (Centre B)	DBD (Centre A)	DBD (Centre B)	p value DCD HOPE vs. DBD (Centre A)	p value DCD HOPE (Centre A) vs. DCD Centre B
Cases outside Milan (n/%)	25/70 (35.7%)	13/70 (18.6%)	26/70 (37.1%)	14/70 (20%)	1.0	0.036
Cases outside UCSF (n/%)	20/70 (28.6%)	6/70 (8.6%)	21/70 (30%)	4/70 (5.7%)	1.0	0.0041
Cases outside Metroticket 2.0 (n/%)	13/70 (18.6%)	1/70 (1.4%)	12/70 (17.1%)	1/70 (1.4%)	1.0	0.0011

F Panel a-e: median and IQR, Mann-Whitney U-test, Table (f): n/%; fisher exact test;

FIGURE 1. Tumor parameter and criteria in liver transplant recipients. The tumor burden, assessed through the overall HCC size (A), the size of the largest HCC (B), the total tumor volume (C), the plasma AFP of the candidate at transplantation (D) and the number of HCCs (E) at explant liver specimen is higher in Center A, when compared to Center B. This becomes more evident through the assessment of the significantly higher number of transplant candidates classified outside currently applied HCC classifications, including Milan, UCSF, and Metroticket 2.0 (F).

TABLE 2. Outcome Parameters Comparing HOPE Treated DCD Liver Recipients With Unperfused Grafts From 2 Different Transplant Centers

Outcome Parameter	DCD HOPE (Center A) (n = 70)	DCD (Center B) (n = 70)	DBD (Center A) (n = 70)	DBD (Center B) (n = 70)	*P* DCD HOPE vs DBD (Center A)	*P* (DCD HOPE Center A vs DCD Center B)
In hospital outcomes						
Duration of transplantation, h	4.76 (3.8–5.553)	4.6 (3.73–5.32)	5 (4.17–6)	4.63 (3.79–5.78)	0.0415	0.5902
No. of. RBC (n)	0 (0–3)	1 (0–3)	0 (0–2)	1.5 (0–4)	0.4909	0.4560
No. of FFP (n)	0 (0)	4 (0–9.5)	0 (0)	4 (0.75–8.5)	0.2226	<0.0001
ICU stay, days	3 (2–4.25)	3 (2–6)	2 (1–4)	2 (2–4.25)	0.2413	0.1903
Hospital stay, days	17 (13–22)	9 (7–15)	15 (12–24)	8 (7–14)	0.8775	<0.0001
INR day 1	1.3 (1.2–1.6)	1.5 (1.3–1.7)	1.4 (1.2–1.6)	1.5 (1.3–1.8)	0.4730	0.0045
ALT day 1, U/L	1305 (773–2044)	1623 (1015–2705)	893 (467.3–1438)	1106 (727–1683)	0.0145	0.0059
Peak creatinine, μmol/L	212.5 (132.8–372.3)	165.5 (102.3–283.3)	131 (97–231)	109 (84–207.3)	0.0002	0.0283
Overall outcomes						
HCC recurrence rate, n (%)	4/70 (5.7%)	10/70 (14.3%)	18/70 (25.7%)	12/70 (17.1%)	0.002	0.1571 (DCD Center A vs DBD Center B: $P = 0.06$)
Time to recurrence, days	529 (376.5–884)	640.5 (388–857.3)	898.5 (578.8–1352)	513.0 (283.8–747.5)	0.2269	0.9451
Recurrence in liver, n (%)	2/4 (50%; 2.9%)	2/10 (20%, 2.86%)	12/18 (66.7%; 17.14%)	6/12 (50%, 8.6%)	0.009	1.0
De novo cancer, n (%)	8 (11.4%; 2× SCLC, 2× skin, 1× bladder, 1× CCC; 2× PTLD)	2 (2.9%; 1××x skin, 1× PTLD)	8 (11.4%; 2× bronchus, 1× SCLC, 1× stomach, 4× other)	7 (10%; 1× gastric, 2× colon, 2× PTLD, 1× skin, 1× prostate)	1.0	0.0552
Acute rejection (biopsy proven)	5 (7.1%)	14 (20%)	14 (20%)	7 (10%)	0.0016	0.0016
Tacrolimus level day 7	5.1 (3.35–7.05)	5.7 (4.15–7.125)	6.1 (4.2–7.875)	5.55 (4.725–7.875)	0.0524	0.1833
Tacrolimus level after 3 mo	7.5 (5.75–9.7)	8 (5.93–9.95)	7.4 (5.9–9.4)	7.35 (5.025–10.08)	0.8256	0.2885
Tacrolimus level after 1 y	6.2 (4.8–7.58)	6.8 (4.9–9.38)	6.3 (4.7–8.2)	6.95 (5.375–8.252)	0.2174	0.1593

Median and IQR, *U* test, n and %, n = 70 per group.

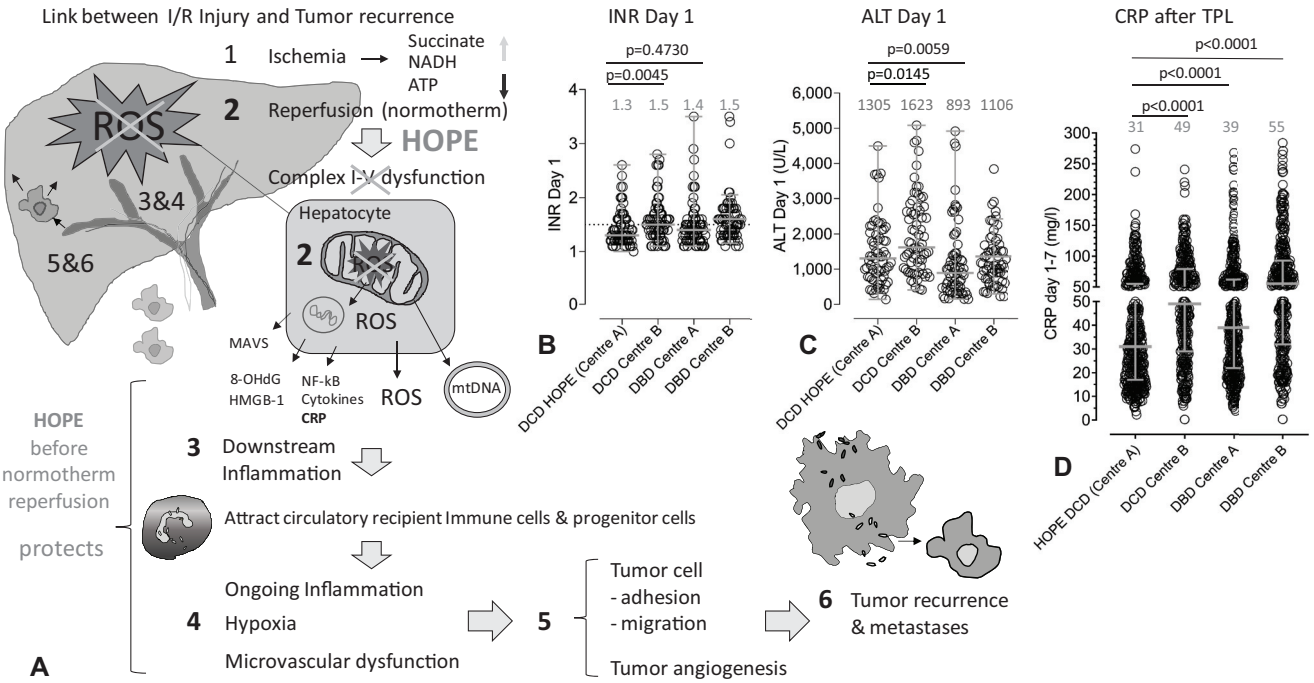


FIGURE 2. Graft function, injury, and inflammation after liver transplantation. Initial ROS release triggers general inflammation and subsequent tumor cell reseeding growth and HCC recurrence (A). HOPE treatment before liver implantation triggers reprogramming of mitochondria, reduces ROS production and downstream injury with protection from tumor recurrence. The immediate liver function and injury are shown during the first week after liver transplantation through assessment of INR (B) and ALT (C). The general inflammation, measured through plasma CRP of the recipient (D) demonstrates significantly lower values after implantation of HOPE-treated DCD grafts, along with better immediate liver function. HOPE perfusion before implantation protects mitochondria from the initial key injury and subsequently prevents ongoing tissue inflammation and hypoxia, with an environment less favorable for tumor cells to resettle and regrow, in contrast to unperfused, cold-stored livers, independent of DBD or DCD (D).

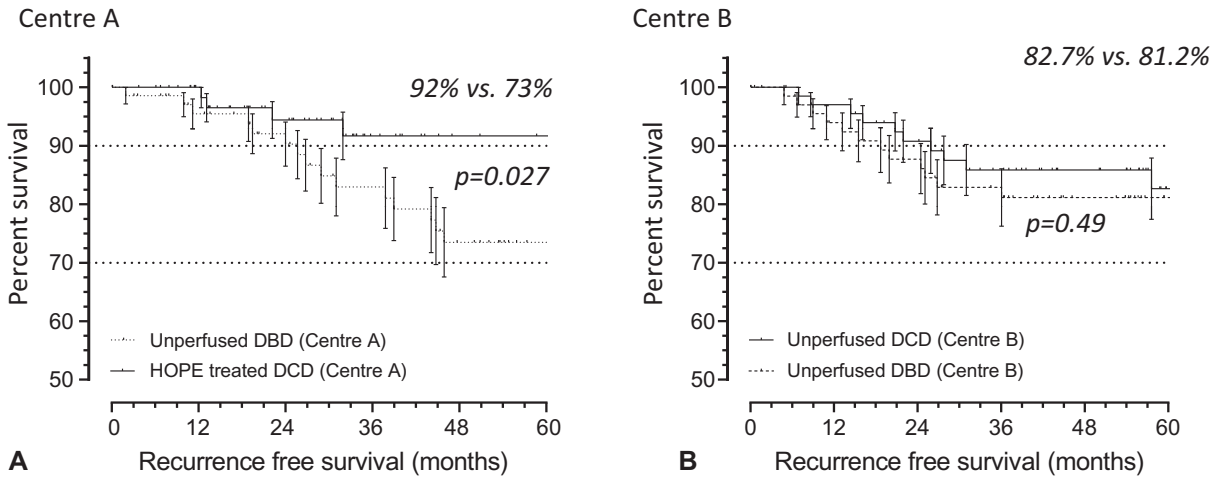


FIGURE 3. Recurrence-free survival after liver transplantation for HCC. HOPE treatment improved recurrence free survival significantly, despite high tumor load and extended criteria DCD grafts when compared to unperfused livers in both centers (A and B).

(73%, 5-year recurrence-free survival, $P = 0.027$) despite the higher donor risk (Fig. 3).

Tumor, Donor, and Recipient Risk Factors, and Related Outcome in Center B

Tumor parameters were more favorable in Birmingham compared to Zurich due to a more restrictive listing policy, with less cases outside Milan, UCSF, or Metroticket 2.0 (Table 1, Fig. 1A–F). Importantly, the waiting time to receive a liver graft was significantly shorter in Birmingham (DCD cohort: 63 vs 155 days, $P = 0.0051$; DBD cohort: 70 vs 263 days, $P < 0.0001$). Additionally, DCD graft quality in Birmingham was better as, for example, shown by a shorter fDWIT (19 vs 31 minutes, $P < 0.0001$) and younger donor age (51 vs 60 years, $P = 0.0077$), resulting in a significantly lower UK DCD risk score (5 vs 9, $P < 0.001$, Table 1).

Despite this, DCD liver recipients in Birmingham showed significantly delayed INR recovery and higher ALT release on day 1 after LT, compared to HOPE-treated DCD liver recipients in Zurich, and higher CRP levels in the first week after transplant (Fig. 2A–D). The biopsy-proven acute rejection rate was also significantly higher (20 vs 7%, $P = 0.0016$), despite similar TAC levels (Table 2). Finally, the tumor recurrence rate in the unperfused DCD cohort in Birmingham was more than twice as high (14%, Center B) when compared to HOPE-treated DCDs (5.7%, Center A), in contrast to significantly lower donor risk and significantly less tumor burden (Table 1, Fig. 1F).

DISCUSSION

HCC is currently the leading indication for LT in liver cirrhosis.[16] Frequently, however, HCC candidates receive extended criteria donor organs, as they may better tolerate ischemia reperfusion injury.[16] We have introduced in 2012 in Zurich a machine perfusion technique for DCD livers, hypothermic oxygenated perfusion, based on the idea to improve graft quality.[17,18] As a main part of this concept, we allocated such HOPE-treated DCD livers preferably to HCC candidates with expected long waiting time, despite relatively advanced tumor stages.[12] In context of the recent discussion regarding a potentially increased tumor recurrence risk triggered by poor graft quality, we were now interested in analyzing tumor-related outcomes in our HCC cohort, comparing DBD and HOPE-perfused DCD liver transplants. The results show acceptable outcomes despite an advanced tumor risk with almost one-third of our HCC recipients, being outside UCSF criteria. Next, we observed a significantly lower recurrence rate in the HOPE-treated DCD cohort compared to a DBD cohort, despite additional donor risk. Third, HCC recurrence appeared even lower compared to an external, nonperfused DCD and DBD liver recipient cohort with a significantly lower tumor burden.

Clinical studies have shown conflicting results with regard to utilization of DCD grafts for HCC recipients. Although some studies report no difference between DCD and DBD livers in terms of HCC recurrence,[19,20] other investigators have raised clear concerns regarding higher HCC recurrence in DCD liver transplants.[21] The limitation of these data is the high variation of donor risk profiles, including warm and cold ischemia times, donor age, or donor BMI.[19–21] In contrast to inconsistent human data, experimental models have confirmed that ischemia reperfusion (I/R) injury of livers and kidneys promotes cancer cell implantation and growth.[6,22–24] Cold storage and recipient warm ischemia were consecutively also both independent predictors of early HCC recurrence in another clinical LT series of DBD livers.[22,24]

A number of biological mechanisms have been suggested for the association between cancer recurrence and I/R injury to the liver. The cascade of I/R induces injury to hepatic sinusoids, which leads to a dysfunction of the hepatic microcirculatory barrier and activates cell signals related to invasion and migration.[6, 25] Hypoxia triggers gene upregulation and release of cytokines, involved in angiogenesis, cellular proliferation, growth, and adhesion. In the absence of oxygen, hypoxia-inducible transcription factor 1 (HIF-1α) binds to hypoxia-response elements, thereby upregulating the hypoxia-response gene expression, including vascular endothelial growth factor.[26, 27]

A correlation between inflammation and cancer has already been identified in 1863, by Virchow, who recognized the inflammatory process as one of the predisposing conditions for tumor development.[28] In response to various tissue injuries, a multifactorial network of chemical signals, initiated and amplified by recruitment and infiltration of leukocytes from the venous system to the sites of damage, initiates and maintains a host response, primarily designed to ''heal'' the damaged tissue. Paradoxically, yet, inflammation is also a major key player for the development of numerous

malignancies through induction of proliferation and migration processes.[25] Accordingly, inflammatory mediators, including reactive oxygen species (ROS) and numerous cytokines (TNFα, IL-1, IL-6, IFN-y) released from various immune cells, induce epigenetic alterations in premalignant lesions and silence tumor suppressor genes.[29,30] Such mediators activate transcription factors in tumor-associated inflammation, for example, in NF-kB, STAT-3, and HIF-1 triggered pathways, which impact on tumor growth during any stage of tumorigenesis.[27,29] In addition, activation of immune checkpoints, such as programmed cell death proteins (PD-1) or cytotoxic T-lymphocyte protein 4 (CTLA-4) by TNF-α, IFN-γ or micro RNAs (miR-20,-21-I30b,-197,-223) and Rho-signaling (Rac1, ROCK, Cdc42) all stimulate tumor growth, which has recently resulted in clinical application of checkpoint inhibitors to prevent HCC growth and recurrence.[6,25] An early inhibition of inflammatory reactions appears therefore important to prevent also tumor growth, especially if injured grafts are implanted in HCC recipients.

HOPE is currently applied on marginal liver grafts, including DCD and steatotic livers,[17,18,31,32] and targets predominantly mitochondria with the aim to minimize upfront release of danger signals, including mitochondrial DNA and ROS, which should decrease inflammasome activation.[18,33,34] Consistently, He et al demonstrate that HOPE treatment conferred protection of DCD livers by inhibition of the oxidative stress dependent TXNIP/NLRP3 inflammasome pathway during reperfusion.[35,36] Our data support this hypothesis, as we found less systemic inflammation in HOPE-treated DCD compared to DBD recipients, correlating with improved mitochondrial function during HOPE[18,34,37,38] (Fig. 2). We demonstrated earlier the effect of HOPE on the innate immune response in livers and kidneys, which underlines the lower activation of T cells by cold oxygenated perfusion before implantation.[3,4] As a next step, we show here for the first time, that strong anti-tumor effects can be expected by HOPE-treatment, which opens the door for the application of this perfusion technique in recipients with risk of tumor recurrence. In this context, we would discuss a routine application of HOPE for all grafts, utilized for candidates with a liver tumor, regardless of the donor type.

The limitation of this study is the retrospective design and consecutive differences in waiting time, post-transplant observation periods, and AFP at transplant (Table 1). However, in a propensity score-matched cohort, adjusted for AFP and all other tumor parameters, we confirmed the significant differences in tumor recurrence between DBD and HOPE-treated DCD transplantations (15/57, 26.3% vs 3/57, 5.3%, $P = 0.004$) (Supplementary Figure 1, http://links.lww.com/SLA/C388). We believe therefore that the observed 4-fold lower tumor recurrence in HOPE-treated DCD transplants is unlikely caused by a selection bias.

A second shortcoming was the shorter follow-up in HOPE-treated DCD compared to DBD recipients (2.6 vs 4.4 years, $P = 0.0008$, Table 1). The median observation time in the HOPE-treated DCD cohort was yet longer than the median time to tumor recurrence in DCD and DBD cohorts of both centers (Table 2). Based on this, the follow-up in our DCD cohort appears long enough to capture tumor recurrences.

We conclude, that these findings are related to the performance of HOPE before liver implantation, and suggest that outcome can be favorable, despite the use of high-risk DCD grafts in a recipient population with high tumor load, when applying modern machine perfusion techniques protecting from mitochondria-induced inflammation after transplantation.

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