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Treating Donation After Circulatory Death Liver Grafts With Alteplase During Ex Situ Normothermic Machine Perfusion

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ABSTRACT

Introduction: Normothermic machine perfusion (NMP) is increasing the safe utilization of donation after circulatory death livers. Historically, tissue plasminogen activator (tPA) has been administered intraoperatively to DCD graft recipients to reduce non-anastomotic biliary complications (NAS). Treating the liver during NMP offers a potentially safer administration, preventing systemic treatment of the recipient. In this retrospective study, we explore our center's experience of giving tPA with FFP during NMP and its effects on clinical chemistries during perfusion, intraoperative transfusions, and post-operative outcomes and clinical chemistries.

Methods: One hundred and twenty-seven livers were perfused using the OrganOx *metra*, including 56 DCD livers (tPA + NMP-DCD) that received a bolus of 10 mg tPA followed by a 90-min infusion of 40 mg. Sixty-five livers from donation after brain death donors underwent NMP without tPA (NMP-DBD). A historical, propensity-matched cohort of 78 livers from non-NMP DCD donor livers ($n = 51$) were an additional comparator group (tPA-DCD).

Results: Intraoperative transfusions, 30-day and 3-month patient survival, and 30-day and 3-month graft survival were not statistically different between the tPA-NMP-DCD and NMP-DBD groups, except for cell saver volumes ($p = 0.0034$). Less platelets and cryoprecipitate transfusions were observed in the tPA-NMP-DCD livers compared to historical tPA-DCD livers ($p = 0.0021$ and 0.0046, respectively). One incidence of primary non-function occurred in the tPA-DCD group, and the tPA-NMP-DCD arm had one case of ischemic cholangiopathy required re-transplant. There was a higher reoperation rate for hematoma evacuation in the NMP-DBD cohort. Minimum follow-up time was 5 months.

Conclusion: Our results continue to lend support to NMP providing a platform for administering tPA to donor livers, curtailing a potential risk to the recipient.

Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; BMI, body mass index; CIT, cold ischemia time; D-HOPE, dual hypothermic oxygenated perfusion; DBD, donation after brain death; DCD, donation after circulatory death; EAD, early allograft dysfunction; fdWIT, functional donor warm ischemia time; FFP, fresh frozen plasma; HTK, histidine-tryptophan-ketoglutarate; IC, ischemic cholangiopathy; NAS, non anastomotic strictures; NMP, normothermic machine perfusion; NRP, normothermic regional perfusion; pRBC, packed red blood cells; tPA, tissue plasminogen activator; IRI, ischemia reperfusion injury.

Summary

Administration of tissue plasminogen activator (tPA) can be safely administered during normothermic machine perfusion. For centers who traditionally deploy tPA in recipients for DCD donor livers, delivering during NMP is an alternative safer option sparing the reciepient from exposure.

1 | Introduction

Liver transplantation is a life-saving therapeutic option for patients with end-stage liver disease [1, 2]. The gap between supply and demand of donor livers continues to push the transplant community to utilize donor livers that may have previously been considered unsuitable for transplant. For example, in recent years, there has been an increase in the utilization of livers from donation after circulatory death donors [3, 4]. Historically, however, livers from these donors have shown to be more susceptible to ischemia-reperfusion injury and less optimal post-transplant outcomes, including decreased 1-year graft survival rates and biliary complications [5–7].

Ex situ normothermic machine perfusion (NMP) is a technique that allows continuous perfusion of a donor graft with an oxygenated blood based perfusate under physiological conditions (35–38°C) to support the metabolic activity of the organ [8]. Multiple studies have shown the feasibility and benefits of NMP for viability assessment as well as for transplant outcomes [9–11]. A major use case for NMP has been its application to increase the safe utilization of DCD donor livers [12, 13]. In addition, NMP is currently being explored as a platform for treating organs in an isolated setting before transplantation [14–18].

One common post transplantation complication particularly seen in DCD grafts is ischemic cholangiopathy (IC) [19]. Non-anastomotic strictures (NAS) constitute a subset of IC and are characterized by multiple diffuse strictures affecting the graft biliary system. NAS are a major cause of graft loss, morbidity, and mortality after liver transplantation, though the exact mechanisms for the various subtypes of this complication are not fully elucidated [20].

Historically, they were thought to occur in part due to the inevitable circulatory disruption during the donor warm ischemia time (WIT) that allows for blood stasis and microthrombi formation within the peribiliary microcirculation [21]. Consequently, this can disrupt the delicate blood supply to the bile duct, leading to ischemia, fibrosis, and stricture formation [22, 23]. In addition, reperfusion injury during OLT, immune-mediated injury to the biliary mucosa, and an increased biliary bile salt/phospholipid ratio post-operatively have been proposed as underlying mechanisms for NAS [24–27]. Biliary epithelia have demonstrated increased generation of toxic oxygen species upon post-anoxic reoxygenation and less glutathione than hepatocytes, making them more susceptible to injury [27].

More recent histologic studies of the common bile duct and common hepatic ducts have shown that the bile ducts of nearly all donor livers suffer significant injury during cold preservation, pointing to differences in biliary regenerative capacities as the underlying reason for whether NAS develops [28]. However, a dose-dependent relationship between bile duct injury and NAS development is still highlighted in the literature [29]. One important limitation of research done thus far is that little histological examination of the higher intrahepatic bile ducts, where NAS develops. Accommodating regeneration and reducing the overall amount of injury to the bile duct wall are two reasons why NMP is considered to provide such protective effects from NAS [30].

In light of previous hypotheses linking NAS to microthrombi, fibrinolytic therapies such as tissue plasminogen activator (tPA) have been administered intra-arterially to DCD graft [31]. The rationale behind this therapy is that the thrombolytic agent may dissolve microthrombi in the biliary microcirculation, thus reducing the risk of IC or NAS development [32]. One meta-analysis on thrombolytic therapy administered during the recipient operation has shown that a thrombolytic flush prior to reperfusion decreases the incidence of ischemic-type biliary lesions in DCD liver transplantation [33]. As such, intra-operative tPA administration is the standard of care at many transplant centers in the United States.

Combining the benefits of NMP and fibrinolytic therapies may be a more precise and effective regimen for treating the graft. Karangwa and colleagues demonstrated fibrinolysis activation during NMP [34], and a compelling study by Watson and colleagues [35, 36] showed that treating livers with tPA during ex situ NMP attenuates fibrin content in the liver, confirming this as a promising therapeutic intervention.

Soon after the initiation of our NMP program, our institution adopted NMP plus tPA + FFP administration as the standard of care for all DCD livers accepted for transplantation. The objective of this paper is to showcase our center's expertise in administering tPA for DCD livers following NMP. To our knowledge, this represents one of the most extensive documented collections detailing the use of tPA during NMP and is one of the first to provide post-transplant recipient chemistries and outcomes.

2 | Materials and Methods

2.1 | Normothermic Machine Perfusion

NMP at our institution is indicated for higher risk donor organs, such as those with macrosteatosis levels >15% and DCD organs, and higher risk recipients, including patients undergoing re-transplantation or multi-organ transplants. Upon arrival at our transplant center, livers undergoing NMP were surgically prepared and cannulated via the hepatic artery, portal vein and inferior vena cava, as described previously [37]. The livers were flushed with 5% albumin (Grifols, Barcelona, Spain) at room temperature to de-air the cannulas before onboarding on the metra system (OrganOx, Oxford, United Kingdom). The bile duct was cannulated after 15 min of perfusion, and livers were perfused at 37°C with a blood-based perfusate [38].

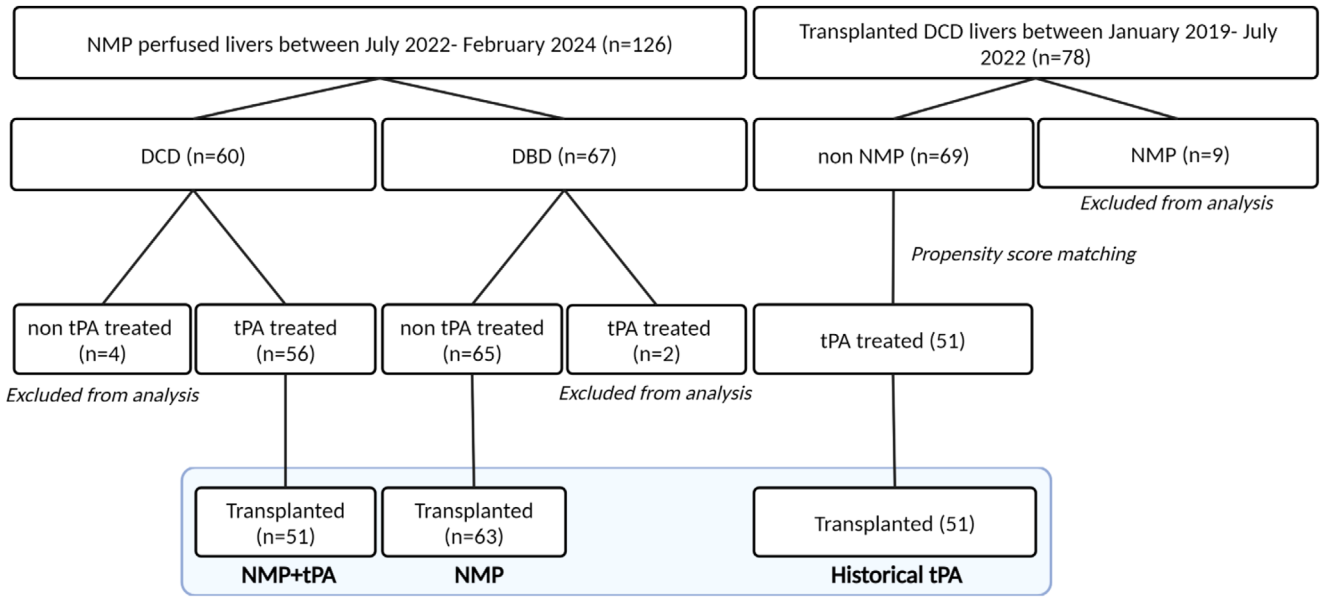

FIGURE 1 | Flow diagram of three groups included in the study. DBD, donation after brain death; DCD, donation after circulatory death; NMP, normothermic machine perfusion; tPA, tissue plasminogen activator.

2.2 | Study Population

In our retrospective study, we included donor livers designated for orthotopic liver transplantation and subjected to NMP from July 2022 to February 2024. We also reviewed transplants from January 2019 to July 2022, prior to our NMP program's initiation. Our study population ($n = 204$) is shown in Figure 1. The study arms were divided into:

- **tPA + NMP-DCD**: This group consisted of DCD livers perfused with tPA ($n = 54$).
- **NMP-DBD**: This group consisted of DBD livers perfused with NMP without tPA administration ($n = 65$).
- **tPA-DCD**: This group included livers from DCD donors which were treated with intra-arterial tPA during the recipient operation but did not undergo NMP ($n = 51$).

Owing to small numbers, cross-over grafts meeting the following criteria were excluded: DCD + NMP organs not administered tPA treatment ($n = 9$), or DBD + NMP livers treated with tPA ($n = 2$).

The historical tPA-DCD cohort was developed to help assess for any changes in transplant outcomes between livers that underwent in vivo versus ex situ NMP tPA treatment. Prior to the start of our institution's NMP program, recipients of DCD livers were treated with a tPA and verapamil into the donor hepatic artery immediately after portal reperfusion. For this cohort, therefore, all DCD donor livers utilized from January 2019 to July 2022 were reviewed for potential inclusion in this historical tPA comparator group (tPA-DCD) (Figure 1). We included 2019 in this historical cohort in an attempt to control for confounders related to the COVID-19 pandemic. Nine historical DCD livers had undergone NMP as part of a clinical trial on a different device and protocol prior to the official initiation of our NMP program in July 2022. Due to the small cohort size and substantial differences in perfusion machines and protocols, these were excluded from the group.

Historical tPA livers were propensity score matched to tPA + NMP-DCD livers using the following 12 covariates: recipient age, recipient sex, recipient race, recipient BMI, recipient MELD at time of transplant, recipient number of OLT, donor age, donor BMI, donor cause of death, donor warm ischemia time, donor functional warm ischemia time (fdWIT), and whether the donor underwent normothermic regional perfusion (NRP) at the time of procurement. WIT was defined as extubation to flush, and fdWIT was defined using the same parameters utilized in the UK-DCD risk score—the time between systolic blood pressure <50 mm Hg and cold aortic organ flush.

2.3 | tPA Administration

In the historical tPA cohort, DCD grafts were treated with 2 mg alteplase (Activase, Genentech, South San Francisco, United States) suspended in 1–2 mL saline, which was injected directly into the donor hepatic artery after reperfusion of the graft, immediately prior to anastomosis of the hepatic artery. The alteplase (Activase, Genentech, South San Francisco, United States) mixture was followed by administration of a suspension of 5 mg of verapamil, followed by approximately 20 mL heparinized saline. All three (alteplase, verapamil, and heparinized saline), were injected directly into the donor hepatic artery, and then artery was then clamped with a vascular bulldog. The arterial anastomosis was completed, and at the time of reperfusion, arterial inflow pushed the alteplase and verapamil through the fully reperfused donor liver.

In the NMP cohort, DCD grafts were treated ex situ with alteplase (Activase, Genentech, South San Francisco, United States) as a fibrinolytic therapy. Fresh frozen plasma (FFP) was also administered as a source of plasminogen, as originally described by

Watson et al. [35]. 10 mg tPA and 100 mL FFP was simultaneously administered into the perfusion circuit via the filling port into the reservoir after reperfusion on NMP had been achieved. Approximate median time for the initiation of the treatment was 15 min after the start of perfusion. This prescribed delay in drug administration was set to standardize timing of the dosage in the setting of differing hemostatic interventions needed for each graft upon reperfusion. That is, some grafts have substantial bleeding that needs to be controlled before tPA is administered. An infusion of 40 mg (26.6 mg/h) tPA and 150 mL (100 mL/h) FFP was started thereafter. This protocol was created based on prior publications demonstrating the safety and efficacy of this dosing regimen [36].

2.4 | Clinical Chemistry

Aspartate aminotransferase (AST), alanine transaminase (ALT), albumin, and d-dimer levels were measured in the perfusate after 2 h of NMP. Recipient plasma levels of AST, ALT, fibrinogen, and total bilirubin were monitored as part of regular post-operative clinical care, and values from the first seven post-operative days were retrospectively reviewed.

2.5 | Viability Assessment, Liver Transplantation, and Post-Operative Outcomes

Viability assessments were performed after 4–6 h of NMP, as described previously [39–42]. The criteria utilized at our center are as follows: perfusate lactate levels <2.2 mmol/L, pH >7.25, bicarbonate administered <70 mL after liver onboarding, glucose levels falling over the course of perfusion, ALAT levels <6000 u/L, and ASAT levels <10,000 u/L. In the case that ASAT or ALAT levels are above these thresholds, reassessment of these values is done at 4 h of perfusion. Before implantation, grafts were flushed with 4–6 L of 4°C Custodiol Histidine-tryptophan-ketolutarate (HTK) solution (Essential Pharmaceuticals, Ewing, United States) to cool the liver and remove perfusate, including any intravascular tPA. Liver transplantation and the use of blood products adhered to conventional surgical procedures.

The post-operative outcomes we monitored included early allograft dysfunction [43], primary non-function, 30-day and 3-month survival, length of hospital admission, and non-anastomotic biliary strictures (NAS) requiring re-listing for liver transplantation. EAD was defined via the Olthoff criteria: a peak AST or ALT >2000 U/L within the first seven post-operative days, or a total bilirubin ≥10 mg/dL or INR ≥ 1.6 on post-operative day seven [44]. Non-anastomotic biliary strictures were diagnosed in patients via MRCP and/or ERCP on suspicion of biliary complications.

2.6 | Data and Statistics

All data were collected retrospectively from donor and recipient charts. This study was approved by our Institutional Review Board (STUDY-24-00135), and patient consent was obtained. Missing data for these cases were not imputed or substituted. For the comparison between tPA + NMP-DCD and historical tPA-DCD cohort, propensity scores were calculated with a random forest method. To accommodate the inclusion of functional donor warm ischemia time into the propensity score, multiple imputation with mice was used to impute the five missing values. Propensity score matching was then performed via greedy nearest neighbor, without replacement. Propensity score calculations and matching were completed using R. GraphPad Prism version 8.0.1 was used for statistical analysis and visualization of graphs. Data were tested for normality and then compared using Mann–Whitney U, Fisher's exact, and Kruskal–Wallis with an uncorrected Dunn's multiple comparison tests, as appropriate. Longitudinal data were analyzed using mixed-effect analysis and Fishers Least Significant Difference for multiple comparison. Results were deemed significant if $p < 0.05$.

3 | Results

3.1 | Donor and Recipient Characteristics

Table 1 illustrates the donor characteristics and recipient characteristics in each cohort. Between the tPA + NMP-DCD and NMP-DBD cohorts, there were no significant differences in donor age, sex, or BMI, cold ischemia time [45], or recipient age or sex. However, the length of NMP was significantly longer ($p < 0.001$) and recipient MELD at transplant was higher ($p = 0.033$) in the tPA + NMP-DCD cohort. When comparing the tPA + NMP-DCD livers to the matched historical tPA-DCD cohort, there was no significant difference in donor sex, BMI, or WIT (defined as extubation to cross clamp), or in recipient age, sex, or MELD at transplant. The donor age was significantly higher in tPA + NMP-DCD group than in the matched control ($p < 0.001$). There were two donors in the tPA + NMP-DCD and seven donors in the historical tPA-DCD cohort who underwent NRP at the time of procurement ($p = 0.083$).

3.2 | Lab Values During NMP

After 2 h of ex situ perfusion, perfusate levels of AST and ALT that serve as markers of hepatocyte injury were both higher in the tPA + NMP-DCD group than the NMP-DBD groups ($p = 0.0034$ and 0.006, respectively) (Figure 2A). These levels were normalized to graft weight prior to analysis to remove graft weight as a potential confounder. However, weight-normalized values are not used at our institution to make clinical decisions on the viability of donor livers. D-dimer levels at 2 h of perfusion, a marker of fibrin degradation, trended higher in the tPA-treated DCD cohort but did not reach significance. Seven grafts, of which five were DCD livers treated with tPA, were not transplanted after failing to meet viability criteria during NMP (Figure 1). Intra-perfusion data of discarded grafts are denoted in Figure 2 by red circles.

3.3 | Intraoperative Transfusion Requirements

Regarding intraoperative transfusion requirements, there was a higher amount of Cell Saver infused for the NMP-DBD cohort as compared to the tPA + NMP-DCD cohort ($p = 0.0034$),

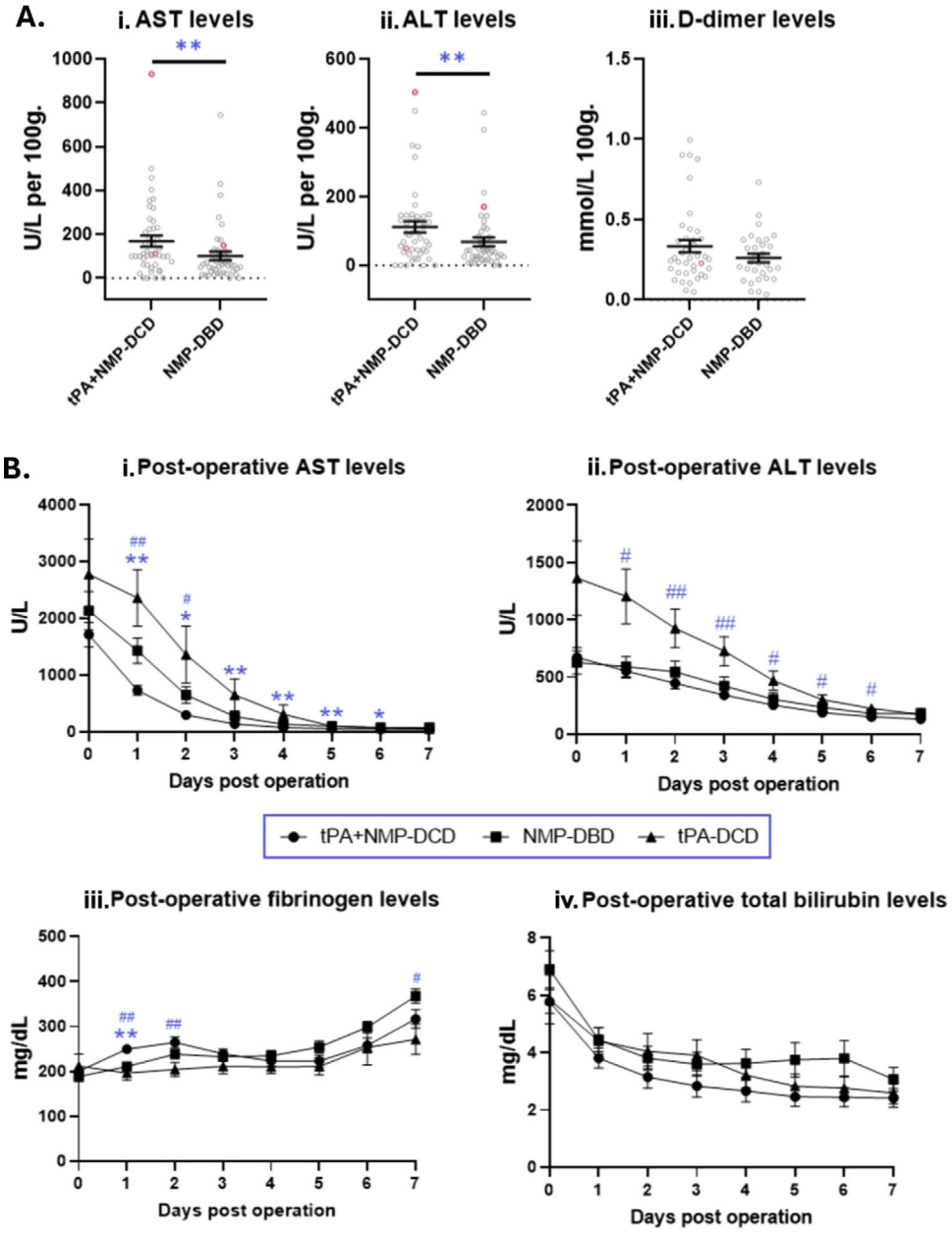

FIGURE 2 | **(A)** Liver injury markers measured in the perfusate after 2 h of normothermic machine perfusion. **(i)** Aspartate aminotransferase (AST) levels in U/L per 100 g of liver weight, $p = 0.0047$. **(ii)** Alanine transaminase (ALT) levels in U/L per 100 g of liver weight, $p = 0.009$. **(iii)** D-dimer levels in mmol/L per 100 g of liver weight. Red dots represent livers that were not transplanted. Values are expressed as the mean ± SEM plus individual values. TPA+NMP-DCD ($n = 56$) and NMP-DBD ($n = 65$) were compared using Mann–Whitney *U* after being tested for normality. No significant differences were observed. **(B)** Liver injury markers for the first 7 days post operation. **(i)** Aspartate aminotransferase (AST) levels in U/L. **(ii)** Alanine transaminase (ALT) levels in U/L. **(iii)** Fibrinogen levels in mg/dL. **(iv)** Total bilirubin levels in mg/dL. Values are expressed over time as the mean ± SEM. NMP-DBD ($n = 65$) and tPA-DCD ($n = 51$) were each compared to tPA+NMP-DCD ($n = 51$) at each individual time point using multiple comparison analysis and Fishers Least Significant Difference (*$p < 0.05$, **$p < 0.01$; ***$p < 0.001$). * = significant differences between tPA + NMP-DCD and NMP-DBD; # = significant difference between tPA + NMP-DCD and tPA-DCD.

though no other blood product showed significant differences between these groups (Table 2). Cryoprecipitate and platelet transfusions were significantly higher in the tPA-DCD group compared to the tPA+NMP-DCD cohort ($p = 0.0046$ and 0.0021, respectively) (Table 2). No differences were found in the intra-operative administration of packed red blood cells (pRBC), FFP, crystalloid, or albumin when comparing the two tPA groups.

3.4 | Transplant Outcomes

To examine transplant outcomes, we first analyzed post-operative lab values up until the seventh day post-transplant (Figure 2B). AST and ALT levels decreased over this window of time in all three groups (Figure 2Bi,ii). AST levels were significantly lower in the tPA + NMP-DCD group on post-operative Days 1–6 when comparing to the NMP-DBD group, and significantly lower on

TABLE 1 | Donor and recipient characteristics.

Donor	tPA + NMP-DCD ($n = 56$)	NMP-DBD ($n = 65$)	tPA-DCD ($n = 51$)	*p* value (*tPA+NMP-DCD* vs. *NMP-DBD*)	*p* value (*tPA+NMP-DCD* vs. *tPA-DCD*)
Age (year)	44 (36–54)	44 (36–54)	35 (28–45)	0.540	**0.00026**
Sex (male)	40 (71.43%)	43 (66.15%)	38 (74.51%)	0.669	0.888
BMI (kg/m^{-2})	27 (22.5–29.5)	25.3 (5.4)	26.0 (23–30)	0.562	0.99
Donor NRP	2 (3.57%)	N/A	7 (13.73%)	—	0.083
Donor WIT (min)	250 (20–28)	N/A	23 (19–27)	—	0.225
Pre-NMP CIT (h)	5.4 (4.8–6.0)	5.6 (5.0–6.3)	5.4 (4.7–5.7)	0.117	0.412
NMP length (h)	10.5 (9.1–11.9)	7.8 (6.6–10.0)	N/A	**2.78×10^{-5}**	N/A
Recipient	**tPA+NMP-DCD ($n = 51$)**	**NMP-DBD ($n = 63$)**	**tPA-DCD ($n = 51$)**	***p* value (*tPA+NMP-DCD* vs. *NMP-DBD*)**	***p* value (*tPA+NMP-DCD* vs. *tPA-DCD*)**
Age (year)	53 (48–62)	57 (46–64)	50 (50–65)	0.914	0.43
Sex (male)	35 (68.62%)	45 (71.43%)	36 (70.59%)	0.905	1.00
HCC	25 (49.02%)	14 (22.22%)	20 (39.22%)	**0.0051**	0.425
MELD score at transplant	20 (11–26)	23 (15–30)	22 (14–28)	**0.033**	0.156

Notes: Data are shown as median (Q1–Q3) or number of participants (percentage). WIT was defined as time from extubation to cross clamp. TPA+NMP-DCD ($n = 56$), NMP-DBD ($n = 65$), and tPA-DCD ($n = 51$) were compared after testing for normality. For normally distributed variables, Fischer's exact, Chi-squared tests, *t*-tests, and one-sided ANOVA test were used. For non-normally distributed variables, Kruskal–Wallis with an uncorrected Dunn's multiple comparison and Mann Whitney *U* were utilized. Significant differences in NMP length between TPA+NMP-DCD and NMP-DBD cohorts and in MELD score at transplant between TPA+NMP-DCD and tPA-DCD were found. There was also a significantly higher donor age in the TPA+NMP-DCD cohort compared to the historical tPA-DCD group. Bold values have reached signficance ($p < 0.05$).

Abbreviations: BMI, body mass index; DBD, donation after brain death; DCD, donation after circulatory death; WIT, warm ischemia time.

Days 1 and 2 when comparing to tPA-DCD (Figure 2Bi). ALT levels significantly differed between the two tPA groups on Days 1–6 (Figure 2Bii). Post-operative fibrinogen levels were analyzed as a marker for liver function. Fibrinogen levels were significantly higher on Day 1 in the tPA + NMP-DCD group compared to the NMP-DBD group, and significantly higher on Days 1 and 2 compared to tPA-DCD (Figure 2Biii). Post-operative total bilirubin levels did not show variance between groups (Figure 2Biv).

Early allograft dysfunction occurred in 11.76% of the tPA+NMP-DCD group, 17.65% of the tPA-DCD group, and 19.05% of the NMP-DBD group (Table 2). One incidence of primary non-function occurred in the matched historical tPA-DCD cohort. One case of NAS that required retransplant occurred in the tPA + NMP-DCD cohort after experiencing irretractable itching despite attempted medical management. Of note, all the donor factors and perfusion characteristics were within the viability criteria of our institution: the donor was a 44-year-old female with a donor risk index of 2.03. Total warm ischemia time was 23 min, with a functional donor warm ischemia time of 12 min from the time of MAP < 50. During NMP, the graft produced bile, utilized glucose, cleared lactate below the 2 mmol/L mark within 90 min of perfusion, and reached a terminal lactate of <0.3 mmol/L. The AST, ALT, and d-dimer at 2 h of perfusion were 1174 U/L, 1614 U/L, and 2.67 mg/L FUE, respectively. This recipient received a DBD-NMP graft that was not treated with tPA for his retransplant organ. There was a significantly higher rate of reoperation for hematoma evacuation in the NMP-DBD cohort compared to tPA + NMP-DCD ($p = 0.0382$).

No significant differences between groups were seen in 30-day or 3-month patient survival, or length of hospital stay (Table 2). There were no patients which had graft loss within the 30-day or 3-month spans; as such, these numbers are the same as patient survival and have not been re-reported. All patients had a minimum follow-up time of 5 months.

4 | Discussion

In addition to serving as an organ viability assessment platform, ex situ NMP can be utilized as a window for drug delivery prior to transplantation—an opportunity that may be leveraged to optimize post-transplant outcomes. To our knowledge, we provide one of the largest reported case series of tPA + FFP administration on this preservation platform, which also reports on clinical chemistry during perfusion and recipients' post-transplant chemistries and outcomes.

4.1 | Cohort Differences

Although the differences in donor age between the two DCD cohorts and MELD between the two perfusion groups may

TABLE 2 | Intraoperative transfusion requirements and post-operative outcomes.

	tPA + NMP-DCD ($n = 51$)	NMP-DBD ($n = 63$)	tPA-DCD ($n = 51$)	*p* value (*tPA+NMP-DCD* vs. *NMP-DBD*)	*p* value (*tPA+NMP-DCD* vs. *tPA-DCD*)
pRBC (units)	7 (4–13)	7 (3–12)	11 (4–16)	0.689	0.175
Cryoprecipitate (units)	0 (0–1)	0 (0–1)	1 (0–2)	0.123	**0.0046**
FFP (units)	6 (4–17)	7 (4–18)	12 (4–19)	0.562	0.167
Platelets (units)	0 (0–1)	1 (0–2)	1 (0–3)	0.402	**0.0021**
Crystalloid (mL)	3000 (2500–3850)	3000 (2000–3000)	3000 (2000–3750)	0.082	0.894
Albumin (mL)	1750 (700–4000)	1500 (375–2625)	1750 (875–2875)	0.215	0.495
Cell saver (mL)	230 (0–1022)	700 (250–1760)	0 (0–1300)	**0.0034**	0.569
	tPA + NMP-DCD ($n = 51$)	**NMP-DBD ($n = 63$)**	**tPA-DCD ($n = 51$)**	***p* value (*tPA+NMP-DCD* vs. *NMP-DBD*)**	***p* value (*tPA+NMP-DCD* vs. *tPA-DCD*)**
Early allograft dysfunction	6 (11.76%)	12 (19.05%)	9 (17.65%)	0.4225	0.576
Primary non-function	0	0	1 (1.96%)	—	—
IC requiring re-listing	1 (1.96%)	0	0	—	—
30-day patient and graft survival	50 (98.04%)	60 (95.24%)	49 (96.08%)	0.627	1.0
3-month patient and graft survival	50 (98.04%)	59 (93.65%)	47 (92.16%)	0.378	0.362
Patients requiring re-operation for hematoma evacuation	5 (9.8%)	17 (26.98%)	8 (15.69%)	**0.0382**	0.553
Length of hospital admission (days)	17 (12–25)	20 (11–42)	18 (112–25)	0.234	0.59

Notes: Data are shown as median (Q1–Q3) or number of participants (percentage). For intraoperative transfusion requirements, TPA+NMP-DCD ($n = 51$), NMP-DBD ($n = 63$), and tPA-DCD ($n = 51$) were compared using Kruskal–Wallis with an uncorrected Dunn's multiple comparison tests, after being testing for normality. The same analyses were conducted to compare TPA+NMP-DCD ($n = 51$) with tPA-DCD ($n = 51$). Significant differences in cryoprecipitate and platelet intraoperative transfusions were found between TPA+NMP-DCD and tPA-DCD, along with a significantly higher Cell Saver infusion in the NMP-DBD group compared with TPA+NMP-DCD. For post-operative outcomes, TPA+NMP-DCD ($n = 51$) and NMP-DBD ($n = 63$) were compared using Fischer's exact tests after being tested for normality. The same analyses were conducted to compare TPA+NMP-DCD ($n = 51$) to tPA-DCD ($n = 51$). A significant difference in reoperation for hematoma evacuation was seen between the TPA+NMP-DCD and NMP-DBD cohorts. Bold values have reached signficance ($p < 0.05$).
Abbreviations: FFP, fresh frozen plasma; IC, ischemic cholangiopathy; pRBC, packed red blood cells.

be considered confounders of our study, these characteristics reflect the changes in institutional practices that have occurred following the initiation of our NMP program. More extended criteria donors and older DCD donors are now accepted. As such, the average MELD at transplant has decreased over time, as illustrated by our groups in this study.

Consequently, the difference in NMP length between the DBD and DCD cohorts may be linked to the differences seen in MELD. Recipient characteristics including logistics and the expected difficulty of the recipient operation are used as indications for NMP. Therefore, the overall perfusion length is shorter in the DBD cohort because this group includes more low-risk livers which were pumped largely for logistics or for minimizing reperfusion injury in the recipient, and to ensure that CIT was kept as short as possible. In turn, our DCD cohort included more livers that were pumped for viability assessment and resuscitation purposes, leading to a longer average perfusion length.

4.2 | Safety and Effects on Blood Transfusion

When comparing the tPA treated DCD group (tPA + NMP-DCD) to our untreated DBD group (NMP-DBD), no significant increases in mean intraoperative transfusions or patient or graft survival were observed. This supports the claim that high dose tPA during NMP does not lead to excessive bleeding during the recipient operation. This observation was anticipated, as tPA is hepatically metabolized with a half-life of 4–10 min and the average duration of the perfusion after cessation of the tPA administration was over 6 h [46]. In addition, all livers were flushed with HTK to wash out tPA prior to implantation.

The significant reduction in the use of platelets and cryoprecipitate in the tPA + NMP-DCD group as compared to the historical tPA-DCD cohort is most likely the effect of NMP. This is an important finding lending support to the potential benefit of our NMP + tPA approach, as cryoprecipitate has been identified

as an independent risk factor for the development of major thromboembolic complications [47].

The increased use of Cell Saver in the NMP-DBD cohort as compared to the tPA + NMP-DCD cohort is likely explained by the increased proportion of HCC recipients in the tPA+NMP-DCD cohort (Table 1). Many patients with HCC receive DCD organs given their lower MELD scores, and Cell Saver is not utilized at our institution for these patients due to the theoretical risk of re-infusing malignant cells present in the operative field.

The higher incidence of reoperation for hematoma evacuation in the perfused DBD grafts compared to tPA-NMP-DCD organs are likely due to the differences in MELD between the two NMP cohorts, and as such, differences in the degree of coagulopathy in the recipient. The patients receiving livers from brain dead donors in the NMP arm (NMP-DBD) have a higher MELD score, reflecting more advanced liver disease and coagulopathy. Nevertheless, these findings continue to lend support to the safety of tPA administration during NMP.

4.3 | Impact on Graft Function and Biliary Complications

For clinical chemistries, the lower AST and ALT values during perfusion in the NMP-DBD group was another expected finding. As noted previously, the NMP-DBD cohort included livers for which the indication for NMP varied greatly and had more grafts perfused for recipient related factors. The post-transplant AST and ALT values being lower in the tPA + NMP-DCD than in the non-perfused tPA-DCD cohort highlight the value of NMP and demonstrate that administering tPA does not adversely impact patients post-operatively. Additionally, the prompter return of fibrinogen levels on post-operative Day 1 in the tPA + NMP-DCD cohort strongly supports the hypothesis that tPA administered during NMP does not lead to increased risk of bleeding post-transplant.

We did not observe differences in symptomatic NAS incidence between the groups compared. However, our study was not powered to detect this difference. IC, and by extension NAS, is one of the most feared biliary complications after the transplantation of DCD livers, stemming from exposure to ischemic events [19, 48]. Patients afflicted by IC have higher rates of readmission, prolonged hospital stays, recurrent radiologic-guided procedures, and a heightened likelihood of re-transplantation [20, 49].

The prevention of IC through fibrinolytic therapy during NMP of DCD livers would require extensive, largely scale evaluation, enrolling hundreds of patients to determine its true value. However, since our findings indicate no potential harm from administration during NMP, the transplant community may consider adopting this as standardized practice, supported by the extensive literature already published on the general value of tPA in DCD grafts [33]. The price of 40 mg tPA and a unit of fresh frozen plasma in relation to the risk of ischemic cholangiopathy and its associated increase in morbidity warrants further evaluation.

4.4 | Fibrinolytic Therapy on the Pump as a Potential Biomarker

D-dimer is a fibrinolytic marker that indicates the activation of coagulation and fibrinolysis [50]. During NMP, elevated D-dimer levels have correlated with poorer ex situ liver function [34, 36]. In our study, d-dimer release was higher in the tPA-treated perfused livers after 2 h of ex situ perfusion, aligning with findings from Watson et al. and Haque et al., though the difference did not reach statistical significance [36, 51]. One major difference is that they compared their Alteplase treated group with DCD livers whilst our NMP comparator cohort comprised of DBD livers. Also, Haque et al. did not transplant the treated livers [51]. Nevertheless, our study was not designed to evaluate d-dimer as a potential biomarker and as such, our conclusions remain limited on this matter.

The initial cold flush during organ procurement potentially diminishes clot burden, and employing cold perfusion strategies like (dual) hypothermic oxygenated perfusion ((D)-HOPE) have demonstrated efficacy in mitigating biliary complications [2, 52]. This is achieved by continuously perfusing the graft with cold, oxygenated solutions, facilitating the removal of microthrombi. However, hypothermic conditions may induce vasoconstriction, making the release of microthrombi more challenging. In contrast, the normothermic conditions during NMP allow for physiological vascular function, enabling fibrinolytic therapy such as Alteplase to reach all microvasculature. In the future, it would be interesting to investigate how various perfusion technologies such as DHOPE alter the biliary system and impact microthrombi formation.

5 | Conclusion

This study marks the report of tPA treatment during NMP for DCD grafts from a single center representing one of the largest reported cohort analyses published to date. Our findings demonstrate the feasibility of administering tPA/FFP to DCD donor livers during NMP in a targeted manner, potentially obviating the necessity for systemic treatment of the recipient post-reperfusion. They also demonstrate the power of NMP to enable utilization of older DCD grafts without increasing complications. Our initial assessment of our center's experience did not uncover any early warning signs associated with this approach in treating DCD livers.

Fibrinolytic therapy is merely one intervention that can be applied during ex situ NMP to mitigate post-transplant complications. A variety of small-molecule drugs, therapeutic proteins, monoclonal antibodies, cell-based therapies, nanoparticles, and gene therapies could be added to the perfusate to repair or resuscitate donor livers prior to transplantation. One major advantage of ex situ treatment, is the ability to use more potent drugs and apply a more targeted approach, thereby eliminating systemic effects [53]. Targeting ischemia reperfusion injury (IRI) with antioxidants, incorporating defatting cocktails to address steatosis, pretreating donor livers with antiviral treatments against hepatitis C or HIV, and rejuvenating livers with stem cell therapies exemplify the spectrum of possibilities for ex situ liver treatment.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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