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Remote ex vivo lung perfusion at a centralized evaluation facility

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KEYWORDS:
EVLP;
lung transplant;
donation after cardiac death;
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PGD3

BACKGROUND: In the US, only 23% of lungs offered for transplantation are transplanted. Ex vivo lung perfusion (EVLP) allows for evaluation of additional donor lungs; its adoption has been limited by resources and expertise. Dedicated facilities with a centralized lung evaluation system (CLES) could expand access to EVLP.

METHODS: In this unblinded, nonrandomized, traditional feasibility study, 7 US transplant centers referred lungs declined for standard transplantation to a dedicated EVLP facility, which utilized a CLES. EVLP was remotely monitored by the transplant teams. CLES lungs were matched with contemporaneous conventional static cold-preserved controls at each center.

RESULTS: A total of 115 recipients were enrolled, and 66 received allografts from 63 donors after EVLP at the dedicated CLES facility. Forty-nine contemporaneous patients served as controls. Primary graft dysfunction grade 3 at 72 hours (PGD3-72 hours) was higher in the CLES group with 16 (24%) vs 2 (4%) in the control (common RD 95% CI, 0.07-0.32; $p = 0.0009$). All recipients survived to 30 days and 1-year survival was similar for both groups (92% controls vs 89% CLES; common RD 95% CI, -0.14-0.08; $p = 0.58$). Total preservation time, hospital and ICU lengths of stay, and time to first extubation were longer in the CLES group.

CONCLUSIONS: Remote *ex vivo* perfusion of lung allografts declined for conventional transplantation at a dedicated CLES facility is feasible and resulted in additional transplants. Recipients of allografts assessed with a CLES had a higher rate of PGD3-72 hours, but similar 30-day and 1-year outcomes compared to conventional lung recipients. (NCT02234128)

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Lung transplantation is the only treatment option for some patients with end-stage lung disease. In the United States, current donor lung utilization rates are 23% and 15% of waitlist registrants die prior to transplantation.[1] Additionally, 33% of candidates removed from the waitlist for being too ill, die within 6 months,[1] while an unknown number of people succumb to end-stage lung disease and are never considered candidates. Increasing the number of transplantable lungs can reduce waitlist mortality and the mortality for end-stage lung disease. *Ex vivo* lung perfusion (EVLP) can save lives and allows for the assessment of additional donor lungs that otherwise would not be considered for transplantation.

In the first prospective clinical trial of EVLP, high-risk donor lungs transplanted after evaluation on the Toronto EVLP system (TES) had similar outcomes to conventionally-selected lungs.[2] In the multicenter NOVEL trial using the XVIVO XPS System, EVLP and control lung recipients had similar 1-year survival.[3] These and other reports demonstrate EVLP utility for assessment of lungs declined for standard transplantation and that lungs transplanted after EVLP at the transplant center have similar short- and long-term outcomes as lungs transplanted after standard static cold storage.[4-6]

A case report of a successful transplant after remote EVLP demonstrated the possibility of off-site EVLP.[7] A dedicated EVLP facility could make this technology safely accessible to transplant centers regardless of infrastructure or expertise. According to the 2020 Organ Procurement and Transplantation Network/Scientific Registry of Transplant Recipients report, the most common reasons for lung discards in the US are “other” and “poor organ function”.[8] It is possible that a group of these lung declines could have been transplanted if better information was available to the transplant centers. Applications for EVLP include evaluation of marginal donor lungs, lungs from donors after circulatory death (DCD), and overcoming logistical challenges that would preclude transplant.[9] This clinical trial was designed to demonstrate the safety and feasibility of utilizing a centralized lung evaluation system (CLES), based on the TES, at a dedicated facility to evaluate lungs that otherwise would not be used for standard transplantation.

Methods

Study design

This was an unblinded, nonrandomized, traditional feasibility study to evaluate the safety of extending preservation and assessment time of donor lungs using normothermic EVLP. EVLP was performed at a dedicated EVLP facility using a centralized lung evaluation system (CLES) based on the TES. The study was conducted at 7 sites in the US between July 2015 and November 2019. Sites were selected based on transplant experience and historical patient outcomes and were within 750 miles of the CLES facility. This study was in strict compliance with the International Society of Heart and Lung Transplantation (ISHLT) ethics statement (ClinicalTrials.gov, number NCT02234128).

The protocol, informed consent form(s), recruitment materials, and all subject materials were submitted to appropriate local institutional review boards (IRBs) for review and approval. Recipients were enrolled in the study at the time of lung transplantation. Consenting recipients receiving a conventional lung transplant were recruited as a contemporaneous control group. The study was initially designed to include 40 subjects in the CLES and 40 subjects in the control group. The sample size was increased to 66 lungs in the CLES group to allow inclusion of lung-grafts deemed not suitable for cold-storage and subsequent transplantation due to expected extended preservation time and/or need for additional assessment in the opinion of the transplant center team. The number of 40 control subjects was deemed appropriate to determine safety. Pending FDA feedback on the change in sample size, enrollment in the control group continued and a total of 49 control subjects were enrolled.

The control group was matched to the CLES group by transplant center, transplant type (single or bilateral), and Lung Allocation Score Disease Diagnosis Group (LAS- DDG).[10] This matching occurred on a subject-by-subject basis, only after a CLES subject was enrolled at the transplant center; and with the next eligible, consented conventional transplant recipient. Study centers were allowed to enroll up to 3 CLES subjects prior to enrolling at least 1 matching control subject.

Inclusion and exclusion criteria

Donor lung criteria

All donor lungs were allocated to transplant centers according to the US Organ Procurement and Transplantation Network (OPTN) policy. Once an acute care hospital identified a potential organ donor, the regional organ procurement organization (OPO) was contacted to coordinate the organ donation. The OPO entered specific donor demographics into a single national database to generate a match run list of patients. In match run order, the OPO contacted a transplant center to offer the matched organ to a specific patient. The transplant center had 1 hour to accept the lung for the matched patient, otherwise the lung offers continued in match run sequence order for additional patients until a center accepted the lung(s), the match run list was exhausted or there were timing issues which may cause the OPO to suspend subsequent lung offers. The lung acceptance by a center was based on a number of factors, but primarily was dependent on the transplant center’s staff decision that a specific donor lung was suitable for the intended recipient. All study lungs were offered through standard allocation. All CLES lungs were referred for EVLP by a study transplant center, indicating that at least 1 US transplant center deemed the lung(s) not suitable for direct transplantation.

Donor lungs were eligible for EVLP if they were considered marginal or met extended donor criteria,[11] required additional assessment or evaluation time in the opinion of the investigator, or when an OPO documented that without EVLP the lungs would not be used for transplantation (Table 1). Donor lungs were excluded from EVLP in cases of confirmed pneumonia/aspiration, persistent purulent secretions, significant mechanical injury/trauma, HIV,

HCV, HBV, or other active infectious disease. (See supplement for details). Lungs were also excluded from EVLP if the time from aortic cross clamp/initial flush to the start of EVLP was expected to exceed 10 hours (Figure S1).

After EVLP, lungs were deemed suitable for consideration for transplantation by the transplant center investigator if they met the following conditions: final partial pressure of oxygen in venous perfusate PvO2/FiO2 $\geq$ 350 mm Hg, < 15% increase in pulmonary vascular resistance (PVR), < 15% increase in pulmonary artery pressure (PAP), and < 15% decrease in static lung compliance (Cstat) from baseline (first hour of EVLP) to the final measurements. The investigator made the final determination for termination of EVLP and proceeding or not proceeding to transplantation after reviewing all data collected during EVLP, donor/recipient information, feedback from EVLP specialists and consultation with an EVLP-experienced surgeon at Toronto General Hospital (TGH).

Recipients

All subjects were at least 18 years-old and underwent a single or bilateral lung transplant. The subject or the subject's representative provided informed consent prior to participating in study-related assessments or procedures. Subjects listed for same-side lung retransplantation, live donor lobar transplant, or multiple-organ transplantation were excluded. Subjects positive for human immunodeficiency virus, active Hepatitis B or C, or *Burkholderia cepacia* infection were excluded. Subjects in the intensive care unit (ICU) at the time of the initial lung offer requiring mechanical ventilation, or extracorporeal life support (ECLS) were excluded.

EVLP logistics and procedure

If a lung allograft was not suitable for conventional transplantation and met the EVLP lung donor criteria described above, the transplant center could refer the lung(s) to the Lung Bioengineering facility in Silver Spring, MD (LB-1). This decision was based on the protocol, the transplant center staff's medical expertise, and the details of the particular case. Once a transplant center referred lung(s) for EVLP, the OPO and the LB-1 staff coordinated transportation. Cold ischemia time 1 (CIT1) was calculated as donor aortic cross clamp/initial flush to the start of EVLP. CIT1 was limited to a maximum of 10 hours. EVLP lasted from 3 to 6 hours per protocol. Cold ischemia time 2 (CIT2) was calculated as the end of EVLP to when the lung(s) were removed from cold storage and was limited to a maximum of 6 hours for the first lung and ten hours for the second lung.

The CLES involved a trained specialist performing EVLP using the TES as previously described (see supplement).[12,13] All collected data was documented and accessed in a web-based electronic chart and available in real time to the transplant team during and after the EVLP procedure. The transplant center, the EVLP facility and an expert consultant surgeon from TGH communicated in real-time using a state-of-the-art audio-visual system.

Ventilation began when the lungs reached 32^{0}C and ventilation parameters were measured hourly after oxygen challenge. Perfusion parameters were collected every 10 minutes during the initial 1-hour warm-up phase and hourly thereafter. Bronchoscopies were performed and observations recorded at the 1-, 3-, and if necessary, 5-hour marks of the EVLP procedure, and as needed to clear airways or upon transplant center request. Digital radiographs of the lung were taken after the first, third, and fifth (if applicable) hour marks to detect edema, consolidations, or other abnormalities.

LB-1 coordinated transportation from the EVLP facility to the transplant center. Total preservation time (TPT) for the first lung transplanted included CIT1, EVLP time, and CIT2 and was limited to a combined 22 hours. TPT for the second lung, if bilateral, was limited to 26 hours.

Endpoints

The primary endpoints were the proportion of recipients with primary graft dysfunction grade 3 at 72 hours (PGD3-72 hours) posttransplant and the 30-day mortality rate posttransplant. PGD3 was graded based on the ISHLT 2005 consensus.[14] If ECMO was required posttransplant, a grade of PGD3 was automatically assigned (Table S1). Secondary endpoints were PGD (Grades 0-3) at 0, 24, 48, and 72 hours, TPT, time to first extubation, ICU length of stay (LOS), and hospital LOS.

Exploratory objectives included longer-term evaluation of adverse events (AEs) and 1-year posttransplant mortality. Additional endpoints included forced expiratory volume in 1 second (FEV_1), bronchiolitis obliterans syndrome (BOS),[15] and rehospitalizations in the first year following transplant [See supplement for details].

Adverse events

All AEs were assessed by the transplant centers for severity, causality, outcome, seriousness, and if unanticipated, documented as such. AEs were managed according to each participating transplant center practice and protocols. Events that were considered inherent to lung transplantation, surgical procedures, and immunosuppression did not require reporting.[16] Investigator-classified lung graft-related or possibly related serious adverse events (SAEs) were grouped posthoc as acute rejection, respiratory failure necessitating prolonged ventilation or reintubation, bronchial anastomotic complications, major lung-related infection, vascular complications, or other.

Statistical analysis

Summaries and analyses were performed using SAS software version 9.4 (Cary, NC). Donor, recipient, and transplant characteristics were summarized and compared across transplant groups (CLES vs. control), using Fisher's Exact Test for categorical data and the Wilcoxon Rank Sum Test for continuous data. Outcomes such as survival at 30 days posttransplant, PGD3-72 hours posttransplant (Y/N), and LOS were also compared across transplant groups. For categorical recipient outcomes, comparisons were performed using the common risk difference (RD), stratifying on transplant center, single lung transplant (SLT)/double lung transplant (DLT) and LAS-DDG. Continuous recipient outcomes, except for FEV1, were compared using the stratified Wilcoxon test, stratifying on transplant center, SLT/DLT and LAS-DDG. The FEV1 was analyzed using a random coefficients model. Sensitivity analyses for matched pairs were also performed and resulted in similar statistical conclusions (Table S2). Time to death was compared across transplant groups using the stratified log-rank test, stratifying on SLT/DLT and LAS-DDG (no stratification on transplant center to avoid sparse strata), and using Cox proportional hazards regression, adjusting for transplant group, transplant center, SLT/DLT, and LAS-DDG. Kaplan-Meier survival curves out to 1 year were plotted for the CLES and control groups along with the p-values from the two analyses of time to death.

Results

One hundred five lung grafts, declined for standard transplant, were transported to the dedicated EVLP facility and

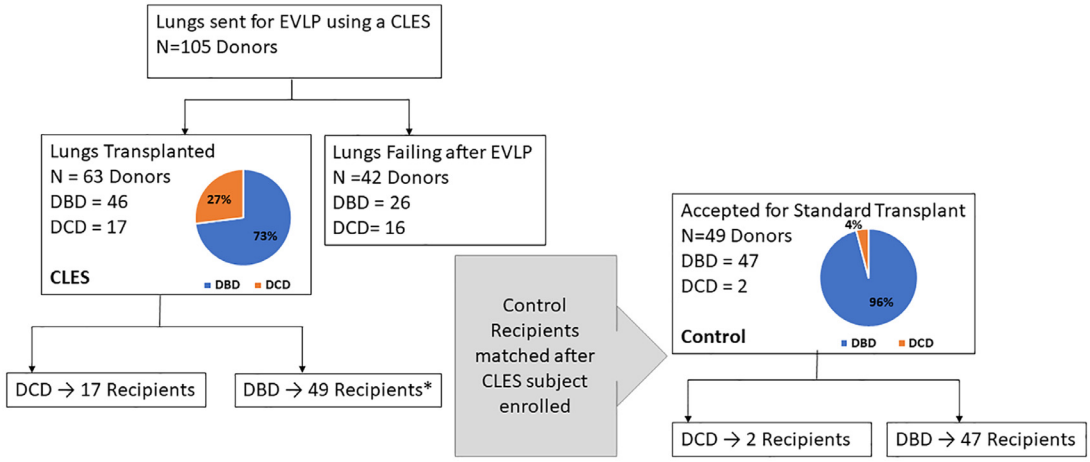

Figure 1 Lung Disposition. Donor lungs were eligible for CLES if they were considered marginal or extended criteria, required additional assessment or evaluation time in the opinion of the investigator, or when an organ procurement organization (OPO) documented that without EVLP the lungs would not be used for transplantation. Standard lung transplant recipients were recruited as a contemporaneous control group. The control group was matched to the CLES group by transplant type (single or bilateral) and Lung Allocation Score Disease Diagnosis Group (LAS-DDG). This matching took place on a subject-by-subject basis and only after an CLES subject had been enrolled at the study site. Up to 3 EVLP recipients could be enrolled at each center prior to a control enrollment. Recipients were enrolled in the study at the time of lung transplantation. A total of 154 donor lungs met the inclusion criteria. *Recipients outnumber donors due to single lung transplants. DBD, donation after brain death; DCD, donation after circulatory death.

underwent EVLP using a CLES. Following EVLP, 63 allografts were accepted for transplantation into 66 recipients, leading to a utilization rate of 60% (Figure 1) for lungs that otherwise were deemed not acceptable for standard cold-storage preservation and subsequent transplantation by the OPO and/or the participating transplant center (Table 1 and 2). The control group ($n = 49$) contains lung(s) accepted for conventional transplantation and were included after matching an CLES recipient for LAS-DDG and transplantation type (single or bilateral) at the same center.

The participants were representative of the US lung transplant population (Table S3). While recipients for CLES and control transplants were well-matched, some differences were seen in the donor pool (Table 2). Donors were significantly older in the control than the CLES group (median 39 vs 33 years, respectively; $p = 0.0252$). Significantly lower final PaO_2/FiO_2 were seen in CLES allografts at referral (median 353 mm Hg) compared to control donors (median 398 mm Hg, $p = 0.0107$). Most notably, the CLES donor pool contained 27% DCD lungs, whereas the standard transplant group contained only 4% DCD lungs (Figure 1, Table 2).

EVLP

As expected, transplanted CLES lungs had a significantly longer total preservation time (median 13 hours) than control lungs (median 3 hours, $p < 0.0001$) (Table 3). CIT1 lasted a median of 4.7 hours (range 2.3-10.0 hours) (Figure S1). CIT2 was a median of 3.7 hours (range 1.4-6.9 hours). Two CIT2s deviated from protocol, exceeding 6 hours for the first lung. One deviation was due to a complex surgery and the other was for an extended back table allograft inspection. No allografts were discarded due to protocol time limits.

Outcomes

The primary study goal was to evaluate the safety and feasibility of assessing lungs rejected for transplantation using a CLES at a dedicated EVLP facility. Grade 3 primary graft dysfunction evaluated at 72 hours posttransplant (PGD3-72 hours) was selected as a primary endpoint, as a predictor of long-term survival in bilateral lung transplant recipients.[17] The PGD3-72 hours incidence was significantly higher in the CLES group (24%) compared to the control recipients (4%) (common RD 95% CI, 0.07-0.32; $p = 0.0009$) (Table 3). PGD3 evaluated at 0, 24, and 48 hours were also higher in CLES recipients than controls, although the numbers declined over time (Table 3). In the CLES group, 13 of 16 subjects with PGD3-72 hours were on ECMO (Table S1). ECMO use was an automatic grade of PGD3.[15]

Since PGD3 may not be predictive for single lung transplants (SLT), which represented 41% of control and 47% of CLES lung recipients (Table 3), 30-day survival was also a primary end-point. All subjects survived to 30 days posttransplant (Table 3). By 1-year posttransplant, 89.4% and 91.8% of recipients survived in the CLES and control groups, respectively (common RD 95% CI, -0.14-0.08; $p = 0.58$). During the 1-year follow-up period, graft in the CLES group failed 6-12 months post-transplant. Survival was similar for both groups out to year ($p = 0.61$) (Figure 2).

During the recipient index hospitalization posttransplant, CLES lung recipients had significantly longer time to extubation after transplant surgery compared to control recipients (median 62.3 vs 21.2 hours; $p < 0.0001$, stratified Wilcoxon) (Table 3). Additionally, posttransplant ICU stay (median 12 vs 5 days; $p < 0.0001$, stratified Wilcoxon) and hospitalization stay were longer (median 29 vs 18 days; $p = 0.0003$, stratified Wilcoxon) for CLES lung than control lung recipients.

Table 1 EVLP Donor Characteristics

Characteristics	Accepted CLES lungs (*n*) N = 63	Rejected CLES lungs[a] (*n*) N = 42
Mean Age ± SD	34.6 ± 12.0	38.0 ± 14.4
Male n (%)	35 (55.6)	26 (61.9)
Mean Height ± SD, cm	170.5 ± 9.0,	173.4 ± 11.9
Weight ± SD, kg	83.7 ± 20.8	86.5 ± 26.6
Race n (%)		
Asian	1 (1.6)	1 (2.4)
Black/African American	6 (9.5)	8 (19.0)
White	50 (79.4)	31 (73.8)
Unknown	6 (9.5)	2 (4.8)
Blood Group *n*(%)		
A	20 (31.7)	11 (26.2)
B	8 (12.7)	7 (16.7)
AB	1 (1.6)	2 (4.8)
O	34 (54.0)	22 (52.4)
Cause of Death n (%)		
Anorexia/Cardiac Arrest	29 (46)	19 (45.2)
Head Trauma	16 (25.4)	15 (35.7)
Cebrovascular/Stroke	16 (25.4)	8 (19.0)
CNS Tumor	1 (1.6)	0 (0)
Other	1 (1.6)	0 (0)
Median Last PaO_2/FiO_2 (range) mm Hg	353 (77-615)	376 (189-751)
Reasons for EVLP Referral		
At the time of referral, the donor PaO2/FiO2 < 300 mm Hg	16	12
Donor received 10 units of blood transfusions	4	4
Pulmonary edema detected via CXR, bronchoscopy or palpation of lungs	11	12
Donation after Circulatory Death (DCD) donor	17	14
Expected cold ischemic time > 6 hours	6	6
Donor age ≥ 55 years old	4	7
Study Center Investigator requires additional assessment ex vivo and/or extended preservation time in situations when not doing so would result in the lung being discarded	63	42
Median Number of Lung Inclusion Criteria Met per Donor	2	2

CXR, chest x-ray.
[a]Rejected CLES lung data was not monitored as part of the trial and has been collected posthoc.

Although early index hospitalization outcomes differed post-transplant between groups, the number of rehospitalizations within a year posttransplant were not significantly different (p = 0.86 stratified Wilcoxon) (Table 3). Change in FEV_1 percent predicted over time was similar between control and CLES transplanted lungs at 1 year (p = 0.91) (Table 3). Obstructive chronic lung allograft dysfunction (CLAD) measured by bronchiolitis obliterans syndrome (BOS) grade was also comparable between groups at 1 year (3.8% CLES vs 4.9% control, common RD 95% CI, -0.10-0.17; p = 0.60).

Serious adverse events (SAEs) related or possibly related to lung-graft were collected and later categorized (Table 4). The distribution of the number of types of categorized lung-graft-related SAEs experienced per patient was similar at 30 days for CLES and control groups (p = 0.13) (Table 4). The distribution was also similar between groups at 1 year. Bronchial anastomotic complications were higher in the CLES group than control, (15% vs 4% at 1 year). In the first 30 days, 4 CLES patients also experienced a vascular complication, none occurred in the controls. Acute cellular rejection (ACR) at 1 year occurred more in the control than the CLES group (24% vs 12%). Differences were not statistically significant between groups (Table 4). Detailed SAE listings provided in the supplement (Table S4 and S5).

Discussion

This is the first prospective, multicenter trial to demonstrate safety and feasibility of a dedicated facility to remotely perfuse and evaluate lungs declined for standard transplantation. Our primary outcomes were PGD3-72 hours and 30-day posttransplant survival. Survival at 30-days and 1-year posttransplant was similar for the CLES and control groups. PGD3-72 hours was higher in the CLES group than controls; the control group (4%) was lower than the national rate reported by the US Lung Transplant Outcomes Group (16.8% +/- 2.1).[18] The rate of PGD 2-3 at 72 hours was 33% for the CLES group, which is similar to the 30% observed in the Toronto trial control group but higher than their EVLP group (15%).[2] This suggests that delayed allograft function and its impact on subsequent clinical course

Table 2 Donors, Recipients, and Transplants Characteristics

Characteristic	CLES DCD	CLES DBD	CLES total	Control DCD	Control DBD	Control total	p[a]
DONORS	$n = 17$	$n = 46$	$n = 63$	$n = 2$	$n = 47$	$n = 49$	
	27%	73%	100%	4·1%	95·9%	100%	
Median Age (range)	32 (11-52)	34 (16-66)	33 (11-66)	36.5 (14-59)	39 (15-68)	39 (14-68)	**0.0252**
Males	$n = 10$	$n = 25$	$n = 35$	$n = 2$	$n = 31$	$n = 33$	0.24
Median Last PaO_2/FiO_2 (range) mm Hg	374 (181-515)	352 (77-615)	353 (77-615)	404 (394-413)	398 (97-562)	398 (97-562)	**0.0107**
RECIPIENTS	$n = 17$	$n = 49$[b]	$n = 66$[b]	$n = 2$	$n = 47$	$n = 49$	
	25.8%	74.2%	100%	4.1%	95.9%	100%	
Median Age (range)	62 (33-72)	63 (20-72)	62.5 (20-72)	57 (56-58)	62 (46-75)	62 (46-75)	1.00
Males	$n = 8$	$n = 27$	$n = 35$	$n = 2$	$n = 33$	$n = 35$	0.0546
UNOS group A	6	24	30	2	20	22	1.00
UNOS group B	0	1	1	0	0	0	
UNOS group C	1	1	2	0	1	1	
UNOS group D	10	23	33	0	26	26	
Median LAS at Transplant (range)	35.35 (32.24-57.07)	35.35 (32.18-78.93)	35.35 (32.18-78.93)	33.21 (32.74-33.68)	37.49 (32.28-88.47)	37.46 (32.28-88.47)	0.46
TRANSPLANT TYPE							
Bilateral	$n = 10$	$n = 25$	$n = 35$	$n = 2$	$n = 27$	$n = 29$	0.57
LUNG PRESERVATION TIME, median (range), hours							
CIT 1	5.3 (2.5-8.0)	4.4 (2.3-10.0)	4.7 (2.3-10.0)	-	-	-	-
EVLP	4.2 (3.5-5.7)	3.9 (3.5-5.8)	4.0 (3.5-5.8)	-	-	-	-
CIT 2	3.9 (1.4-5.4)	3.4 (1.4-6.9)	3.7 (1.4-6.9)	-	-	-	-
Total	13.9 (8.2-15.3)	12.2 (8.5-19.8)	13.0 (8.2-19.8)	5.1(4.4-5.9)	3.0 (0.68-6.0)	3.0 (0.68-6.0)	**<0.0001**[c]

CLES, centralized lung evaluation system; CIT1, cold ischemia time 1; CIT2, cold ischemia time 2; DCD, donation after circulatory death; DBD, donation after brain death; EVLP, *ex vivo* lung perfusion; LAS, lung allocation score; UNOS group A, obstructive lung disease; UNOS group B, pulmonary vascular disease; UNOS group C, cystic fibrosis; UNOS group D, restrictive lung disease.

Percentages are calculated within total treatment group. Transplantation percentages calculated from total recipients in treatment group.

Bolded p-values are significantly different ($P > 0.05$).

[a] p compares CLES Total to Control Total, using either Fisher's Exact Test (for categorical data) or the Wilcoxon Rank Sum Test (for continuous data).

[b] Number of recipients is greater than number of donors due to single lung transplants.

[c] p-value calculated using the stratified Wilcoxon test; estimate and 95% CIs for the location shift calculated using the Hodges Lehmann estimation: 9.0 (95% CI 8.1-9.9). Cold ischemia time 1 (CIT1) was calculated at donor aortic cross clamp/initial flush to the start of EVLP. Cold ischemia time 2 (CIT2) and total preservation time were calculated based on the times for the first implanted lung. Time ends, as per protocol, when the lungs are removed from the ice in the operating room at the recipient's hospital.

Table 3 Recipient Outcomes

End-point	CLES DCD N = 17	CLES DBD N = 49	CLES Total N = 66	Control DCD N = 2	Control DBD N = 47	Control Total N = 49	Treatment Difference (95 % CI)[a]	*p* value[a]
Primary end-points *n* (%)								
Survival 30 days[b]	17 (100)	49 (100)	66 (100)	2 (100)	47 (100)	49 (100)	-	-
PGD3 at 72 hours[b]	3 (18)	13 (27)	16 (24)	0 (0)	2 (4)	2 (4)	0.21 (0.07-0.32)	**0.0009**
Additional end points								
Survival[b], *n* (%)								
90 days	17 (100)	48 (98)	65 (99)	2 (100)	47 (100)	49 (100)	-0.01 (-0.03-0.02)	0.46
6 months	16 (94)	46 (94)	62 (94)	2 (100)	45 (96)	47 (96)	-0.02 (-0.10-0.07)	0.60
12 months	14 (82)	45 (92)	59 (89)	2 (100)	43 (91)	45 (92)	-0.03 (-0.14-0.08)	0.58
PGD3[b], *n* (%)								
0 hours	11 (65)	29 (59)	40 (61)	1 (50)	9 (19)	10 (20)	0.40 (0.23-0.54)	**<0.0001**
24 hours	4 (24)	17 (35)	21 (32)	0 (0)	2 (4)	2 (4)	0.27 (0.13-0.39)	**<0.0001**
48 hours	3 (18)	12 (24)	15 (23)	0 (0)	3 (6)	3 (6)	0.16 (0.02-0.28)	**0.01**
PGD2, *n* (%)								
0 hours	2 (12)	6 (12)	8 (12)	1 (50)	8 (17)	9 (18)		
24 hours	0 (0)	8 (16)	8 (12)	1 (50)	3 (6)	4 (8)		
48 hours	1 (6)	6 (12)	7 (11)	1 (50)	3 (6)	4 (8)		
72 hours	1 (6)	5 (10)	6 (9)	0 (0)	2 (4)	2 (4)		
PGD1, *n* (%)								
0 hours	2 (12)	6 (12)	8 (12)	0 (0)	9 (19)	9 (18)		
24 hours	11 (65)	18 (37)	29 (44)	1 (50)	22 (47)	23 (47)		
48 hours	12 (71)	21 (43)	33 (50)	1 (50)	23 (49)	24 (49)		
72 hours	9 (53)	20 (41)	29 (44)	2 (100)	20 (43)	22 (45)		
PGD0, *n* (%)								
0 hours	2 (12)	8 (16)	10 (15)	0 (0)	21 (45)	21 (43)		
24 hours	2 (12)	6 (12)	8 (12)	0 (0)	20 (43)	20 (41)		
48 hours	1 (6)	10 (20)	11 (17)	0 (0)	18 (38)	18 (37)		
72 hours	4 (24)	11 (22)	15 (23)	0 (0)	23 (49)	23 (47)		
Transplantation Hospitalization								
Time to Extubation[c]								
Median, hours	50	63	62	40	21	21	29.8 (15.8-72.9)	**<0.0001**
IQR, hours	(24-377)	(30-266)	(26-287)	(9-71)	(14-40)	(14-40)		
ICU Stay[c]								
Median, day	11	12	12	6	5	5	5 (2.5-10)	**<0.0001**
IQR, dau	(4-22)	(6-28)	(5-28)	(1-11)	(3-10)	(3-10)		
Total Hospital Stay[c]								
Median, day	31	29	29	15.5	18	18	8 (3-14.5)	**0.0003**
IQR, day	(17-49)	(19-48)	(18-49)	(10-21)	(13-31)	(13-29)		
Additional Outcomes at 1 Year								
Rehospitalizations[c] Median	1	2	1.5	5	1	1	0 (-0.5-0.5)	0.86

(*continued on next page*)

Table 3 (*Continued*)

End-point	CLES DCD N = 17	CLES DBD N = 49	CLES Total N = 66	Control DCD N = 2	Control DBD N = 47	Control Total N = 49	Treatment Difference (95 % CI)[a]	*p* value[a]
IQR	(1-3)	(1-3)	(1-3)	(1-8)	(2-3)	(1-3)		
FEV_1 % predicted[d]								
Median	75	66	69.5	67	82	79	-0.009 (-0.16-0.14)	0.91
IQR	(67-105)	(56 -91)	(57-95)	(59-75)	(64-92)	(64-92)		
BOS 1,2 or 3[b,e]	N=14	N=39	N=53	N=2	N=39	N=41	0.04 (-0.10-0.17)	0.60
n (%)	0 (0.0)	2 (5)	2 (4)	0 (0)	2 (5)	2 (5)		

BOS, bronchiolitis obliterans syndrome; CLES, centralized lung evaluation system; DBD, donation after brain death; DCD, donation after circulatory death; FEV_1, forced expiratory volume in the first second; ICU, intensive care unit; PGD, primary graft dysfunction

Percentages calculated within each group.

Bolded p-values are significantly different ($P > 0.05$).

[a]95% CI and *p*-values compare CLES Total to Control Total.

[b]*p*-value, estimate, and 95% CI for common risk difference.

[c]*p*-value calculated using the stratified Wilcoxon test; estimate and 95% CIs for the location shift calculated using Hodges-Lehmann estimation.

[d]FEV fit to a random coefficients model. Estimate, 95% CI, and *p*-value are for mean slope difference.

[e]Worse case imputed in case of death; unknown BOS subjects excluded.

may differ for remote EVLP. This could be the result of higher quality allografts in the control group, postoperative ECMO automatically graded as a PGD3[14] (Table S1), higher proportion of DCD lungs in the CLES group,[19] longer preservation times including 2 cold-storage periods in the CLES group, and probable alternative mechanisms for the development and resolution of PGD in CLES lungs perfused with an acellular solution.

In our population, PGD3-72 hours did not predict recipient survival or development of ACR or BOS. ACR at 1 year posttransplant in the CLES group was half of the control group (12% vs 24%). At 1-year, both groups had lower incidence of BOS (3.8% CLES, 4.9% control) than the US national average (7.1%).[1] It is possible, PGD3-72 hours may not be a strong predictor for remote EVLP outcomes. Possible mechanisms for this these findings require further investigation in future trials. The number of subjects experiencing specific categories of SAEs were not significantly different between groups at 30-day and 1 year (Table 4). Some SAEs were more commonly observed in the CLES group, like bronchial anastomotic and vascular complications at 30-day and 1-year, respiratory failure at 30-day and major respiratory-related infections at 1 year. The small number of subjects in the trial and the fact that the management of complications was based on each center's expertise and protocols may have prevented the detection of any significant associations, if they exist.

Although short-term transplant metrics, such as hospital LOS, were longer for CLES allograft recipients, the number of rehospitalizations and survival rates 1 year after transplant were not significantly different across groups. These findings are indicative of the relative safety of CLES lungs compared to standard transplants. We continue to follow study participants to assess long-term outcomes.

In the US, the proportion of lung transplants from DCD increased from 2.3% in 2014 to 5.7%[1] in 2019. This proportion is lower than the observed in Australia, Europe and Canada.[20] Given the limited experience with DCDs at some centers in the US, there is less comfort in accepting these lungs which can result in unnecessary discards. In our study, 27% of the CLES group received DCD lung transplants, compared to only 4.1% in controls. With CLES available as an assessment tool, transplant teams can be less constrained by warm ischemia time in DCD cases, and hopefully leading to future increases in DCD organ utilization with or without the use of EVLP.

CLES allows for single allograft perfusion and assessment, especially when the contralateral lung is unsuitable, maximizing the utilization of available lung allografts. In our study, 47% of transplanted EVLP lungs were SLT, compared to 25% in 2019 in the US.[1] Bilateral EVLP was performed on 11 of the 31 single lungs transplants. One set of lungs were bilaterally perfused then split at the CLES facility and sent to 2 different transplant centers; another bilateral set was split at the transplant center and transplanted into 2 different recipients. In the other 9 cases of bilateral EVLP, one of the lungs was deemed not suitable for transplantation and only 1 lung allograft was transplanted. In 1 case, the lungs from the same donor were

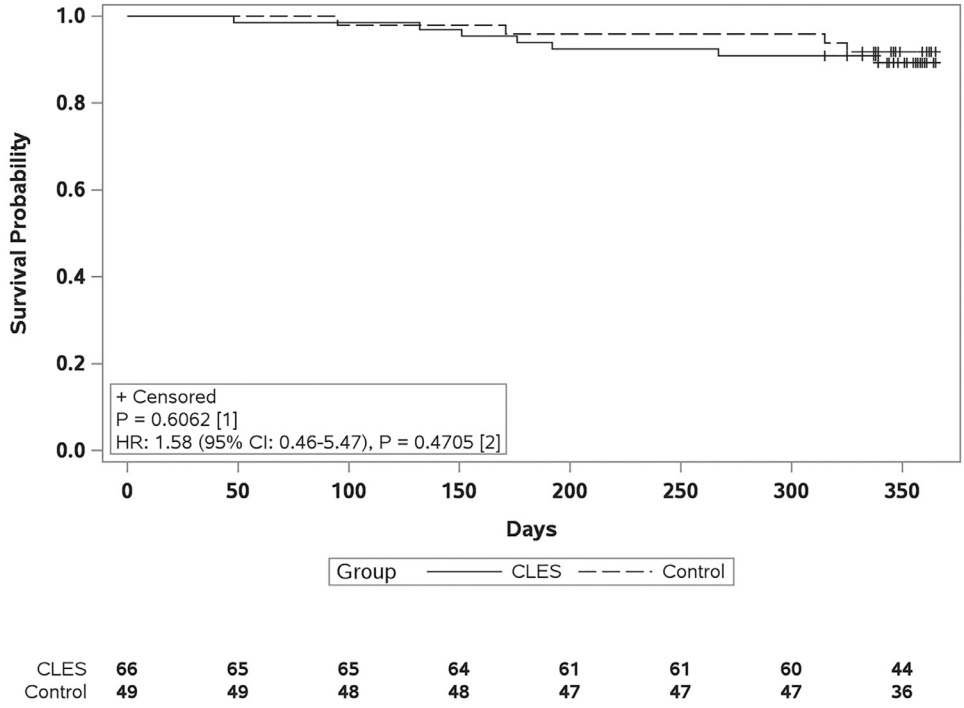

Figure 2 One year survival is not significantly different between CLES and Control lung transplant recipients. Kaplan-Meier survival curves out to one-year posttransplant, comparing CLES to Control (standard transplant) lungs. [1] P-value based on the stratified log-rank test, with transplant type and LAS-DDG as the strata. [2] Hazard ratio, 95% CI, and *p*-value calculated with the Cox proportional hazards model with transplant group, transplant center, transplant type, and LAS-DDG as explanatory variables.

perfused separately at the CLES facility for 2 different transplant centers. Both lung allografts proceeded to be transplanted. Our data suggests that DCD lungs and single lungs can be safely transplanted after using a CLES, which could increase transplants by hundreds per year in the US.

For our study we only utilized lungs that would have been discarded without EVLP, which resulted in a similar 1-year survival to the US national average (89%) and previous multicenter trials.[3,21,22] Capturing lungs that would have otherwise been declined for standard transplant involved collaborations between the organ procurement organization (OPO), the transplant center and the dedicated EVLP facility. Effective communication between all parties allowed referral of lung allografts at different times of the recovery process, from the initial match-run to last minute declines in the operating room. This intervention features not only extending the window for lung utilization via EVLP, but a service model that could augment the number of lungs available for transplantation. DEVELOP-UK, a 5-center trial that was terminated early, had a slow EVLP enrollment and low utilization rate (34%), and demonstrated the logistical and staff availability challenges of running an EVLP service alongside an active clinical transplant program.[23] Our EVLP utilization rate was 60%, compared to 69% for the Toronto group,[2] supporting the concept that a CLES at a dedicated facility can help mitigate limitations in resources, logistics and staffing challenges.

The study is limited by the lack of randomization, which is ethically challenging with perfusion of marginal organs. We partially mitigated this limitation by matching CLES transplants with contemporaneous control, by LAS-DDG, transplant type (single vs bilateral) and transplant center. The donor characteristics collected for the trial (Table 2) were limited and prevents a more detailed description of the quality of lungs used in the trial. Another limitation is the small number of subjects that prevents more accurately assessing the relationship between PGD and survival at 1-year in the CLES group. This data was affected by outdated definitions for PGD and chronic lung allograft dysfunction.[14,24] Changes for both definitions were published after the trial design was completed and enrollment for the trial had begun.[25,26] This may have resulted in over grading of PGD given that post-operative ECMO use was assigned as PGD3 in the 2005 criteria and used in occasions for nonhypoxic indications. We report the number of patients on ECMO at 72-hour (see supplement), although the indication and ECMO settings were not collected for the study. A CLES clinical trial is currently being conducted (NCT03641677) and data is being collected to report PGD and CLAD according to the latest definitions. Future studies of the CLES model should focus on factors that influence the utilization of lungs referred for EVLP, effects of total preservation/ischemia times on transplant outcomes, predictive biomarkers for organ quality, and cost-effectiveness.

Although prepandemic US lung transplant volume was increasing, only 23% of the lungs offered for transplantation are transplanted.[27] Implementation of technologies like EVLP are essential for increasing the number of transplanted lungs. Dedicated EVLP facilities can provide transplant centers with the resources and expertise to adopt or expand their access to this technology. In other words,

Table 4 Lung-Allograft Related SAEs

Distribution of the number of lung allograft-related SAE categories experienced by subjects

	n (%) of subjects experiencing 0, 1, 2, 3, 4, 5, and 6 of the SAE categories							
	0	1	2	3	4	5	6	*p*-value[a]
CLES 30 days (N = 66)	46 (70%)	17 (26%)	3 (5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.13
Control 30 days (N = 49)	40 (82%)	8 (16%)	0 (0%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	
CLES 1 year (N = 66)	33 (50%)	15 (23%)	12 (18%)	5 (8%)	1 (2%)	0 (0%)	0 (0%)	0.57
Control 1 year (N = 49)	25 (51%)	16 (33%)	5 (10%)	3 (6%)	0 (0%)	0 (0%)	0 (0%)	

Number of lung allograft-related serious adverse events

n (%) [events]	CLES 30 days (events = 41)	Control 30 days (events = 21)	*p*-value[a] 30 days	CLES 1 year (events = 114)	Control 1 year (events = 60)	*p*-value[a] 1 year
Acute Rejection	2 (3%) [2]	2 (4%) [2]	1.00	8 (12%) [8]	12 (24%) [13]	0.13
Respiratory Failure[b]	14 (21%) [15]	7 (14%) [7]	0.4651	22 (33%) [28]	14 (29%) [16]	0.69
Bronchial anastomotic complication	2 (3%) [3]	0	0.5066	10 (15%) [15]	2 (4%) [2]	0.07
Major pulmonary-related infection	1 (2%) [1]	2 (4%) [2]	0.5743	14 (21%) [20]	7 (14%) [8]	0.47
Vascular complication	4 (6%) [4]	0	0.1349	4 (6%) [4]	0	0.13
Other	11 (17%) [16]	8 (16%) [10]	1.00	17 (26%) [39]	15 (31%) [21]	0.67

CLES, centralized lung evaluation system; SAE, serious adverse event.

Table represents SAEs that were related or possibly related to the lung-graft, which were further categorized by the authors (See supplement for details). Data are n (%) [events], where *n* represents the number of recipients experiencing the event, % represents the percent of patients experiencing an event type, and events represents total number of events, unless otherwise stated. SAEs were managed according to each participating transplant center practice and protocols.

[a]*p*-values calculated using Pearson's exact Chi-square test comparing CLES and Control groups.

[b]Need for reintubation, the inability to discontinue ventilator support within 4 days posttransplant, or tracheostomy.

remote EVLP at dedicated centers represents a viable way to utilize EVLP as an alternative or in addition to "in-house" EVLP which requires additional resources and staff.[28]

Other potential benefits of CLES include relying more on regional recovery teams, simultaneous perfusion of multiple organs and perfusion of lungs from the same donor for 2 different recipients. Having access in real time to experts in EVLP, surgeons from Toronto General Hospital in our case, allow the participating transplant centers teams to build experience in the technology. Soon, EVLP will allow for longer and more precise assessment of lung allografts and provide opportunities for therapeutic interventions.[9,29-36] We believe that dedicated perfusion centers with experienced staff and standard protocols can provide consistency and facilitate targeted therapy for various graft injuries.

In conclusion, we demonstrated that remote EVLP can be performed at a dedicated facility utilizing lungs rejected for standard transplant, and that recipients of CLES lungs have similar 1-year survival to recipients of standard lung transplants. As *ex vivo* machine perfusion is adopted for other organs, centralized facilities with the appropriate infrastructure and expertise will provide access to this technology, and in turn benefit patients with end-stage organ failure listed at transplant centers across the US. Centralized lung evaluation systems (CLES) will have an important role as this technology transitions from organ assessment to a routine therapeutic platform.

Author contributions

Conception and design of the work: JMM, MR. Conception of the protocol: JMM, MR. Acquisition, analysis, and/or interpretation of data: All authors. Writer: JMM, NEP. Critical review: All authors. Approval final draft: All authors

Disclosure statement

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Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.healun.2022.09.006.

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