ORIGINAL ARTICLE

Lung transplant after prolonged *ex vivo* lung perfusion: predictors of allograft function in swine

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SUMMARY

Portable normothermic EVLP has been evaluated in clinical trials using standard and extended-criteria donor lungs. We describe a swine model of lung transplant following donation after circulatory death using prolonged normothermic EVLP to assess the relationship between EVLP data and acute lung allograft function. Adult swine were anesthetized and heparinized. In the control group ($n = 4$), lungs were procured, flushed, and transplanted. Treatment swine underwent either standard procurement ($n = 3$) or agonal hypoxia followed by 1 ($n = 4$) or 2 hours (H) ($n = 4$) of ventilated warm ischemia. Lungs were preserved for 24H using normothermic blood-based EVLP then transplanted. Recipients were monitored for 4 H. After 24H of preservation, mean pulmonary artery pressure (mPAP), pulmonary vascular resistance (PVR), and dynamic compliance (C_{dyn}) were improved in all EVLP groups. After transplant, EVLP groups showed similar allograft oxygenation. EVLP PVR, mPAP, and lung block weights had significant negative correlations with post-transplant allograft oxygenation. EVLP P:F ratio did not correlate with acute post-transplant allograft function until 24H of preservation. Data measured in the first 8H of EVLP were sufficient for predicting acute post-transplant allograft function. This study provides a benchmark and platform for evaluation of therapies for donor-related allograft injury in injured lungs treated with prolonged normothermic EVLP.

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Introduction

The current supply of donor lungs from standard donors (donation after brain death, DBD) is insufficient for the number of patients in need of lung transplantation [1]. Donation after circulatory death (DCD) can occur either after elective withdrawal of support in an operating room (controlled DCD, Maastricht III) or

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following unsuccessful resuscitation of a patient suffering a witnessed cardiac arrest (uncontrolled DCD, Maastricht II) and allows donation without declaration of brain death [1]. However, concerns about donor-related warm ischemic/hypoxic insult and inability to assess allograft function following procurement limit widespread adoption of this practice in the United States [2–4]. *Ex vivo* lung perfusion (EVLP) allows preservation of allografts for longer periods than are possible with standard cold storage and for functional evaluation immediately before implantation [5–8].

There is growing experience with various methods of EVLP for preserving and evaluating DBD and DCD lungs, including those from 'extended-criteria' donors, which has generated interest in new EVLP-based methods of allograft evaluation and reconditioning [5,7–17]. Allograft evaluation using EVLP is predicated on an understanding of the value of physiologic data collected during EVLP in predicting post-transplant allograft function. These markers include pulmonary vascular resistance (PVR), mean pulmonary artery pressure (mPAP), compliance, the PaO_2:FiO_2 (P:F) ratio, cytokine expression, and pulmonary edema. Currently, the most prominent criterion for allograft acceptance on EVLP is a P:F ratio ≥300–400 mmHg [7,18]. However, experimental models of EVLP have demonstrated the value of other measures, including airway pressure, PVR, and P:F ratio response to varying FiO_2 [19–21]. These criteria will evolve as more extended-criteria lungs are evaluated with EVLP.

Our group has reported a methodology for preserving swine lungs for ≤24 hours (H) using blood-based normothermic EVLP and have studied and reconditioned lungs subjected to severe DCD insults (≤2H of unventilated warm donor ischemia) [5,22]. We have found that, despite a persistent oxygenation deficit, DCD lung physiology improves to levels equivalent to beating-heart (analogous to DBD) donor lungs during prolonged preservation [22]. The current study sought to characterize the correlation between data available during EVLP and acute post-transplant allograft function.

Materials and methods

Animal model and study design

Adult male Yorkshire swine (70–80 kg) were used. All animals received humane care in compliance with the 'Principles of Laboratory Animal Care', formulated by the National Society for Medical Research, and *The Guide for the Care of Laboratory Animals*, published by the National Institutes of Health. This research protocol was approved by our local Institutional Animal Care and Use Committee.

Donors were divided into four groups. The control group ($n = 4$) underwent standard procurement and immediate single lung transplantation, simulating a 'best-case' scenario. The 'standard donor' group ($n = 3$) underwent standard procurement with no warm ischemia, 24H EVLP, and single lung transplantation. The '1H DCD' group ($n = 4$) underwent agonal hypoxia resulting in cardiac arrest. Lungs were left in the chest for 1H of ventilated warm ischemia, followed by procurement, 24H EVLP, and transplantation. The '2H DCD' group ($n = 4$) underwent 2H of ventilated warm ischemia but was otherwise identical to the 1H DCD group (Fig. 1).

Donor lung procurement

Our methods for donor anesthesia, blood collection, and procurement have been described in detail [5]. Briefly, all donors underwent general anesthesia, sternotomy, and full heparinization. In the control ($n = 4$) and standard ($n = 3$) donor groups, cardiac arrest was achieved by aortic clamping and cardioplegia administration. Antegrade flush with 2 L chilled OCS Lung solution (TransMedics, Inc., Andover, MA, USA) was delivered via the main PA. Donor whole blood (1.5 L) was collected, followed by en bloc lung procurement and retrograde flush with 1 L OCS Lung solution via the pulmonary vein (PV) ostia.

In the 1H DCD and 2H DCD groups, hypoxic cardiac arrest was achieved via prolonged expiratory hold, which marked the start of a 15-min 'no touch' period, regardless of the time to arrest. The expiratory hold was continued into the no-touch period, preventing spontaneous respiration. All donors expired within this interval; median time from the start of the expiratory hold to cardiac arrest was 8.5 min. Autologous whole blood was then collected as above and the chest was temporarily closed. Donors underwent either 1H (analogous to controlled DCD with prolonged agonal phase) or 2H (analogous to uncontrolled DCD, e.g. witnessed cardiac arrest, although no resuscitation was provided in this model) of warm ischemia with post-mortem mechanical ventilation [tidal volume (TV) 3–4 ml/kg, positive end-expiratory pressure (PEEP) 5 mmHg, and FiO2 100%]. This postmortem ventilation strategy is consistent with that described in

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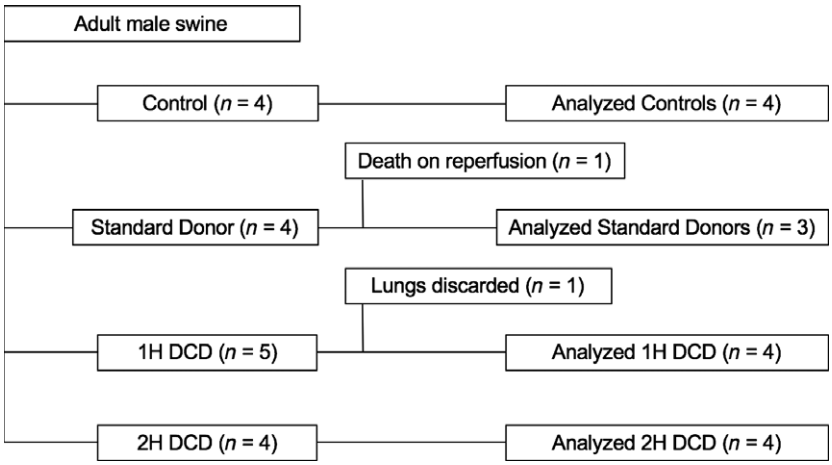

Figure 1 Study design and final analyzed cohort. One 1 H DCD lung block was discarded prior to transplant and one recipient from a standard donor experienced terminal arrhythmia on reperfusion. Data from these experiments were not included in the final analysis. DCD, donation after circulatory death; H, hour.

clinical reports of uDCD lung transplantation [23,24]. Intrapleural temperature monitoring was performed; no animal had a temperature decrease to <35 °C during this time. The chest was reopened and the lungs were flushed and procured as above.

Ex vivo lung perfusion

Normothermic EVLP was performed in the noncontrol groups as reported previously [5]. Briefly, the bilateral lung block was connected to the OCS Lung device (TransMedics, Inc., Andover, MA, USA), which was primed with 1600 ml whole blood, 700 ml OCS Lung solution, and standard additives. The lungs underwent 24H of normothermic EVLP. PVR, mPAP, peak airway pressure (PAWP), TV, PEEP, and hematocrit were measured every 2 min. Dynamic compliance (C_{dyn}) was calculated *post hoc* using the formula:

$$\left(C_{dyn} = \frac{\text{tidal volume(ml)}}{\text{PAWP (mmHg)} - \text{PEEP (mmHg)}},\right)$$

Dedicated assessments of allograft oxygenation via perfusate arterial blood gas (ABG) analysis, described previously, were performed at 30 min (considered the '0H' point) and 2, 4, 6, 8, and 24H [5]. Flexible bronchoscopy followed each assessment. Supplemental $NaHCO_3$ (goal >20 mmol/l) and glucose (goal >120 mg/dl) were administered as needed. OCS Lung solution was added to the reservoir as needed to maintain a volume of ≥500 ml. After 24H, the lung block was flushed antegrade with 1L of chilled OCS Lung solution and decannulated.

Lung transplant procedure and recipient data collection

Single left lung transplant was performed via thoracotomy using standard methods [13]. The right lung was left in situ, undisturbed. Methylprednisolone 1 g was given at anastomosis-start for immunosuppression. Following implantation, a Swan-Ganz catheter, directed medially, was placed directly into the transplanted PA. Additionally, a 3F sampling catheter was introduced into the transplanted left atrial (LA) cuff and directed into an allograft PV (APV) to allow dedicated ABG analysis of blood returned from the transplanted lung. The recipient was observed for 4H. Hourly samples were drawn from systemic arterial and APV lines for ABG and cytokine analysis. Vital signs, PAP, and C_{dyn} were recorded every 15 min. After final sample collection, the donor superior and inferior PVs and donor LA cuff were directly aspirated for confirmatory ABG analysis and the recipient was sacrificed.

Measurement of pulmonary edema

The development of pulmonary edema on EVLP was measured using lung block weights (pre- and post-EVLP), hematocrit, and total reservoir volume replacement (RVR). Increases in weight and hematocrit and elevated RVR were indicative of edema. Allografts were weighed before implantation and after recipient sacrifice.

Measurement of inflammatory expression

Cytokine levels were measured in perfusate samples at 24H of EVLP and hourly blood samples following

implantation. Levels of IL-4, IL-6, IL-8, and IL-10 were measured on the Luminex platform (Luminex Corporation, Austin, TX, USA), using bead sets from EMD Millipore (Billerica, MA, USA).

Statistical analysis

Physiologic data (e.g., C_{dyn}, hematocrit, PVR, and mPAP) collected during EVLP and after transplant were analyzed using hourly measurements over 24H and P:F ratio was analyzed using all available measurements to compare the groups. Each analysis used a mixed linear model implemented in SAS's MIXED procedure (v.9.4, SAS Institute Inc., Cary, NC, USA), with an exponential correlation structure (SP(EXP) in the MIXED procedure's RANDOM statement) to account for temporal dependence between measurements on a pair of lungs. Some outcomes were transformed before analysis based on results of so-called diagnostic tests, which permitted us to use parametric methods and thus preserve statistical power. Adjusted averages and standard errors at each time point were calculated. Plots display adjusted averages ± one standard error.

For data collected during EVLP and after transplant, we used standard tests in the context of the mixed linear model (i.e., SAS's type III tests of the group and time main effects and their interaction) to assess differences between all groups in group averages over all time points ('group' effects in Table 1 and 2), differences between all groups in the pattern over time ('interaction' effects), and differences between times, averaging over groups ('time' effects). Baseline was defined as 0H for P:F ratio and 1H for all other outcomes.

Cytokine expression at the end of EVLP and over the post-transplant observation period was compared between groups using one- and two-way analysis of variance (ANOVA), as appropriate. Correlations between EVLP and recipient data were analyzed using Pearson's correlation (r). GraphPad Prism 7 (GraphPad Software, La Jolla, CA, USA) was used for these tests. $P < 0.05$ was considered significant for all analyses with no adjustment for multiple comparisons. Numerical data are presented in the text as mean ± standard deviation.

Results

EVLP function and inflammation

Physiology: PVR and mPAP decreased significantly (Fig. 2a and c) and C_{dyn} increased significantly (Fig. 2d) during preservation in all groups. Groups did not differ significantly in these trends during preservation (Table 1).

Table 1. *Ex vivo* lung perfusion global effect testing.

Outcome	Effect	*P*-value
Vascular resistance	Time	**<0.0001**
	Group	0.4681
	Interaction	0.9377
Mean PA pressure	Time	**<0.0001**
	Group	0.3464
	Interaction	0.7319
Dynamic compliance	Time	**<0.0001**
	Group	0.9614
	Interaction	0.461
Hematocrit	Time	**0.0195**
	Group	0.8353
	Interaction	0.8736
P:F Ratio	Time	0.3867
	Group	0.2274
	Interaction	0.3352

Table 2. Recipient global effect testing.

Outcome	Effect	*P*-value
Mean PA pressure	Time	0.3316
	Group	0.1563
	Interaction	0.6452
Dynamic compliance	Time	0.3771
	Group	**0.0237**
	Interaction	0.5214
Systemic P:F ratio	Time	**0.0054**
	Group	**0.0011**
	Interaction	0.3426
Allograft PV P:F ratio	Time	0.2369
	Group	**0.0005**
	Interaction	0.1941

PA, pulmonary artery; P:F, PaO_2:FiO_2.
Only *P*-values displayed. Significant values in bold.

Oxygenation: At the start of EVLP, standard donors had P:F ratios ≥350 mmHg, higher than both DCD groups. Oxygenation in the DCD groups was unchanged throughout preservation. By 24 h, P:F ratios were similar for all groups. (standard donor 253 ± 43 mmHg, 1H DCD 261 ± 89 mmHg, 2H DCD 261 ± 45 mmHg, Fig. 2b).

Pulmonary Edema: Edema was similar in all EVLP groups. Prepreservation lung block weights from DCD donors were greater than standard donors, but weights in all groups increased significantly to similar levels during preservation (Fig. 2f). Perfusate hematocrit decreased significantly during preservation in all groups (Fig. 2e,

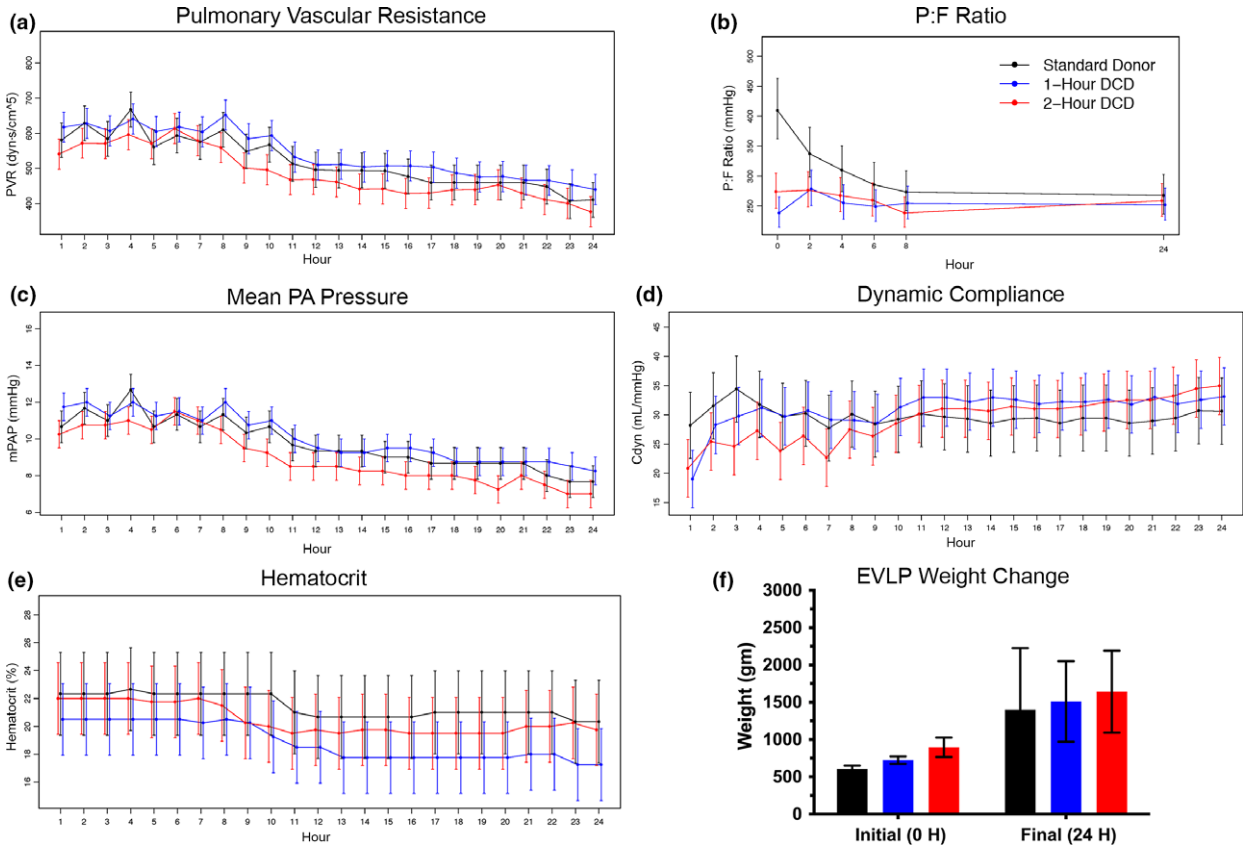

Figure 2 *Ex vivo* lung perfusion physiology. (a) Pulmonary Vascular Resistance; (b) P:F ratio; (c) Mean PA Pressure; (d) Dynamic Compliance; (e) Hematocrit; (f): EVLP Weight Change. Note that PVR, mPAP, and C_{dyn} remain relatively stable over the first approximately 8H but all tend to improve uniformly across groups beyond this point. Standard donor lungs show superior oxygenation at the start of EVLP but this difference disappears by 24 H of preservation. C_{dyn}, dynamic compliance; EVLP, *ex vivo* lung perfusion; H, hour; mPAP, mean pulmonary artery pressure; PVR, pulmonary vascular resistance.

Table 1). RVR was lowest in the standard group (174 ± 120 ml) compared with the 1H DCD (408 ± 425 ml) and 2H DCD (252 ± 112 ml) groups, but this difference was not statistically significant ($P = 0.5383$).

One 1H DCD block developed severe edema and parenchymal injury with inability to maintain reservoir volume during preservation. This block was discarded before transplant and its EVLP data excluded given the lack of post-transplant data for correlation (Fig. 1). *Post hoc* review of the EVLP data from this run revealed that early PVR and mPAP were notably higher than the others in this experimental group. More detailed preservation data are displayed in Fig. S1.

Inflammatory Expression: Perfusate levels of IL-4, IL-8, and IL-10 were significantly higher across groups in a comparison of standard and DCD donors after prolonged preservation. IL-6 was also elevated in DCD donors compared with standard, but this difference did not reach statistical significance (Fig. 3).

Post-transplant function and inflammation

Survival: All control recipients and all but one EVLP recipient survived observation. A fatal arrhythmia was encountered on reperfusion in the fourth standard donor recipient. The analyzed cohort is described in Fig. 1.

Physiology: Recipients of control or standard donor lungs tended to have lower mPAP following reperfusion compared with DCD recipients, a nonsignificant trend that was consistent throughout observation (Fig. 4a, Table 2). C_{dyn} remained stable during observation; 2H DCD recipients had a significant relative deficit (Fig. 4b, Table 2). There was a nonsignificant decrease in allograft weight in all EVLP groups during observation (Fig. 4c).

Oxygenation: There were inter-group differences between systemic arterial and APV oxygenation corresponding to the level of preprocurement donor insult, likely driven by the excellent performance of control

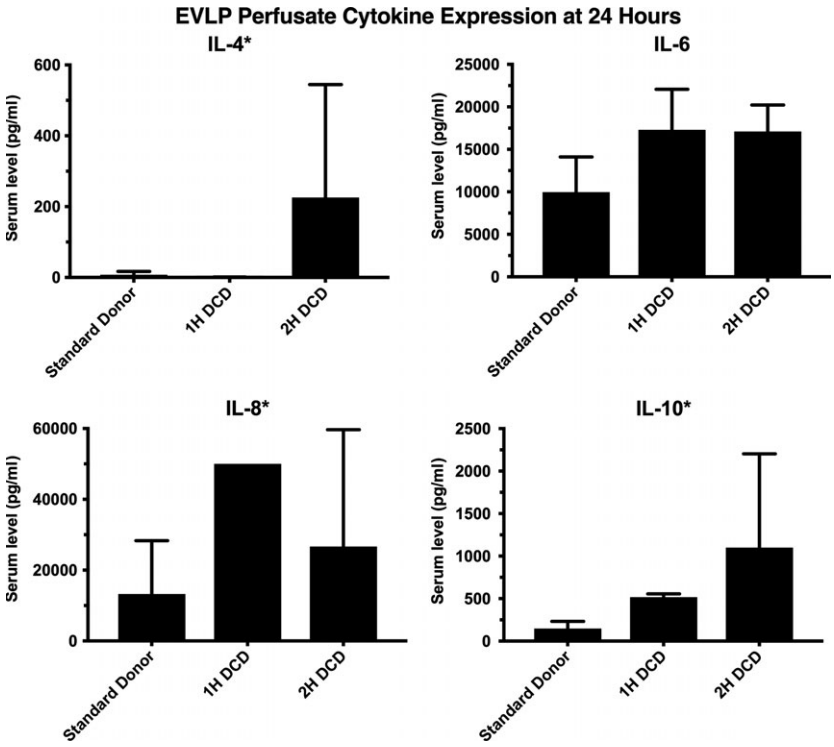

Figure 3 *Ex vivo* lung perfusion perfusate cytokine expression at 24 h. Perfusate levels of IL-4, IL-8, and IL-10 were proportional to level of donor ischemic injury. (*) indicates $P < 0.05$ for one-way ANOVA of between-group comparison. DCD, donation after circulatory death; EVLP, *ex vivo* lung perfusion; IL, interleukin.

lungs (Table 2). EVLP recipient groups had equivalent oxygenation during observation. (Fig. 4d and e).

Cytokine Expression: Figure 5 shows initial (0H) and final (4H) cytokine levels in systemic arterial and APV circulation. Systemic and APV cytokine expression did not vary significantly.

EVLP versus Post-transplant functional correlation

Ex vivo lung perfusion measurements at 0H, 8H, and 24H were correlated with recipient data measured at the end of observation to identify predictors of acute post-transplant allograft performance, which was the central analysis of our study. The primary outcome of this analysis was the APV P:F ratio which provides data unique to the transplanted allograft. EVLP PVR, mPAP, P:F ratio, and lung block weight had substantial correlations with this outcome (Fig. 6, Table 3).

Ex vivo lung perfusion PVR ($r = -0.7846$, $P = 0.0042$) and mPAP ($r = -0.7860$, $P = 0.0041$) at 8H had a significant negative correlation with final APV P:F. Final (24H) EVLP P:F ratio was positively correlated with final APV P:F ($r = 0.5759$, $P = 0.0637$). Prepreservation lung block weight was not significantly correlated with the primary outcome. However, lung block weight gain during preservation and final post-EVLP lung block weight showed significant negative correlations with final APV P:F (Fig. 6).

Ex vivo lung perfusion predictors of other post-transplant recipient variables (C_{dyn}, mPAP, systemic P:F ratio) were also analyzed. Recipient C_{dyn} had a significant positive correlation with 1H EVLP DC and significant negative correlation with prepreservation lung block weight (Table 3). Post-transplant recipient mPAP and systemic oxygenation were not correlated with any EVLP measure.

Discussion

Before EVLP, the only testing platform for extended-criteria donor lungs was the recipient. EVLP now allows evaluation, preservation, and reconditioning of these organs prior to transplant [22,25]. We have previously demonstrated that the hemodynamics and compliance of swine DCD lungs normalize relative to standard donors during 24H normothermic EVLP, although more injured lungs had worse oxygenation [22]. We performed the present study to identify EVLP data predictive of post-transplant allograft function.

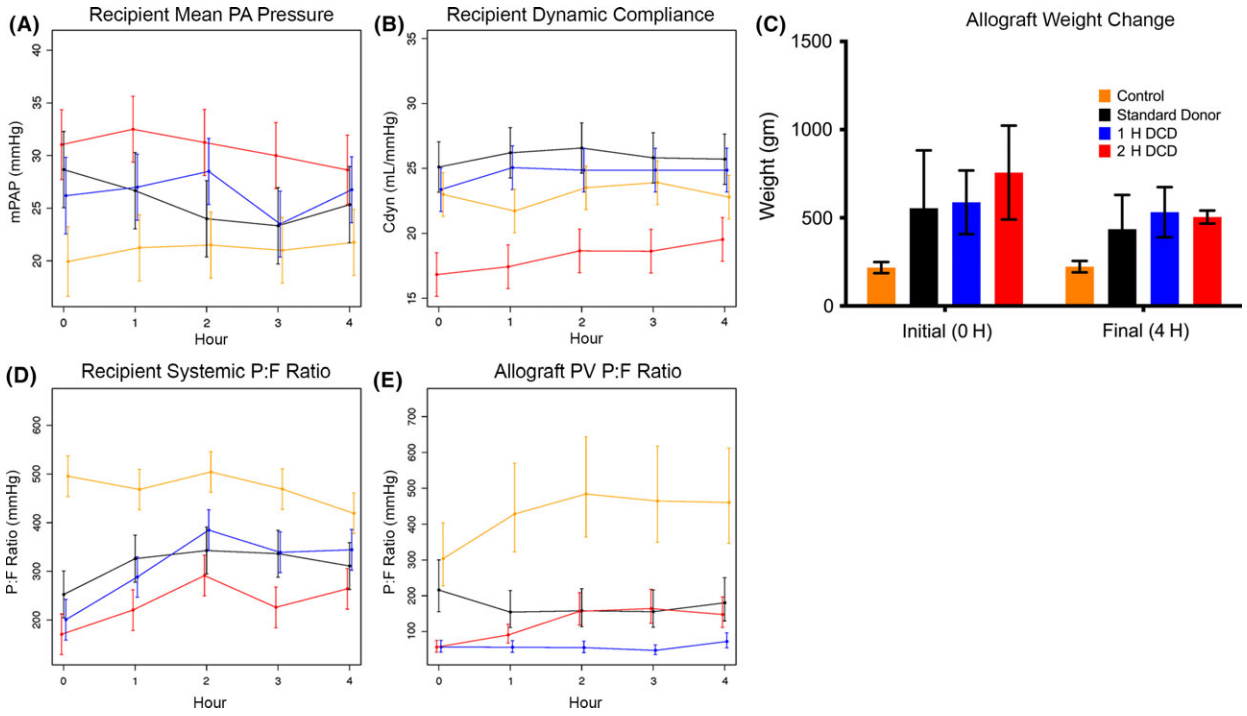

Figure 4 Post-transplant recipient physiology, oxygenation, and pulmonary edema. (a) Recipient mean PA pressure; (b) Recipient dynamic compliance; (c) Allograft weight change; (d) Recipient systemic P:F ratio; (e) Allograft PV P:F ratio. Recipient PA pressure corresponded to level of donor ischemic injury; it was lowest in control allografts and highest in 2 H DCD. Compliance was notably decreased in 2 H DCD recipients but was otherwise similar across groups. Systemic and allograft oxygenation was best in control recipients but was similar in all EVLP groups. Allograft weight decreased slightly during observation. The differences between final weight after EVLP (Fig. 2f) and the initial weights described here are accounted for division of the bilateral lung block to prepare the L lung for implantation. C_{dyn}, dynamic compliance; DCD, donation after circulatory death; H, hour; mPAP, mean pulmonary artery pressure; P:F, PaO_2:FiO_2.

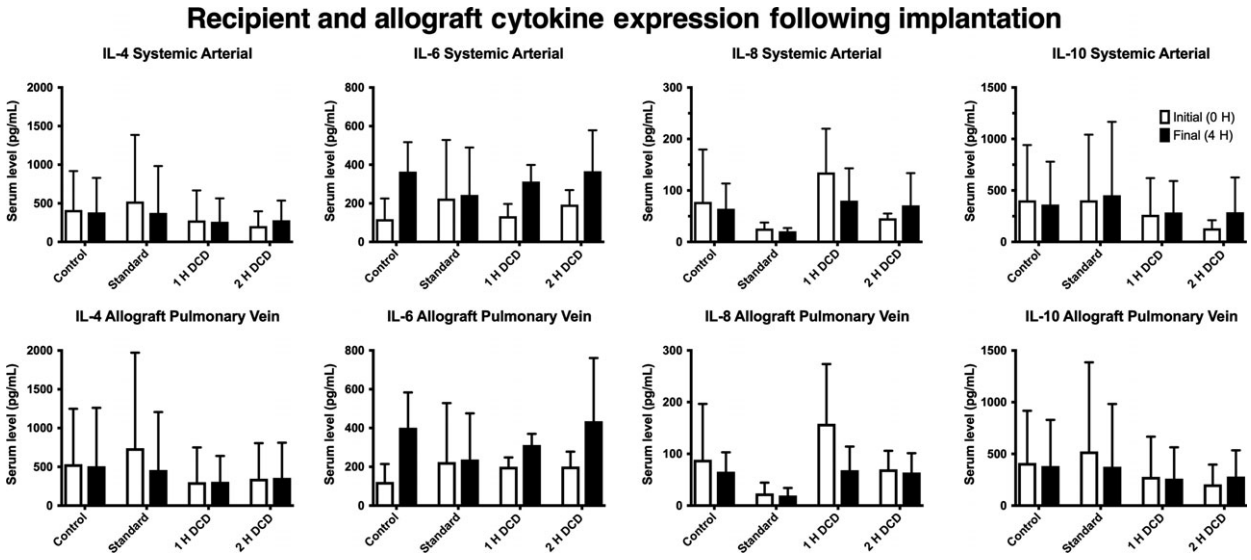

Figure 5 Recipient cytokine expression after implantation. There was a significant time-dependent increase in IL-6 expression across groups on two-way ANOVA ($P < 0.05$). There were no other group-, source- (systemic versus APV), or time-dependent differences in cytokine expression. ANOVA. analysis of variance; APV, allograft pulmonary vein; DCD, donation after circulatory death; H, hour; IL, interleukin.

Mid-preservation (8H) PVR and mPAP and the development of pulmonary edema were important predictors of post-transplant oxygenation. The most intuitively appealing marker of successful recovery, EVLP P:F ratio, also had a positive correlation with APV P:F ratio by the end of preservation but failed to

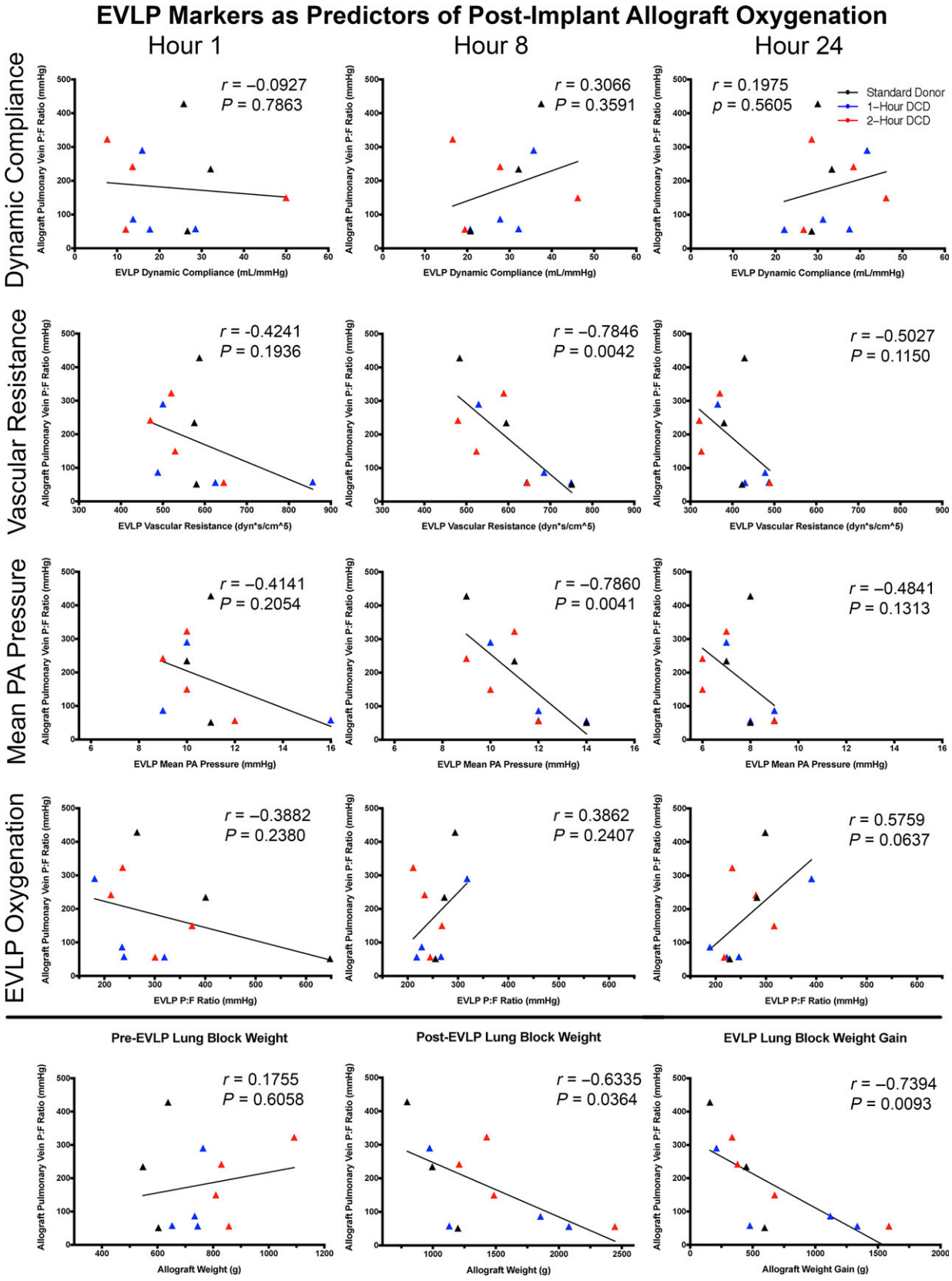

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Figure 6 *Ex vivo* lung perfusion markers of allograft oxygenation after implantation. 'Hour 1', 'Hour 8' and 'Hour 24' refer to time on EVLP. $P < 0.05$ considered significant. Note significant relationships between 8H PVR and mPAP and APV P:F ratio. Post-EVLP lung block weight and overall weight gain during EVLP also significantly correlated. APV, allograft pulmonary vein; DCD, donation after circulatory death; EVLP, *ex vivo* lung perfusion; mPAP, mean pulmonary artery pressure; PA, pulmonary artery; P:F, PaO_2:FiO_2; PVR, pulmonary vascular resistance; R, correlation coefficient.

reach statistical significance, likely because of the lone recipient death. There was a significant negative correlation ($r = -0.6768$, $P = 0.0222$) between prepreservation lung block weight, highest in the 2H DCD group, and recipient C_{dyn}, suggesting a lack of recovery from the severe ischemic injury (Table 4).

The fact that most parameters improved after 24 h of EVLP, but lost their predictive value, suggests two possible explanations. On the one hand, it is conceivable that all injured lungs stabilize their perfusion and ventilation characteristics by 24 h but that the level of peak injury at 8 h is most predictive of their ultimate performance. This is akin to the phenomenon of 'transient primary graft dysfunction' whereby transient acute lung injury that resolves still has lasting effect on the allograft after transplant [26].

Alternatively, PVR and mPAP data at 24 h can be misleading. Muir and colleagues demonstrated in a pair of canine studies that pulmonary edema, particularly intra-alveolar fluid, raises the resistance of adjacent pulmonary vascular beds, leading to overall increased PVR [27,28]. This may have been responsible for early elevations in EVLP PVR, after which flow may have been shunted to lower-resistance vascular beds and larger vessels after approximately 8–10H of preservation, lowering the overall PVR.

With some exceptions, the study of EVLP in DCD lungs has generally focused on controlled DCD (Maastricht III), involving only short periods of donor warm ischemia [1,7,17,29]. The recovery of injured donor lungs may require extended preservation, which was the impetus for our attempt at 24H of preservation and reconditioning. Our demonstration of prolonged preservation and good acute survival of recipients of allografts subject to longer (≤2H) pre-procurement ischemia suggest that such a model of preservation, evaluation, and possible reconditioning could eventually promote the use of lungs from uncontrolled DCD (Maastricht II) donors, substantially enlarging the donor pool [29]. However, the substandard oxygenation after transplant demonstrated by such lungs in our study suggests that additional intervention may be required to salvage such lungs, if possible.

Donor- and EVLP-based therapies with this goal have been described. In situ topical hypothermia via intrapleural instillation of cold saline slush is effective in preserving DCD lungs before transplant [30]. EVLP perfusate delivery of stem cells, adenosine receptor antagonists, gene therapy, and plasmin have all been shown to be beneficial, as have intrabronchial delivery of surfactant and bronchodilators [9,12–14,31,32]. Perfusate analysis in this study found elevations of proinflammatory cytokines corresponding to the level of DCD injury, suggesting an opportunity for targeted therapy or, at minimum, an assay for evaluating therapeutic efficacy of other adjunct interventions beyond organ-level physiologic data. Our model of preservation and transplantation provides a baseline for comparison for future studies, along with a platform for translational evaluation of new donor and allograft management strategies aimed at mitigating donor-related allograft injury, some of which may require a prolonged interval for maximum effect.

The relationship between EVLP data and post-transplant allograft function has been investigated. Okamoto and colleagues used a model of cellular EVLP to examine the relationship between EVLP P:F ratio and other

Table 3. Lung block weights and postimplant allograft function.

	R	*P*-value
Allograft PV P:F ratio		
Pre-EVLP lung block weight	0.1755	0.6058
Post-EVLP lung block weight	**−0.6335**	**0.0364**
EVLP lung block weight gain	**−0.7394**	**0.0093**
Recipient dynamic compliance		
Pre-EVLP lung block weight	**−0.6768**	**0.0222**
Post-EVLP lung block weight	−0.3168	0.3425
EVLP lung block weight gain	−0.1290	0.7055
Recipient PA pressure		
Pre-EVLP lung block weight	0.0415	0.9036
Post-EVLP lung block weight	−0.0461	0.8930
EVLP lung block weight gain	−0.0628	0.8544
Recipient systemic P:F ratio		
Pre-EVLP lung block weight	−0.0259	0.9398
Post-EVLP lung block weight	−0.3735	0.2579
EVLP lung block weight gain	−0.3952	0.2290

EVLP, *ex vivo* lung perfusion; P:F, PaO_2:FiO_2; PA, pulmonary artery; *R*, correlation coefficient.

Results of Pearson's correlation analysis between lung weights and recipient measures at the end of observation. Significant values in bold.

Table 4. *Ex vivo* lung perfusion predictors of post-implant allograft function.

	1 h		8 h		24 h	
EVLP marker	*R*	*P*-value	*R*	*P*-value	*R*	*P*-value
Allograft PV P:F ratio						
Dynamic compliance	−0.0927	0.7863	0.3066	0.3591	0.1975	0.5605
Vascular resistance	−0.4241	0.1936	**−0.7846**	**0.0042**	−0.5027	0.1150
Mean PA pressure	−0.4141	0.2054	**−0.7860**	**0.0041**	−0.4841	0.1313
P:F ratio	−0.3882	0.2380	0.3862	0.2407	*0.5759*	*0.0637*
Recipient dynamic compliance						
Dynamic compliance	**0.6464**	**0.0316**	0.5346	0.0901	0.1500	0.6598
Vascular resistance	0.0652	0.8489	0.2084	0.5386	0.0401	0.9069
Mean PA pressure	0.0434	0.8991	0.3660	0.2683	0.0462	0.8926
P:F ratio	0.3708	0.2615	0.3317	0.3189	0.1360	0.6901
Recipient PA pressure						
Dynamic compliance	0.0402	0.9066	0.0158	0.9632	0.2890	0.3887
Vascular resistance	−0.2262	0.5037	−0.1994	0.5566	−0.5181	0.1026
Mean PA pressure	−0.2429	0.4716	−0.1905	0.5747	*−0.5425*	*0.0847*
P:F ratio	−0.0522	0.8788	−0.3403	0.3058	−0.0010	0.9976
Recipient systemic P:F ratio						
Dynamic compliance	−0.3534	0.2864	−0.1573	0.6442	0.1318	0.6992
Vascular resistance	0.0318	0.9261	−0.2084	0.5386	−0.1529	0.6536
Mean PA pressure	0.0181	0.9579	−0.4649	0.1496	−0.1367	0.6886
P:F ratio	−0.2434	0.4708	0.3039	0.3636	0.3584	0.2792

EVLP, *ex vivo* lung perfusion; PA, pulmonary artery; P:F, PaO_2:FiO_2; *R,* correlation coefficient.

Results of Pearson's correlation analysis between EVLP measures and recipient measures at the end of observation. '1 h', '8 h', and '24 h' refer to EVLP time points. Significant values in bold. Values approaching statistical significance in italics.

data and concluded that EVLP P:F ratio alone was insufficient for assessing an allograft's suitability for transplantation [19,20]. Similarly, Yeung and colleagues demonstrated in a model of acellular EVLP and transplantation that EVLP P:F may be a lagging indicator of injury and that edema and discouraging trends in other measurements (particularly airway pressures and compliance) should be considered when determining suitability for transplant [21]. Our findings of the importance of a spectrum of EVLP measures in determining the suitability of an allograft for transplant are consistent with this work.

Some donor lungs studied and treated on EVLP will remain unsuitable for transplant despite attempts at salvage, particularly those that demonstrate early and persistent hemodynamic derangement and edema, emphasizing the importance of sound clinical judgment (in addition to valid interpretation of EVLP data) on the part of transplant teams when evaluating such lungs. Our 1H DCD block that was discarded is one example. Notably absent from our study are donors subjected to unventilated warm ischemia. This decision was made based on our previous data demonstrating superior EVLP performance of donor lungs preserved following ventilated warm ischemia [22]. We do not believe that the procurement, preservation, and transplant of lungs from a donor that experiences prolonged warm unventilated ischemia comports to any realistic clinical scenario.

However, based on our results, we believe lungs from a wide range of potential donors that may otherwise be declined based on standard criteria (e.g. P:F ratios <300 mmHg, elevated PA pressures, uncontrolled DCD) should be evaluated using EVLP with the possibility of deferring allocation or decision of acceptability until composite assessment of hemodynamics, ventilation, and oxygenation can be satisfactorily performed. Although we studied 24 h of preservation, many such assessments can be performed in less time, even in extended-criteria donors. Our data suggest that a determination about suitability for transplant can be made relatively early (e.g. 8 h) in the preservation process. Once this determination is made, longer periods of preservation may be used as dictated by the requirements of adjunct therapies delivered during EVLP or by clinical/ logistical constraints.

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Limitations

Our study has important limitations. The use of 24 h of normothermic preservation and delay of transplantation until this time, intended to characterize the lung recovery and provide a platform for reconditioning, almost certainly impacted our overall findings. Transplant at earlier intervals would help to determine whether some lungs were unsalvageable or if donor-related injury of otherwise-viable lungs was exacerbated by prolonged EVLP. The impaired performance of the standard donor group compared with our previously-reported results was surprising and likely also had a significant impact on our results [5]. The similarity of post-transplant performance between this group and the DCD groups was also surprising. Nonetheless, they contributed to the robustness of the data that allowed us to correlate important trends between EVLP parameters and acute allograft function. The effect of perfusate hemolysis, which was not directly quantified in this study, is unclear but should have been similar across groups. Finally, pulmonary shunt fraction and relative distribution of cardiac output to each lung, which may have provided more granular insight into the functional trajectory of the allografts during preservation and after implantation, were not measured.

The donor whole blood used in the perfusate was collected immediately following cardiac arrest, regardless of group assignment. This would not always be feasible in a clinical uncontrolled DCD donor. Furthermore, donor animals in this study were not subject to the metabolic sequelae of preprocurement brain injury and withdrawal of therapy, an important difference between our study and a beating-heart clinical donor. However, our group has previously demonstrated the utility of autologous donor whole blood for prolonged preservation of lungs from standard and DCD donors and the current study was performed to characterize the translational effects of prolonged preservation as a proof of concept. It is possible that banked blood products (e.g. packed red blood cells, plasma, platelets) could be combined to create a perfusate similar to autologous donor whole blood. However, this may raise immunologic concerns and the ideal combination is unknown; further study is required.

The normal right lung was undisturbed at transplant to facilitate allograft evaluation for the entire observation period. This allowed some compensation for dysregulated allograft function, including impaired oxygenation. However, we believe that the comparative post-transplant differences in recipient physiology corresponding to the level of donor injury, described above, indicated incomplete compensation and allow meaningful comparison between donor groups (Fig. 4). Furthermore, although contralateral pulmonary exclusion was not performed, the use of selective APV blood gas sampling, which has been previously reported, allowed dedicated analysis of allograft oxygenation [13,33]. In our experience, contralateral pulmonary exclusion significantly impacts the animal's physiology and disrupts consistent data collection. While selective APV blood sampling is not perfect, it circumvents this issue and facilitates consistent data collection.

The 4H observation period may have been insufficient to detect complete reversal of donor- and EVLP-related allograft injury. The milieu of noncardiogenic pulmonary edema, impaired oxygenation, and elevated pro-inflammatory cytokine expression is analogous to primary graft dysfunction and/ or acute respiratory distress syndrome, both of which require an extended period for complete recovery [34]. The tendency of allograft weight to decrease during observation suggests that such recovery may occur after transplant. The relatively brief (4H) period of post-transplant observation was chosen because this was a pilot study intended to evaluate the feasibility of lung transplantation in this setting (uDCD followed by 24H normothermic EVLP) and to understand how lungs procured and preserved in this manner would function after transplant. A chronic survival model would demonstrate whether such injured lungs ultimately regain normal function and allow correlation between EVLP data and long-term physiologic function. Relatively small group sizes ($n = 4$) may have also limited our ability to detect intergroup differences in function.

In order to isolate the effect of prolonged blood-based normothermic EVLP on the recovery of injured lungs and characterize their post-transplant performance as a baseline for future studies, none of the adjunct therapies described above were deployed. Any combination of these likely would have improved post-transplant allograft performance.

Future directions

Our results raise questions requiring further investigation. As mentioned above, whole blood collected from donors with prolonged warm ischemia is likely to have greater levels of thrombus, hemolysis, and metabolic waste compared with beating-heart donors. Future studies would hold the preprocurement ischemic interval constant while varying the timing of blood collection after arrest to characterize the effect of downtime on

the quality of the blood perfusate, including baseline and time-varying levels of hemolysis over the course of EVLP. The effects of transplant at earlier intervals and the use of adjunct pharmacologic or cellular therapies aimed at improving the quality of preservation in this setting merit investigation.

Conclusions

We report a novel translational model of 24H normothermic blood-based EVLP followed by single lung transplantation with acceptable acute survival. Allograft oxygenation on EVLP at 24H was predictive of post-transplant function. High levels of allograft edema and unfavorable early hemodynamics generally predicted poor allograft oxygenation after transplant. Although transplantation at earlier intervals may ultimately be beneficial, this model provides a benchmark and a platform for translational evaluation of new therapies aimed at mitigating donor-related allograft injury.

Authorship

JRS, PAI, AP-M, GL, LMM, CM and TI: conceived and planned the project. JRS: performed extensive literature review. PAI: provided study animals, lab space, and ancillary staff support. JRS, PAI, GL, LMM, CM, and TI: collected data. JRS, AP-M, RZB and LMM: analyzed data. JRS: performed principal manuscript composition. JRS and RZB: figure and table construction. All authors participated in manuscript editing and provided final approval of the text of the manuscript.

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Conflict of interest

Nonclinical grade disposable perfusion equipment was provided courtesy of TransMedics (Andover, MA, USA). This included the blood collection chambers, reusable modules for animal work, and OCS Lung solution. Dr. Loor is a co-investigator in the INSPIRE and EXPAND trials and receives grant support for these trials from TransMedics.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Physiologic review of severely injured 1H DCD lung block rejected for porcine transplant.

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