Supplementary Information

Machine perfusion prevents early tumor recurrence in liver transplantation for hepatocellular carcinoma: a multicenter retrospective cohort study

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Ethics Statement

The study was conducted in accordance with the Declaration of Helsinki 2013 and approved by local relevant institutional review boards (approval number: 24312-4-02).

HCC recurrence

Based on the published literature, a physician conducted a review of liver malignancy follow-up data and cause of death variables to identify cases of tumor recurrence. Records showing recurrence of pre-transplant malignancy or a cause of death related to HCC or metastatic malignancy were classified as cases of HCC recurrence. For post-transplant HCC recurrence, patient follow-up was measured from the date of LT to either HCC recurrence or HCC related death. Patients were censored at the date of non-HCC related death or at their last follow-up[1].

Method for handling missing values

To address missing data in the dataset, the multiple imputation (MI) function in SPSS (version 26.0) was employed. For continuous variables, linear regression models were used for imputation, while logistic regression models were applied to categorical variables. Variables included in the multiple imputation models are listed in Table S1.

Propensity score matching

The sample for propensity score matching (PSM) included 434 MP patients and 12,730 SCS patients. Variables included in the PSM models are listed in Table S2. PSM was performed using SPSS statistics (version 26.0) to minimize selection bias and ensure comparability between groups. A matching tolerance (caliper) of 0.02 was applied to enhance precision, and an optimal matching algorithm without replacement was employed.

Definition of the beneficial subgroup

To further identify subgroups with more significant benefits from MP, we hypothesized that MP would demonstrate stronger protective effects in specific subgroups while showing weaker or no protective effects in others. Through subgroup analyses of prognostic factors, we evaluated the HRs of MP across different subgroups. The "beneficial group" was subsequently established by combining factors where MP showed statistically significant protective effects.

Sensitivity analyses

To ensure the robustness of the primary analysis, we conducted three sets of sensitivity analyses. First, considering that MP was primarily implemented after 2022 (Figure S1), we excluded participants enrolled prior to 2022 and reassessed the association between MP and HCC recurrence. We also excluded participants within the first six months of follow-up and repeated the analysis. To address the potential impact of MI for missing data, we performed a complete-case analysis by deleting missing data and reanalyzing the impact of MP on HCC recurrence. Second, to account for the impact of competing events (non-tumor related death) on the results, we applied the Fine and Gray competing risks model to ensure the robustness of the findings. Additionally, we used the Fine and Gray's sub-distribution hazards regression model to verify the association between MP and HCC recurrence. Finally, given that MP prevents HCC recurrence by mitigating IRI and improving graft quality, we accounted for potential effect modification by donor-related confounders (graft type, steatosis, donor age) in subgroup analyses. we explored potential effect from donor related confounding risk factors by performing stratified analyses.

Statistical analysis

All statistical analyses were conducted using GraphPad Prism (Version 8.0.2), SPSS statistics (Version 26.0), and R (Version 4.2.2, R Foundation). Continuous variables were reported as median

values with interquartile ranges (IQR) and were analyzed using the Mann-Whitney U test for comparisons. Categorical variables were evaluated using either the chi-squared test or Fisher's exact test, depending on the data distribution. RFS or overall survival (OS) were assessed using the Kaplan-Meier method, with comparisons made via the log-rank test.

Cox proportional hazards models were employed to calculate hazard ratios (HR) with 95% confidence intervals (CI), assessing the association between variables and the events of interest. Univariate analyses were conducted to identify potential risk factors for RFS or OS. Variables with *P*-value < 0.05, along with variables of particular interest, were subsequently included in the forward stepwise multivariate analyses. We constructed three models to analyze the data. Model 1 adjusted for all variables with *P*-value < 0.05. Model 2 further adjusted for tumor burden factors based on Model 1. Finally, Model 3 incorporated hemodynamic factors in addition to the Model 2. Results with *P*-value < 0.05 were deemed statistically significant.

1. Mehta N, Dodge JL, Grab JD, et al. National Experience on Down-Staging of Hepatocellular Carcinoma Before Liver Transplant: Influence of Tumor Burden, Alpha-Fetoprotein, and Wait Time. Hepatology 2020;71:943-954.

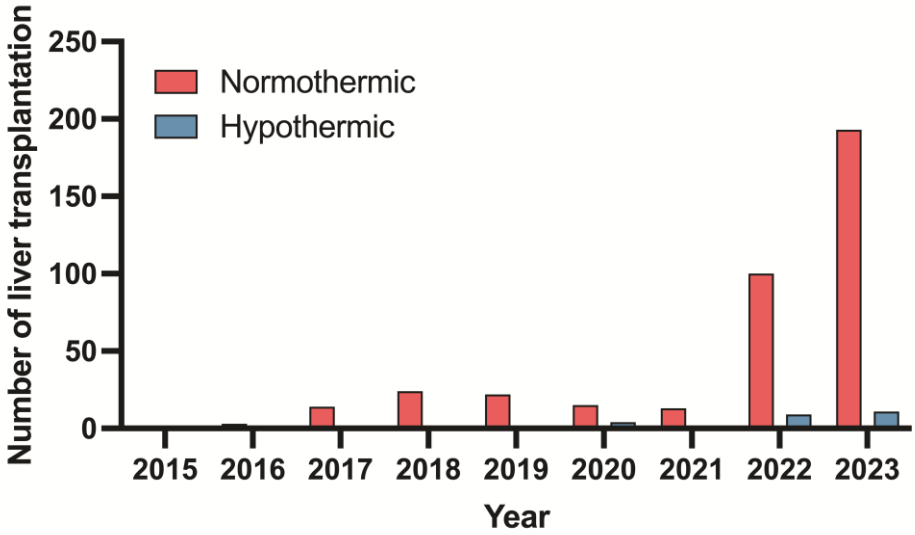

Figure S1. Trends in the use of machine perfusion from 2015 to 2024.

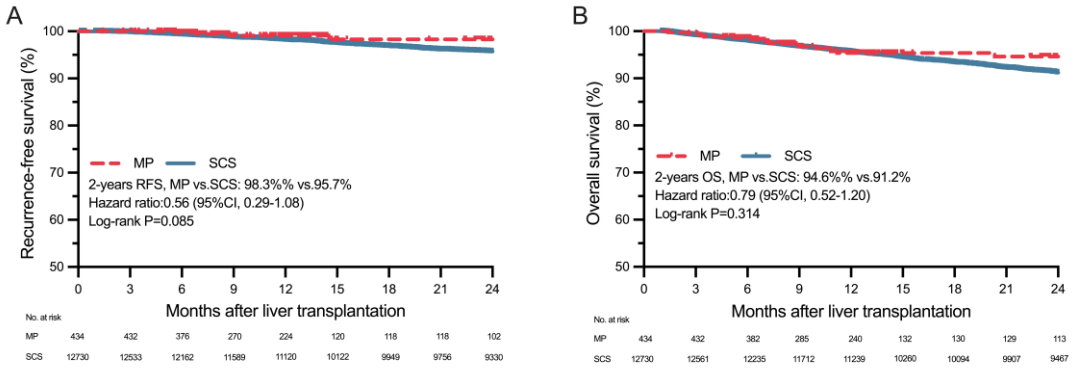

Figure S2. Recurrence and overall survival curves for MP vs. SCS preserve before the propensity score matched. (A) (B). RFS and OS curves in recipients MP vs. SCS using log-rank tests.

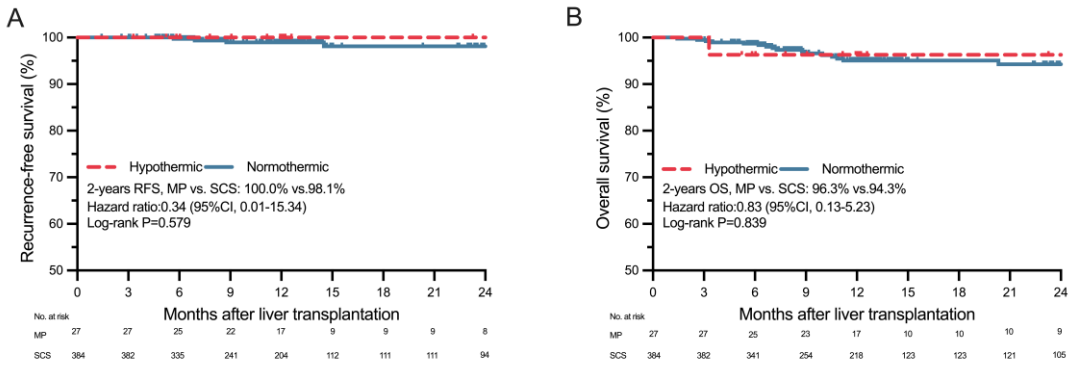

Figure S3. Recurrence and overall survival curves for hypothermic vs. normothermic preserve matched. (A) (B). RFS and OS curves in recipients hypothermic vs. normothermic using log-rank tests.

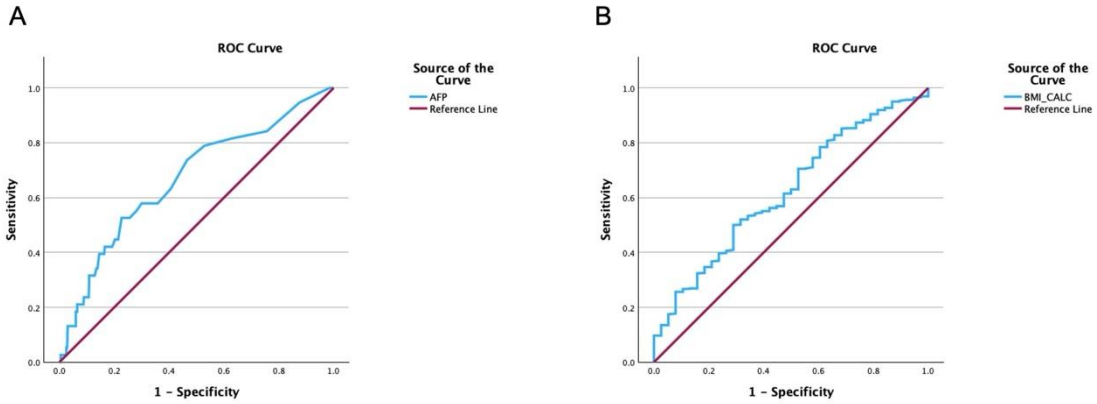

Figure S4. Receiver operating characteristic (ROC) curves for RFS. (A). ROC curve of AFP for 2-year RFS; (B). ROC curve of BMI for 2-year RFS.

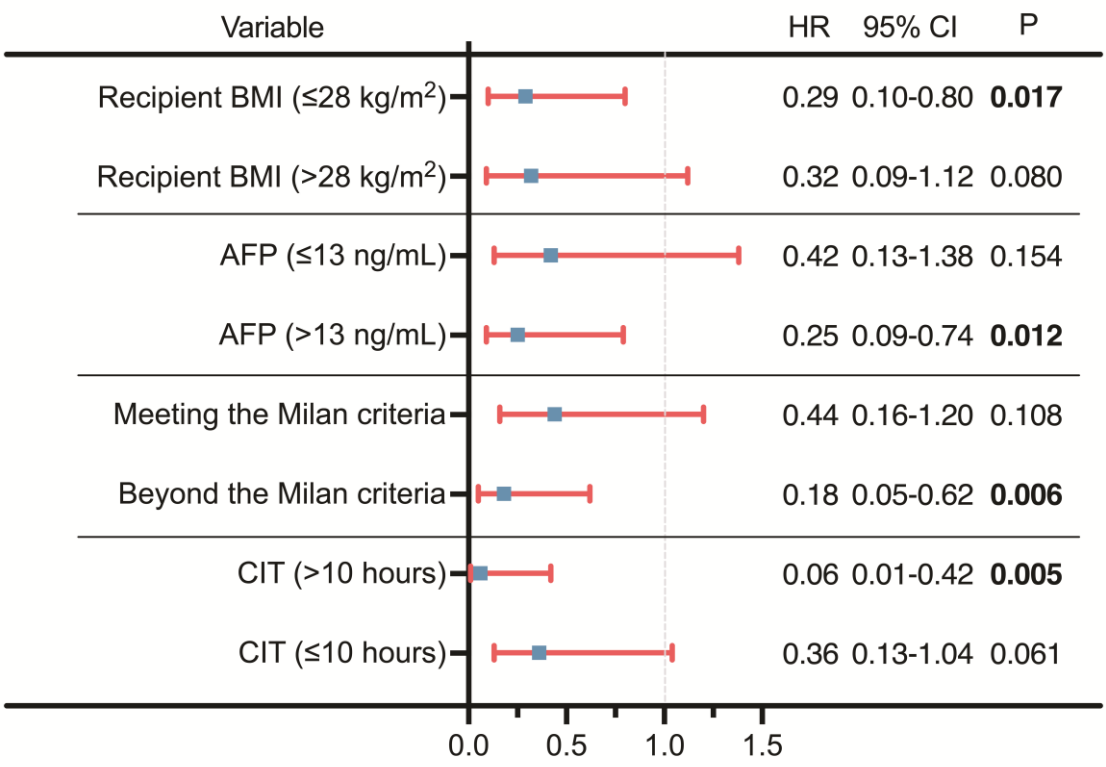

Figure S5. Subgroup analysis showing that MP improves RFS.

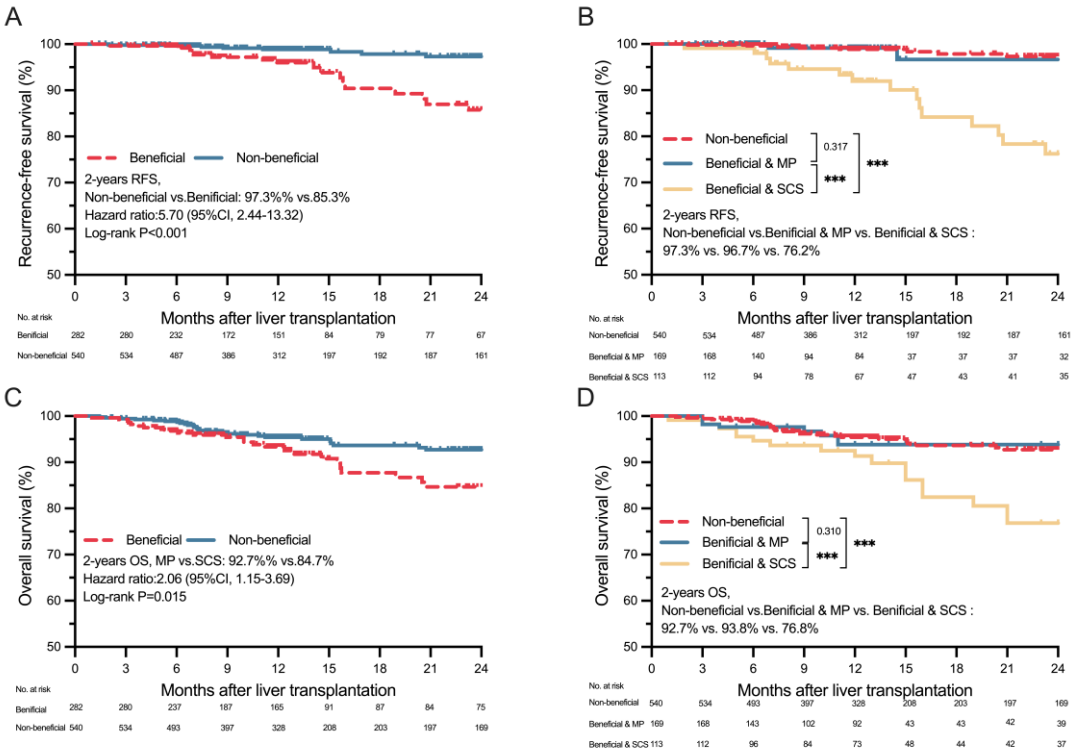

Figure S6. Recurrence survival curves for the benefit group and non-benefit group. (A). RFS for benefit and non-benefit groups; (B). RFS for benefit & MP, benefit & SCS and non-benefit groups; (C). OS for benefit and non-benefit groups; (D). OS for benefit & MP, benefit & SCS and non-benefit groups. *: <0.05; **: <0.01; ***: <0.001.

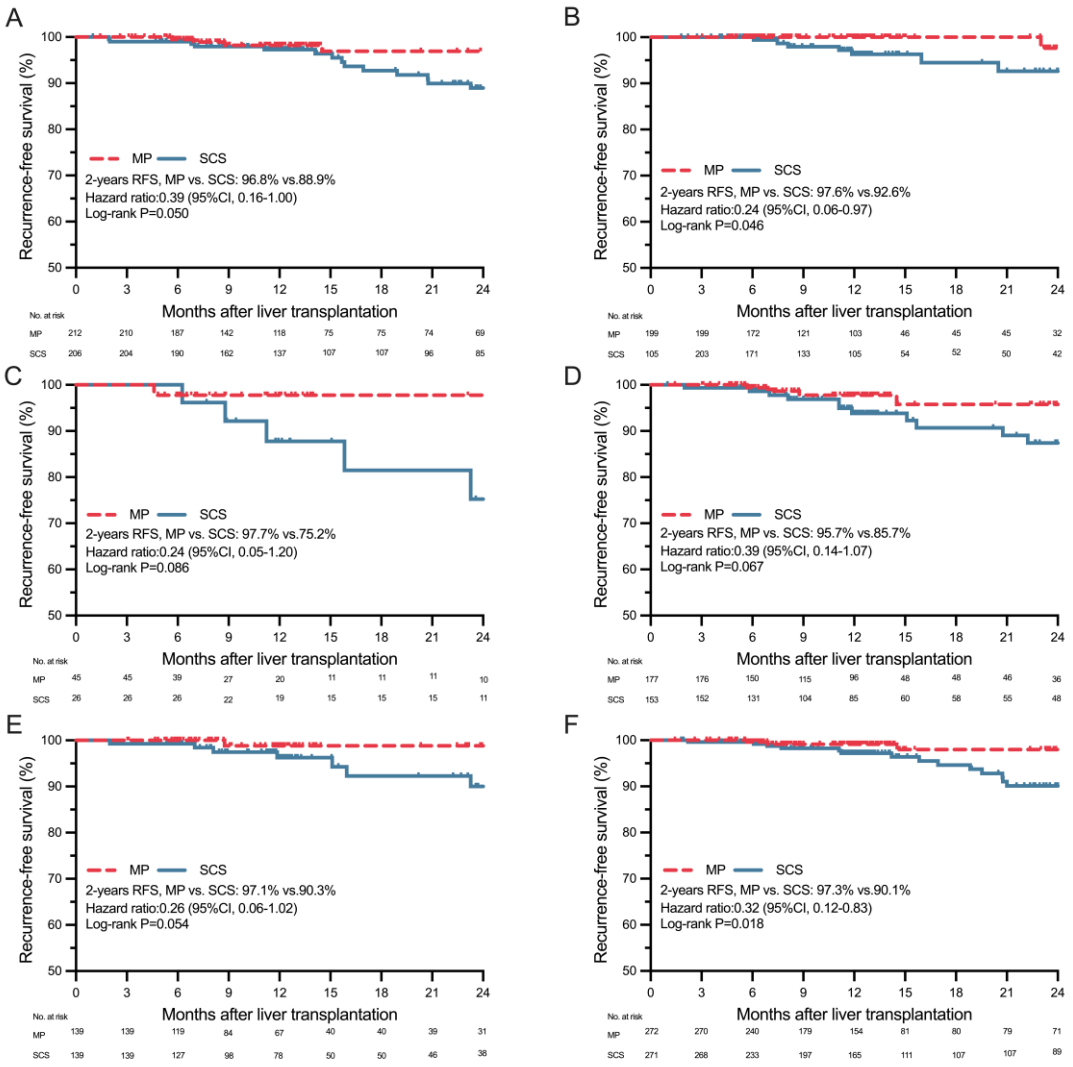

Figure S7. Sensitivity analysis of stratified analyses. (A) (B). RFS in the DBD and DCD subgroups; (C) (D). RFS in the steatotic and non-steatotic donor subgroups; (E) (F). RFS in the donor age ≥55 years and < 55 years subgroups.

Table S1. Variables included in the multiple imputation models

Variables
Donor BMI (kg/m^2)
Recipient BMI (kg/m^2)
Donor BUN (mg/dL)
Recipient BUN (mg/dL)
Donor serum creatinine (mg/dL)
Recipient hospital stay (day)
Donor serum sodium (mmol/L)
Donor total bilirubin (umol/L)
Recipient total bilirubin (umol/L)
Tumor differentiation
Satellite lesions
Vascular invasion

Abbreviations: BMI, body mass index; BUN, blood urea nitrogen.

Table S2. Variables included in the PSM models for the final cohort

Variables
Pretransplant AFP (ng/mL)
Donor age (years)
Recipient age (years)
Donor gender
Recipient gender
DCD donors
Size biggest HCC lesion (cm)
Overall size all HCCs (cm)
Tumor differentiation
Satellite lesions
Vascular invasion
Transplantation year

Abbreviations: AFP, alpha-fetoprotein; DCD, donation after circulatory death; HCC, hepatocellular carcinoma.

Table S3. Univariable Cox regression analyses for the recurrence-free survival and overall survival.

	RFS			OS		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Preservation method (MP vs. SCS)	0.221	0.076-0.645	**0.006**	0.527	0.295-0.942	**0.031**
Donor age (years)	1.003	0.977-1.030	0.834	1.001	0.983-1.020	0.879
Donor gender (male)	0.433	0.198-0.949	**0.037**	0.713	0.410-1.241	0.232
Donor antihypertensives (yes vs. no)	2.043	0.932-4.478	0.075	1.359	0.777-2.337	0.282
Donor BMI (kg/m^2)	0.967	0.908-1.031	0.308	1.009	0.973-1.047	0.619
Donor serum creatinine (mg/dL)	0.846	0.619-1.157	0.296	0.886	0.728-1.077	0.225
Donor total bilirubin (mg/dL)	1.407	0.908-2.180	0.127	0.935	0.586-1.491	0.778
Donor serum sodium (mmol/L)	0.979	0.928-1.032	0.429	0.978	0.943-1.014	0.230
DCD donors	0.552	0.230-1.328	0.185	0.717	0.407-1.264	0.251
Donor CPR	1.175	0.727-4.230	0.211	1.027	0.781-1.350	0.851
Age at transplant (years)	1.011	0.953-1.071	0.723	1.022	0.980-1.066	0.314
Gender (male)	1.458	0.500-4.250	0.490	1.340	0.653-2.749	0.425
Recipient BMI (kg/m^2)	0.913	0.840-0.993	**0.033**	0.952	0.901-1.005	0.076
Preoperative MELD score	0.993	0.931-1.059	0.829	1.023	0.984-1.064	0.247
Recipient total bilirubin (mg/dL)	1.005	0.889-1.136	0.936	1.093	1.068-1.118	**<0.001**
Recipient antihypertensives (yes vs. no)	0.496	0.147-1.677	0.259	0.380	0.150-0.963	**0.041**
Wait time (days)	1.000	0.998-1.001	0.822	1.000	1.000-1.001	0.365
CIT (hours)	0.992	0.901-1.093	0.871	1.009	0.952-1.068	0.771
Size biggest HCC lesion (cm)	1.158	0.929-1.443	0.192	0.901	0.747-1.086	0.274
Overall size all HCCs (cm)	1.118	0.980-1.274	0.096	0.917	0.789-1.066	0.258
Number of lesions (single)	2.651	1.143-6.149	**0.023**	0.924	0.417-2.050	0.847
Pretransplant AFP (ng/mL)	1.002	1.000-1.004	0.083	0.999	0.995-1.003	0.526
Meeting the Milan criteria	3.197	1.436-7.119	**0.004**	1.296	0.666-2.522	0.445
Satellite lesions	0.798	0.108-5.903	0.825	0.400	0.055-2.895	0.364
Tumor differentiation	-	-	-	-	-	-
Complete tumor necrosis	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Well	1.516	0.306-7.511	0.610	2.171	0.873-5.397	0.095
Moderate	2.903	0.846-9.964	0.090	1.842	0.837-4.054	0.129
Poor	5.847	1.197-28.998	**0.031**	4.170	1.445-12.033	**0.033**
Vascular invasion	-	-	-	-	-	-
None	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Microvascular invasion	2.791	1.165-6.682	**0.021**	1.864	0.958-3.627	0.067
Macrovascular	-	-	-	-	-	-

Abbreviations: BMI, body mass index; DCD, donation after circulatory death; CPR, cardiopulmonary resuscitation; MELD, model for end-stage liver disease; AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma.

Table S4. Multivariable Cox regression analyses for the overall survival.

	Model 1			Model 2			Model 3		
	HR	95%CI	P	HR	95%CI	P	HR	95%CI	P
Preservation method (MP vs. SCS)	0.426	0.225-0.805	**0.009**	0.426	0.225-0.805	**0.009**	0.316	0.160-0.627	**<0.001**
Recipient total bilirubin (umol/L)	1.131	1.096-1.167	**<0.001**	1.131	1.096-1.167	**<0.001**	1.142	1.105-1.180	**<0.001**
Tumor differentiation	-	-	-	-	-	-	-	-	-
Complete tumor necrosis	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Well	4.489	1.479-13.630	0.008	4.489	1.479-13.630	**0.008**	4.781	1.566-14.598	**0.006**
Moderate	3.995	1.384-11.303	**0.010**	3.995	1.384-11.303	**0.010**	4.045	1.402-11.672	**0.010**
Poor	9.899	2.635-37.193	**<0.001**	9.899	2.635-37.193	**<0.001**	10.608	2.800-40.192	**<0.001**
CIT (10 hours)	-	-	-	-	-	-	2.292	1.128-4.661	**0.022**

Note: Values in bold represent statistically significant differences.

Model 1: Adjusted for Preservation method, Recipient age, Recipient total bilirubin, Recipient serum creatinine, Tumor differentiation

Model 2: Adjusted for Model 1 + Pre-transplant AFP + Meeting the Milan criteria + Satellite lesions + Vascular invasion

Model 3: Adjusted for Model 2 + CIT + DCD donor + Donor CPR

Abbreviations: LT, liver transplantation; MP, machine perfusion; SCS, static cold storage; DCD, donation after circulatory death; AFP, alpha-fetoprotein; CIT, cold ischemia time

Table S5. Scoring of Subgroups Where MP Improves HCC Transplantation Outcomes

	0 point	1 point
Pretransplant AFP (ng/mL)	< 13	≥ 13
Recipient BMI (kg/m^2)	≥ 28	< 28
Meeting the Milan criteria	No	Yes
CIT (10 hours)	< 10	≥ 10

Abbreviations: AFP, alpha-fetoprotein; CIT, cold ischemia time; BMI, body mass index.

Table S6. Patient characteristics of the beneficial subgroups

	Entire cohort (n=282)	Machine perfusion (n=169)	Static cold storage (n=113)	*P*-value
Donor characteristics				
Donor age (years)	47.0 (38.8-57.0)	47.0 (38.0-56.5)	47.0 (39.0-58.5)	0.529
Gender (male)	182 (64.5%)	113 (66.9%)	69 (61.1%)	0.374
Donor BMI (kg/m^2)	27.1 (23.5-32.6)	27.2 (23.9-33.2)	27.0 (23.2-31.5)	0.093
Donor serum creatinine (mg/dL)	1.0 (0.7-1.8)	1.0 (0.7-1.9)	0.9 (0.6-1.6)	0.228
Donor total bilirubin (mg/dL)	0.5 (0.3-0.8)	0.5 (0.3-0.8)	0.5 (0.4-0.7)	0.438
Donor serum sodium (mmol/L)	146.5 (141.0-151.3)	148.0 (142.0-152.0)	146.0 (141.0-151.0)	0.183
DCD donors	144 (51.1%)	89 (52.7%)	55 (48.7%)	0.544
Donor CPR	145 (51.4%)	82 (48.5%)	63 (55.8%)	0.421
Donor antihypertensives	94 (33.3%)	60 (35.5%)	34 (30.1%)	0.369
Cold ischemia time (hours)	11.2 (6.0-15.0)	12.9 (10.3-16.5)	5.6 (4.5-10.6)	**<0.001**
Recipient characteristics				
Age at transplant (years)	65.0 (60.0-68.0)	64.0 (59.0-68.0)	65.0 (60.0-68.0)	0.250
Gender (male)	217 (77.0%)	131 (77.5%)	86 (76.1%)	0.775
Recipient BMI (kg/m^2)	26.1 (23.7-28.1)	26.4 (24.4-29.3)	25.8 (23.1-27.7)	0.056
Recipient total bilirubin (mg/dL)	0.6 (0.4-0.8)	0.5 (0.4-0.8)	0.6 (0.4-1.0)	**0.023**
Preoperative MELD score	11.0 (8.8-16.0)	11.0 (9.0-16.0)	10.0 (8.0-15.0)	0.161
Waiting time (days)	237.0 (133.8-423.5)	229.0 (114.5-485.5)	238.0 (161.5-343.5)	0.658
Tumor parameter				
Pretransplant AFP (ng/mL)	14.0 (5.0-34.5)	9.0 (4.0-27.5)	10.0 (11.0-25.0)	0.098
Size biggest HCC lesion (cm)	1.7 (0.0-2.4)	1.6 (0.0-2.3)	2.0 (0.0-2.7)	0.365
Overall size all HCCs (cm)	2.0 (0.0-3.1)	2.0 (0.0-2.9)	2.0 (0.0-3.4)	0.361
Number of lesions (single)	229 (81.2%)	142 (84.0%)	87 (77.0%)	0.162
Meeting the Milan criteria	189 (67.0%)	120 (71.0%)	69 (61.1%)	0.093
Satellite lesions	16 (5.7%)	9 (5.3%)	7 (6.2%)	0.796
Tumor differentiation				0.766
Complete tumor necrosis	54 (19.1%)	29 (17.2%)	25 (22.1%)	
Well	48 (17.0%)	29 (17.2%)	19 (16.8%)	
Moderate	150 (53.2%)	93 (55.0%)	57 (50.4%)	
Poor	30 (10.6%)	18 (10.7%)	12 (10.6%)	
Vascular invasion				0.764
None	199 (70.6%)	122 (72.2%)	77 (68.1%)	
Microvascular invasion	74 (26.2%)	42 (24.9%)	32 (28.3%)	
Macrovascular	9 (3.2%)	5 (3.0%)	4 (3.5%)	
Duration of follow-up (months)	12.2 (12.0-12.5)	12.1 (12.0-12.2)	14.1 (12.4-16.9)	0.143

Note: Results are presented as n (%) or median (IQR). Values in bold represent statistically significant differences.
Abbreviations: BMI, body mass index; DCD, donation after circulatory death; CPR, cardiopulmonary resuscitation; MELD, model for end-stage liver disease; AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma.

Table S7. Patient characteristics of the non-beneficial subgroups

	Entire cohort (n=540)	Machine perfusion (n=242)	Static cold storage (n=298)	*P*-value
Donor characteristics				
Donor age (years)	46.0 (33.0-58.0)	48.0 (34.0-59.0)	45.0 (32.0-57.0)	0.217
Gender (male)	370 (68.5%)	165 (68.2%)	205 (68.8%)	0.926
Donor BMI (kg/m^2)	27.3 (23.9-32.1)	27.8 (24.1-32.9)	26.9 (23.8-31.9)	0.135
Donor serum creatinine (mg/dL)	1.1 (0.7-2.0)	1.1 (0.7-1.8)	1.2 (1.0-1.6)	0.672
Donor total bilirubin (mg/dL)	0.6 (0.4-0.9)	0.6 (0.4-0.9)	0.6 (0.4-0.8)	0.194
Donor serum sodium (mmol/L)	147.0 (142.0-153.0)	147.0 (140.0-152.0)	147.0 (142.0-153.0)	0.234
DCD donors	260 (48.1%)	110 (45.5%)	150 (50.3%)	0.259
Donor CPR	305 (56.5%)	141 (58.3%)	164 (55.0%)	0.303
Donor antihypertensives	171 (31.7%)	78 (32.2%)	93 (31.2%)	0.852
Cold ischemia time (hours)	6.8 (5.1-9.5)	8.9 (6.9-12.6)	5.5 (4.4-7.0)	**<0.001**
Recipient characteristics				
Age at transplant (years)	64.0 (59.3-68.0)	64.0 (59.0-68.0)	64.0 (60.0-68.0)	0.286
Gender (male)	418 (77.4%)	182 (75.2%)	236 (79.2%)	0.270
Recipient BMI (kg/m^2)	30.3 (28.1-33.7)	27.8 (24.1-32.9)	30.1 (27.0-33.5)	0.053
Recipient total bilirubin (mg/dL)	0.6 (0.4-0.9)	0.6 (0.4-0.9)	0.6 (0.4-1.0)	0.750
Preoperative MELD score	11.0 (8.0-16.0)	11.0 (8.0-16.0)	11.0 (8.0-16.0)	0.386
Waiting time (days)	249.5 (193.3-391.5)	266.5 (195.5-444.5)	244.0 (190.0-358.3)	**0.033**
Tumor parameter				
Pretransplant AFP (ng/mL)	5.0 (3.0-8.0)	5.0 (3.0-8.0)	5.0 (3.0-9.0)	0.071
Size biggest HCC lesion (cm)	1.5 (0.0-2.4)	2.0 (0.0-2.5)	1.0 (0.0-2.4)	0.083
Overall size all HCCs (cm)	1.6 (0.0-2.4)	2.1 (0.0-3.1)	1.0 (0.0-2.7)	0.102
Number of lesions (single)	483 (89.4%)	217 (89.7%)	266 (89.3%)	0.878
Meeting the Milan criteria	496 (91.9%)	225 (93.0%)	271 (90.0%)	0.390
Satellite lesions	21 (3.9%)	10 (4.1%)	11 (3.7%)	0.792
Tumor differentiation				0.948
Complete tumor necrosis	165 (30.6%)	72 (29.8%)	93 (31.2%)	
Well	95 (17.6%)	45 (18.6%)	50 (16.8%)	
Moderate	257 (47.6%)	115 (47.5%)	142 (47.7%)	
Poor	23 (4.3%)	10 (4.1%)	13 (4.4%)	
Vascular invasion				0.624
None	504 (93.3%)	227 (93.8%)	277 (93.0%)	
Microvascular invasion	31 (5.7%)	12 (5.0%)	19 (6.4%)	
Macrovascular	5 (1.0%)	3 (1.2%)	2 (0.7%)	
Duration of follow-up (months)	12.7 (12.0-13.3)	12.7 (12.0-13.3)	12.6 (11.7-13.5)	0.631

Note: Results are presented as n (%) or median (IQR). Values in bold represent statistically significant differences.
Abbreviations: BMI, body mass index; DCD, donation after circulatory death; CPR, cardiopulmonary resuscitation; MELD, model for end-stage liver disease; AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma.

Table S8. Patient characteristics of excluding HCC patients who underwent LT prior to 2022

	Entire cohort (n=580)	Machine perfusion (n=313)	Static cold storage (n=267)	*P*-value
Donor characteristics				
Donor age (years)	47.0 (35.0-58.0)	47.0 (35.0-59.0)	47.0 (35.0-58.0)	0.982
Gender (male)	388 (66.9%)	207 (66.1%)	181 (67.8%)	0.723
Donor BMI (kg/m^2)	27.2 (23.7-32.2)	27.2 (23.9-33.2)	27.2 (23.6-31.9)	0.201
Donor serum creatinine (mg/dL)	1.0 (0.7-1.9)	1.0 (0.7-1.9)	1.0 (0.7-1.8)	0.877
Donor total bilirubin (mg/dL)	0.6 (0.4-0.8)	0.6 (0.4-0.8)	0.5 (0.3-0.8)	0.181
Donor serum sodium (mmol/L)	146.0 (141.0-152.0)	147.0 (141.0-152.0)	146.0 (142.0-152.0)	0.820
DCD donors	335 (57.8%)	173 (55.3%)	162 (60.7%)	0.206
Donor CPR	310 (53.4%)	168 (53.7%)	142 (53.2%)	0.865
Donor antihypertensives	191 (32.9%)	111 (35.5%)	80 (30.0%)	0.184
Cold ischemia time (hours)	8.9 (5.5-14.2)	12.2 (8.7-15.9)	5.8 (4.5-8.1)	**<0.001**
Recipient characteristics				
Age at transplant (years)	65.0 (60.0-68.0)	65.0 (59.0-68.0)	65.0 (60.0-69.0)	0.190
Gender (male)	439 (75.7%)	236 (75.4%)	203 (76.0%)	0.923
Recipient BMI (kg/m^2)	29.0 (25.6-32.6)	29.3 (25.7-32.6)	28.7 (25.6-32.3)	0.447
Recipient total bilirubin (mg/dL)	0.6 (0.4-0.8)	0.5 (0.4-0.8)	0.6 (0.4-0.8)	0.068
Preoperative MELD score	11.0 (8.0-16.0)	12.0 (9.0-17.0)	11.0 (8.0-15.0)	**0.040**
Waiting time (days)	249.0 (186.3-416.3)	247.0 (158.0-474.0)	250.0 (193.0-371.0)	0.799
Tumor parameter				
Pretransplant AFP (ng/mL)	6.0 (3.0-12.0)	5.0 (3.0-11.0)	6.0 (4.0-13.0)	0.258
Size biggest HCC lesion (cm)	1.7 (0.0-2.4)	1.8 (0.0-2.4)	1.4 (0.0-2.5)	0.407
Overall size all HCCs (cm)	1.9 (0.0-2.9)	2.0 (0.0-2.9)	1.5 (0.0-2.8)	0.233
Number of lesions (single)	513 (88.4%)	275 (87.9%)	238 (89.1%)	0.398
Meeting the Milan criteria	490 (84.5%)	264 (84.3%)	226 (84.6%)	1.000
Satellite lesions	22 (3.8%)	13 (4.2%)	9 (3.4%)	0.669
Tumor differentiation				0.822
Complete tumor necrosis	157 (27.1%)	80 (25.6%)	77 (28.8%)	
Well	102 (17.6%)	55 (17.6%)	47 (17.6%)	
Moderate	279 (48.1%)	154 (49.2%)	125 (46.8%)	
Poor	42 (7.2%)	24 (7.7%)	18 (6.7%)	
Vascular invasion				0.588
None	498 (85.9%)	265 (84.7%)	233 (87.3%)	
Microvascular invasion	74 (12.8%)	44 (14.1%)	30 (11.2%)	
Macrovascular	8 (1.4%)	4 (1.3%)	4 (1.5%)	
Duration of follow-up (months)	11.9 (11.8-12.1)	11.9 (11.6-12.3)	12.0 (11.8-12.1)	0.712

Note: Results are presented as n (%) or median (IQR). Values in bold represent statistically significant differences.
Abbreviations: BMI, body mass index; DCD, donation after circulatory death; CPR, cardiopulmonary resuscitation; MELD, model for end-stage liver disease; AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma.

Table S9. Patient characteristics of excluding HCC patients with a follow-up time of less than 6 months

	Entire cohort (n = 720)	Machine perfusion (n = 360)	Static cold storage (n = 360)	*P*-value
Donor characteristics				
Donor age (years)	47.0 (34.3-58.0)	47.0 (34.3-58.0)	46.0 (34.3-58.0)	0.917
Gender (male)	485 (67.4%)	247 (68.6%)	238 (66.1%)	0.525
Donor BMI (kg/m^2)	27.4 (23.9-32.1)	27.9 (23.9-33.1)	27.0 (23.8-31.9)	0.074
Donor serum creatinine (mg/dL)	1.0 (0.7-2.0)	1.1 (0.7-2.0)	1.0 (0.7-1.9)	0.877
Donor total bilirubin (mg/dL)	0.6 (0.4-0.8)	0.6 (0.4-0.9)	0.6 (0.4-0.8)	0.135
Donor serum sodium (mmol/L)	147.0 (142.0-152.0)	148.0 (141.0-152.0)	147.0 (142.0-153.0)	0.533
DCD donors	338 (46.9%)	170 (47.2%)	168 (46.7%)	0.940
Donor CPR	394 (54.7%)	196 (54.4%)	198 (55.0%)	0.562
Donor antihypertensives	235 (32.6%)	120 (33.3%)	115 (31.9%)	0.751
Cold ischemia time (hours)	7.3 (5.3-11.5)	10.2 (7.6-13.8)	5.5 (4.5-7.1)	**<0.001**
Recipient characteristics				
Age at transplant (years)	64.0 (60.0-68.0)	64.0 (59.0-68.0)	64.5 (60.0-68.0)	0.298
Gender (male)	555 (77.1%)	277 (76.9%)	278 (77.2%)	1.000
Recipient BMI (kg/m^2)	28.9 (25.5-32.4)	29.2 (25.7-32.5)	28.7 (25.4-32.4)	0.330
Recipient total bilirubin (mg/dL)	0.6 (0.4-0.9)	0.6 (0.4-0.8)	0.6 (0.4-1.0)	0.083
Preoperative MELD score	11.0 (8.0-16.0)	11.0 (8.0-16.0)	11.0 (8.0-16.0)	0.200
Waiting time (days)	249.0 (190.3-401.5)	266.0 (192.0-457.8)	242.5 (187.5-343.0)	**0.027**
Tumor parameter				
Pretransplant AFP (ng/mL)	6.0 (3.0-13.0)	6.0 (3.0-12.0)	6.5 (4.0-15.8)	0.088
Size biggest HCC lesion (cm)	1.6 (0.0-2.5)	2.0 (0.0-2.5)	1.2 (0.0-2.4)	0.080
Overall size all HCCs (cm)	1.9 (0.0-2.9)	2.0 (0.0-3.0)	1.3 (0.0-2.8)	0.074
Number of lesions (single)	621 (86.3%)	314 (87.2%)	307 (85.3%)	0.262
Meeting the Milan criteria	599 (83.2%)	303 (84.2%)	296 (82.2%)	0.550
Satellite lesions	31 (4.3%)	15 (4.2%)	16 (4.4%)	1.000
Tumor differentiation				0.501
Complete tumor necrosis	194 (26.9%)	88 (24.4%)	106 (29.4%)	
Well	123 (17.1%)	63 (17.5%)	60 (16.7%)	
Moderate	363 (50.4%)	189 (52.5%)	174 (48.3%)	
Poor	40 (5.6%)	20 (5.6%)	20 (5.6%)	
Vascular invasion				0.507
None	617 (85.7%)	307 (85.3%)	310 (86.1%)	
Microvascular invasion	91 (12.6%)	45 (12.5%)	46 (12.8%)	
Macrovascular	12 (1.7%)	8 (2.2%)	4 (1.1%)	
Duration of follow-up (months)	13.2 (12.5-13.8)	12.7 (12.1-13.3)	14.5 (9.5-19.5)	0.086

Note: Results are presented as n (%) or median (IQR). Values in bold represent statistically significant differences.
Abbreviations: BMI, body mass index; DCD, donation after circulatory death; CPR, cardiopulmonary resuscitation; MELD, model for end-stage liver disease; AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma.

Table S10. Patient characteristics of excluding HCC patients with missing data

	Entire cohort (n = 538)	Machine perfusion (n = 269)	Static cold storage (n = 269)	*P*-value
Donor characteristics				
Donor age (years)	48.0 (35.0-59.0)	50.0 (35.0-58.5)	48.0 (35.0-59.0)	0.799
Gender (male)	360 (66.9%)	181 (67.3%)	179 (66.5%)	0.927
Donor BMI (kg/m^2)	27.4 (23.9-32.6)	27.6 (24.1-33.2)	27.2 (23.7-32.0)	0.254
Donor serum creatinine (mg/dL)	1.0 (0.7-2.0)	1.1 (0.7-2.0)	1.1 (0.7-2.0)	0.484
Donor total bilirubin (mg/dL)	0.6 (0.4-0.9)	0.6 (0.4-0.9)	0.6 (0.4-0.9)	0.875
Donor serum sodium (mmol/L)	147.0 (141.0-152.0)	148.0 (141.0-152.0)	146.0 (142.0-151.0)	0.189
DCD donors	258 (48.0%)	122 (45.4%)	136 (50.6%)	0.262
Donor CPR	287 (53.3%)	146 (54.3%)	141 (52.4%)	0.930
Donor antihypertensives	161 (30.0%)	88 (32.7%)	73 (27.1%)	0.187
Cold ischemia time (hours)	7.7 (5.7-11.5)	9.9 (7.6-13.7)	6.0 (4.9-7.9)	**<0.001**
Recipient characteristics				
Age at transplant (years)	65.0 (60.0-69.0)	65.0 (60.0-69.0)	65.0 (60.5-69.0)	0.656
Gender (male)	419 (77.9%)	205 (76.2%)	214 (80.0%)	0.406
Recipient BMI (kg/m^2)	29.2 (25.7-33.0)	29.4 (26.0-32.5)	29.0 (25.4-33.4)	0.796
Recipient total bilirubin (mg/dL)	0.6 (0.4-1.0)	0.6 (0.4-0.9)	0.6 (0.4-1.2)	0.114
Preoperative MELD score	11.0 (8.0-15.0)	10.0 (8.0-14.0)	11.0 (8.0-15.0)	0.071
Waiting time (days)	271.0 (207.0-446.5)	304.0 (213.0-477.5)	254.0 (198.0-399.0)	**0.002**
Tumor parameter				
Pretransplant AFP (ng/mL)	6.0 (3.0-13.0)	5.0 (3.0-10.5)	6.0 (3.0-14.5)	0.090
Size biggest HCC lesion (cm)	2.0 (0.0-2.5)	1.9 (0.0-2.4)	2.0 (0.0-2.6)	0.165
Overall size all HCCs (cm)	2.1 (0.0-3.0)	2.0 (0.0-3.1)	2.1 (0.0-3.0)	0.328
Number of lesions (single)	469 (87.2%)	235 (87.4%)	234 (87.0%)	0.922
Meeting the Milan criteria	529 (98.3%)	265 (98.5%)	264 (98.1%)	1.000
Satellite lesions	23 (4.3%)	8 (3.0%)	15 (5.6%)	0.200
Tumor differentiation				0.181
Complete tumor necrosis	104 (19.3%)	62 (23.0%)	42 (15.6%)	
Well	105 (19.5%)	51 (19.0%)	54 (20.1%)	
Moderate	298 (55.4%)	142 (52.8%)	156 (58.0%)	
Poor	31 (5.8%)	14 (5.2%)	17 (6.3%)	
Vascular invasion				0.297
None	465 (86.4%)	234 (87.0%)	231 (85.9%)	
Microvascular invasion	57 (10.6%)	30 (11.2%)	27 (10.0%)	
Macrovascular	16 (3.0%)	5 (1.9%)	11 (4.1%)	
Duration of follow-up (months)	12.5 (12.0-12.9)	12.3 (11.8-12.8)	12.8 (12.3-13.4)	0.110

Note: Results are presented as n (%) or median (IQR). Values in bold represent statistically significant differences.
Abbreviations: BMI, body mass index; DCD, donation after circulatory death; CPR, cardiopulmonary resuscitation; MELD, model for end-stage liver disease; AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma.

Table S11. Association between preservation method and recurrence of HCC in sensitivity analyses

	Excluding HCC patients who underwent liver transplantation prior to 2022 (n = 580)			Excluding HCC patients with a follow-up time of less than 6 months (n = 720)		
Model 1	HR	95%CI	*P*-value	HR	95%CI	*P*-value
Preservation method (SCS)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Preservation method (MP)	0.094	0.012-0.736	**0.024**	0.246	0.084-0.720	**0.010**
Model 2						
Preservation method (SCS)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Preservation method (MP)	0.096	0.012-0.749	**0.025**	0.224	0.076-0.666	**0.007**
Model 3						
Preservation method (SCS)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Preservation method (MP)	0.121	0.015-0.962	**0.046**	0.231	0.077-0.689	**0.009**
	Excluding HCC patients with missing values in key variables (n = 538)			Propensity-matched cohort* (n = 822)		
Model 1	HR	95%CI	*P*-value	HR	95%CI	*P*-value
Preservation method (SCS)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Preservation method (MP)	0.162	0.048-0.552	**0.004**	0.226	0.077-0.668	**0.007**
Model 2						
Preservation method (SCS)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Preservation method (MP)	0.162	0.048-0.552	**0.004**	0.201	0.064-0.636	**0.006**
Model 3						
Preservation method (SCS)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Preservation method (MP)	0.162	0.048-0.552	**0.004**	0.172	0.047-0.625	**0.008**

Note: Values in bold represent statistically significant differences.

Model 1: Adjusted for Preservation method, Recipient BMI, Donor gender, Tumor differentiation, Meeting the Milan criteria, Vascular invasion

Model 2: Adjusted for Model 1 + Pre-transplant AFP + Satellite lesions

Model 3: Adjusted for Model 2 + CIT + DCD donor + Donor CPR

Abbreviations: HCC, hepatocellular carcinoma; LT, liver transplantation; MP, machine perfusion; SCS, static cold storage; BMI, body mass index; DCD, donation after circulatory death; CPR, cardiopulmonary resuscitation; AFP, alpha-fetoprotein; CIT, cold ischemia time.

*: We used the Fine and Gray's sub-distribution hazards regression model.

Table S12. Association between preservation method and recurrence of HCC in subgroup sensitivity analyses

	Donor type					
	DCD			**DBD**		
Model 1	HR	95%CI	*P*-value	HR	95%CI	*P*-value
Preservation method (SCS)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Preservation method (MP)	0.150	0.018-1.254	0.080	0.366	0.120-1.116	0.077
Model 2						
Preservation method (SCS)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Preservation method (MP)	0.150	0.016-0.436	0.100	0.366	0.120-1.116	0.077
Model 3						
Preservation method (SCS)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Preservation method (MP)	0.071	0.004-1.131	0.061	0.385	0.125-1.185	0.096
	Steatotic donors					
	Steatotic*			**Non-steatotic**		
Model 1	HR	95%CI	*P*-value	HR	95%CI	*P*-value
Preservation method (SCS)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Preservation method (MP)	-	-	-	0.424	0.130-1.342	0.155
Model 2						
Preservation method (SCS)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Preservation method (MP)	-	-	-	0.424	0.130-1.342	0.155
Model 3						
Preservation method (SCS)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Preservation method (MP)	-	-	-	0.420	0.129-1.368	0.150
	Donor age					
	≥ 55 years			**< 55 years**		
Model 1	HR	95%CI	*P*-value	HR	95%CI	*P*-value
Preservation method (SCS)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Preservation method (MP)	0.165	0.020-1.340	0.090	0.232	0.066-0.809	0.022
Model 2						
Preservation method (SCS)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Preservation method (MP)	0.205	0.025-1.712	0.143	0.232	0.066-0.809	0.022
Model 3						
Preservation method (SCS)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Preservation method (MP)	0.247	0.029-2,125	0.203	0.223	0.064-0.781	0.019

Model 1: Adjusted for Preservation method, Recipient BMI, Donor gender, Tumor differentiation, Meeting the Milan criteria, Vascular invasion

Model 2: Adjusted for Model 1 + Pre-transplant AFP + Satellite lesions

Model 3: Adjusted for Model 2 + CIT + DCD donor + Donor CPR

Abbreviations: HCC, hepatocellular carcinoma; MP, machine perfusion; SCS, static cold storage; BMI, body mass index; DCD, donation after circulatory death; CPR, cardiopulmonary resuscitation; AFP, alpha-fetoprotein; CIT, cold ischemia time.

*: Due to the limited sample size, Cox regression analysis yielded numerically unstable estimates. These data were therefore omitted from the final multivariate model to avoid overinterpretation.