

Clinical Trial Protocol: PXUS 14-001

Study Title: A Phase 2, Multicenter, Open-Label Study to Measure the Safety of Extending Preservation and Assessment Time of Donor Lungs Using Normothermic Ex Vivo Lung Perfusion and Ventilation (EVLV) as Administered by the Sponsor Using the Toronto EVLP System

Study Number: PXUS 14-001

Study Type: Traditional Feasibility

Product Name: Toronto EVLP System

IDE Number: G140021

Indication: Normothermic *ex vivo* ventilation and perfusion of lungs for transplantation

Investigators: Multicenter

Sponsor: Lung Bioengineering Inc.

Medical Director: Jordan Shin, MD

Sponsor Contact: Michael Roberts

	Date
Original Protocol (v1.0)	31 January 2014
Amendment 1 (v2.0)	04 May 2014
Amendment 2 (v2.2)	22 May 2014
Amendment 3 (v2.4)	24 July 2014
Amendment 4 (v2.5)	05 March 2015
Amendment 5 (v5.0)	02 May 2016
Amendment 6 (v6.0)	03 November 2017

Statement of Compliance

The study will be conducted in accordance with the International Conference on Harmonisation (ICH) guidelines for Good Clinical Practice (GCP) (ICH E6), the Code of Federal Regulations (CFR) on the Protection of Human Subjects (21 CFR Part 50), the Investigational Device Exemption (IDE) requirements under 21 CFR 812, and the SPONSOR Clinical Terms of Award. All personnel involved in the conduct of this study have completed human subjects protection training.

Confidentiality Statement

The concepts and information contained herein are confidential and proprietary and shall not be disclosed in whole or part without the express written consent of Lung Bioengineering, Inc.

List of Sponsor Contacts

Study Sponsor Medical Director:	Jordan Shin, MD, PhD 1040 Spring Street Silver Spring, MD 20910 Phone: 301.578.1438 Email: jshin@lungbiotechnology.com
Sponsor Contact:	Michael Roberts Director, Regulatory Affairs Lung Bioengineering, Inc. 1015 Spring Street Silver Spring, MD 20910 Phone: 240.650.2261 Email: mroberts@lungbioengineering.com
EVLP Facility:	Lung Bioengineering - 1 ("LB-1") EVLP Facility 1015 Spring Street Silver Spring, MD 20910 Phone: 844.367.3857

See [Appendix 2](#) for other key study personnel contacts.

SYNOPSIS

Sponsor:

Lung Bioengineering Inc.

Description of Intervention:

Normothermic EVLP for extended preservation, assessment and transplantation of donor lungs.

Study Title:

A Phase 2, Multicenter, Open-Label Study to Measure the Safety of Extending Preservation and Assessment Time of Donor Lungs Using Normothermic Ex Vivo Lung Perfusion and Ventilation (EVLP) as Administered by the Sponsor Using the Toronto EVLP System

Study Number:

PXUS 14-001

Study Type: Traditional Feasibility

Primary Objective(s):

The primary objective of this study is to evaluate the short-term safety of subjects receiving a lung transplant, where the lung(s) was perfused by the Sponsor using the Toronto EVLP System (TES). Assessment of Primary Graft Dysfunction (PGD) Grade 3 at 72 hours post-transplant (T72) and 30-day mortality will represent the outcome measures, and the primary endpoints are the following two measures:

- The proportion of recipients with PGD Grade 3 (PGD3) assessed at T72
- 30-day mortality post-transplant.

The above information will also be collected on patients enrolled in a contemporaneous control group to provide context for EVLP results and to inform control measures for future research.

Secondary Objective(s):

The secondary objectives of this study are to evaluate the short-term safety of subjects receiving a lung transplant where the lung(s) has been perfused via the TES performed by the Sponsor by assessment of the following:

- PGD Score (Grades 0-3) measured at 0, 24, 48 and 72 hours post-transplant
- Time to first extubation
- Intensive care unit (ICU) length of stay (LOS), measured as total number of days post-transplant in the ICU until first hospital discharge, inclusive of ICU readmissions during that hospitalization.
- Hospital LOS measured as total number of days in the hospital prior to discharge post-transplant
- Total preservation time (TPT), defined as the elapsed time between first allograft lung removal from cold storage time prior to transplant in the recipient and the donor aortic cross clamp time at procurement
- Assessment of the overall safety of TES lung transplants during subject's participation in the study, as reported through adverse events (AEs).

The above information will also be collected on patients enrolled in a contemporaneous control group to provide context for EVLP results and to inform control measures for future research.

Study Design:

This is a Phase 2, unblinded, non-randomized, traditional feasibility study to evaluate the safety of subjects undergoing lung transplant using lungs after EVLP, and performed exclusively by the Sponsor at the Sponsor's facility using the Toronto EVLP System (TES).

Once the donor lung is accepted following EVLP, the eligible recipient, who has provided written informed consent, and receives the lung transplant, is enrolled into the study. Patients who consent for the current EVLP

study (PXUS 14-001) but receive a conventional (i.e., non-EVLP) lung transplant will be considered for a contemporaneous control group matched to the EVLP treatment group (66 subjects each). This matching will take place on a patient-by-patient basis and only after an EVLP subject has been enrolled at that Study Center. Investigators and their team will be notified by the Sponsor on a real-time basis of the specific matching criteria required for a control subject as EVLP subjects are enrolled. In order to be considered for eligibility, the control patient must “match” a priori to at least one EVLP subject who has already been enrolled at that Study Center based on the following criteria: SLT versus DLT and Lung Allocation Score Disease Diagnosis Group (LASDDG).

The post-intervention phase of the study will then be split into two phases: 1) the Analysis Phase, which includes follow-up within one year post-intervention and 2) Long-term Follow-up, which will capture outcome data annually from 1-year post-intervention until 5-years post intervention. Although the study will continue after one year, the database will be locked for analyses of endpoints after the last subject has his/her study visit at 1 Year. The Long-term Follow-up data will be summarized when the study has completed in support of long-term evaluation.

Analysis Phase

Following transplantation, subjects will be admitted to the ICU, and the initial PaO₂ and PGD Score will be recorded. The subject’s PGD Score will be recorded every 24 hrs for the next 72 hrs. The ICU discharge date will be documented. Study visits will occur 30 days (±5), 90 days (±10), 6 months (±2 weeks), and 1 year (±4 weeks) post-transplant. Study assessments will be performed as outlined in the Schedule of Events ([Appendix 1, Table 3](#)).

Long-term Follow-up

Although the database will be locked and analyses of endpoints performed after the last subject has his/her study visit at 1 Year, the Sponsor will continue to collect specified outcome parameters that are required to be reported by transplant centers to OPTN/ United Network for Organ Sharing (UNOS). Collection will be annually for 5-years post-intervention; outcome collection is outlined in the Schedule of Events ([Appendix 1, Table 4](#)).

Study Population:

The study will be open to all eligible male and female patients, aged 18 years or older, with end-stage lung disease listed for lung transplantation at a participating Study Center. These participants may not be receiving mechanical ventilation, ECMO or ECLS, at the time of initial lung offer prior to EVLP.

A sample size of 132 subjects, receiving either SLT or DLT, is planned for this study, where 66 subjects receive donor lung(s) after EVLP (EVLP Group) and 66 subjects are in a contemporaneous control group (Control Group). No more than two-thirds of the subjects who are enrolled (44 subjects per group) will have received a single-lung transplant (SLT) or double-lung transplant (DLT).

Subject (recipient) Inclusion Criteria

Each patient must meet the following criteria to be enrolled in this study:

1. Male or female patients.
2. All patients, 18 years of age or older.
3. Patient already on or added to the active waiting list for a single or bilateral lung transplant.
4. Patient or patient’s representative provides informed consent for participation in the study prior to participating in any study-related assessments or procedures.
5. Patient or patient’s representative reconfirms informed consent for the study on the day of lung transplant if initial consent is > 24 hrs.
6. Patient matches with and undergoes a transplant with a Donor Lung.

Subject (recipient) Exclusion Criteria

Patients who meet any of the following criteria will be excluded from the study:

1. Patients listed for same-side lung re-transplantation.
2. Patients listed for multiple organ transplantation including lung and any other organ.
3. Patients listed for live donor lobar transplant.
4. Patients positive for human immunodeficiency virus, active Hepatitis B or C, or *Burkholderia cepacia* infection.
5. Patients in the ICU at the time of the initial lung offer requiring ventilation, ECMO or other extracorporeal life support (ECLS). [Note: The decision to place the recipient on ECMO or other ECLS for post-transplant prophylaxis at time of or immediately prior to transplant is not considered an exclusion]
6. Patient receives a conventional (non-EVLP) lung transplant but does NOT match to an EVLP subject based on the criteria for control matching outlined above.

Study Intervention:

EVLP is a novel technology that allows extended assessment of lungs whose suitability for transplantation is initially uncertain. By protocol design, EVLP-treated lungs undergo a longer period of total preservation, including an initial cold ischemic time (CIT-1), a period of normothermic EVLP assessment, and a final round of CIT (CIT-2). The extended donor lung preservation time may enable better logistical coordination of the recipient and donor hospital transplant teams.

Upon retrieval, donor lungs will be packaged and transported to the Sponsor's EVLP facility. The EVLP procedure will be performed by specifically-trained *Ex Vivo* Lung Specialists under remote video supervision by one of the Sponsor's Expert Medical Consultants. The Sponsor will perfuse the lung using the TES for up to 6 hrs, collecting and relaying lung function assessment data hourly, or as requested by the Study Center Investigator/team. Additionally, the Study Center surgeon will have access to remote monitoring capabilities at the Sponsor EVLP Center for evaluating lung function data and monitoring the procedure through a dedicated audio/video link.

The Study Center Investigator/team, together with the Sponsor's Expert Medical Consultants, will monitor the donor lung(s) for EVLP inclusion criteria and collaborate to determine the timing of EVLP termination. However, final decisions reside with the Study Center Investigator/team. Upon acceptance of an EVLP donor lung by the Study Center, the single lung or lung block is cooled according to TES methodology to 10°C, and perfusion and ventilation are stopped. This point marks the start of CIT-2. The end of CIT-2 is defined as the time the organ is removed from cold storage to begin the implantation phase of the transplantation procedure. The time between the organ's removal from cold storage to reperfusion in the recipient is known as warm ischemic time (WIT). Total preservation time for the first lung transplanted from donor lung retrieval to the end of CIT-2 must not be greater than a combined 22 hrs and individual phase limits as follows:

$$(\leq 10 \text{ hrs CIT-1}) + (3 - 6 \text{ hrs EVLP}) + (\leq 6 \text{ hrs CIT-2}) \leq 22 \text{ hrs}$$

Total preservation time for the second lung transplanted from donor lung retrieval to the end of CIT-2 must not be greater than a combined 26 hrs and individual phase limits as follows:

$$(\leq 10 \text{ hrs CIT-1}) + (3 - 6 \text{ hrs EVLP}) + (\leq 10 \text{ hrs CIT-2}) \leq 26 \text{ hrs}$$

Duration of Subject Participation:

Data will be collected from the organ donor, EVLP procedure (if applicable), and each subject from the time of lung transplantation and up to one year post-transplant for the Analysis Phase. In addition, outcome data will be collected annually up to 5 years post-transplant.

The study duration for the Analysis Phase (from first patient, first visit [FPFV] to last patient, last visit [LPLV] prior to database lock) is expected to be 3 years. The estimated time to complete enrollment is approximately 24 months.

Safety Assessments:

Medical History and concomitant medications will be collected for each subject. Physical examinations, including specific tests to assess lung function, will be performed at Baseline, Day 30, and Month 6. Vital signs will be assessed at each study visit.

The subject's PGD Score is to be determined by the Investigator at Baseline (Day 0) after transplantation and upon admission to the ICU, as well as 24, 48, and 72 hrs post-transplant. Subject arterial blood gases must be determined by the Investigator, in order to calculate PGD grade. Graft survival will be evaluated by the Investigator (or his/her designee) at Day 30, Day 90, Month 6, Year 1 post-transplant, and premature termination (if applicable).

Subject survival, measured as living or dead, will be recorded Day 30, Day 90, Month 6, and Year 1 post-transplant. If the subject expires within first year post-transplant, the date of death and primary cause of death will be collected.

Evaluation of Bronchiolitis Obliterans Syndrome (BOS), physical capacity, and work status will be conducted at Day 90, Month 6, Year 1 post-transplant, and premature termination (if applicable).

In addition, after completion of the Analysis Phase, survival will be recorded annually until 5-year post-transplant. If survival is confirmed, then FEV₁ and oxygen at rest will be collected annually, along with BOS, physical capacity, work status, graft survival, by the Sponsor for the Long-term Follow-up Phase (Year 2, 3, 4, and 5).

Statistical Methods:

Statistical analysis will be performed primarily on data collected during the Analysis Phase (1 year post-intervention). The database will be locked for analysis of endpoints after the last subject completes his/her study visit at Year 1, and all queries have been resolved.

All subjects are included in the analyses to the extent data are available.

The primary endpoint of PGD3 T72 will be calculated from PGD T72 as follows: PGD T72 = {0, 1, & 2} will be coded to PGD3 T72 = 0 and PGD T72 = {3} will be coded to PGD3 T72 = 1. The primary analysis will be descriptive as the proportion of subjects with PGD3 T72 by Transplant Group (EVLP and Control). A one-sided 95% CI will be calculated around the proportion. A two-sided 95% CI will also be calculated.

The primary endpoint of 30-Day mortality will be derived as follows: Living at Day 30 will be coded to 0, and Dead before or on Day 30 will be coded to 1. The primary analysis will be descriptive as the proportion of subjects with 30-Day mortality, summarized by Transplant Group. A one-sided 95% CI will be calculated around the proportion. A two-sided 95% CI will also be calculated.

All secondary endpoints will be presented using descriptive statistics along with two sided 95% CIs by Transplant Group. Other supportive endpoints may be provided to supplement the secondary analyses. Results may be displayed graphically. There will be no inferential testing conducted.

All exploratory endpoints will be presented using descriptive statistics along with two sided 95% CIs by Transplant Group. There will be no inferential testing conducted.

A contemporaneous Control group will be entered into this study. This Control group will provide context of the EVLP results and estimation of Control group parameters for design of future studies. All analyses of the Control group will be descriptive and no formal statistical comparison will be made between the EVLP and Control groups.

Date of Original Approved Protocol: 31 January 2014

Date of Most Recent Protocol Amendment: 02 May 2015

Prepared in: Microsoft Word 2010

Table of Contents

List of Sponsor Contacts	2
Synopsis.....	3
List of In-Text Tables	11
List of In-Text Figures.....	11
List of Appendices.....	11
List of Abbreviations and Definitions of Terms.....	12
1 Introduction.....	15
1.1 Lung Transplantation and Ex Vivo Lung Perfusion Background	15
1.1.1 Toronto Clinical Experience: Toronto EVLP System	16
1.1.2 Sponsor Clinical Experience: Toronto EVLP System	17
1.1.3 Report of Prior Investigations.....	17
1.2 Potential Risks and Benefits	17
1.2.1 Potential Risks	18
1.2.2 Potential Benefits	18
1.2.2.1 Increased Donor Lung Utilization	18
1.2.2.2 Shorter Recipient Waiting Times	19
2 Study Objectives.....	20
2.1 Primary Objective(s).....	20
2.2 Secondary Objective(s).....	20
2.3 Other Objective(s).....	21
3 Investigational Plan	22
3.1 Overall Study Design and Plan.....	22
3.1.1 Lung Collection and EVLP Procedure	24
3.1.1.1 Lung Referral.....	24
3.1.1.2 Lung Retrieval	25
3.1.1.3 Transfer of EVLP Data to the Study Center	26
3.1.1.4 Acceptance of EVLP Lung for Transplant	27
3.1.1.5 Request for Reallocation of Lungs After EVLP	28
3.2 Rationale for Study Design and Control Group.....	29
3.3 Study Duration and Dates	30
4 Study Population Selection.....	31
4.1 Study Population.....	31
4.2 Inclusion Criteria	31
4.3 Exclusion Criteria	31
4.4 Strategies for Recruitment and Retention.....	32
5 Study Intervention	33
5.1 Description of Intervention.....	33
5.2 Lung Transplantation.....	34
5.2.1 Donor Lung Inclusion Criteria (Pre-EVLP)	34
5.2.2 Donor Lung Exclusion Criteria (Pre-EVLP)	35
5.2.3 Donor Lung Criteria Post-EVLP Assessment.....	35
5.2.3.1 Donor Lung Inclusion Criteria (Post-EVLP).....	35

5.3	Method of Assigning Patients to Intervention	36
5.4	Blinding.....	37
5.5	Restrictions	37
5.6	Intervention and Study Procedure Compliance	37
5.7	Packaging and Labeling.....	37
5.8	Storage and Accountability.....	37
5.8.1	STEEN Solution.....	38
5.8.2	EVLP Disposables	38
5.9	Investigational Product Retention at Study Center.....	38
6	Study Procedures	39
6.1	Donor and Donor Lung Information.....	39
6.2	Informed Consent.....	39
6.3	Medical History	39
6.4	Concomitant Medications	40
6.5	Physical Examination.....	40
6.6	Vital Signs.....	40
6.7	Primary Graft Dysfunction Score	40
6.8	Survival.....	41
6.9	Bronchiolitis Obliterans Syndrome Grade.....	41
6.10	Physical Capacity.....	42
6.11	Work Status.....	42
6.12	Clinical Laboratory Tests.....	43
6.12.1	Laboratory Parameters.....	43
6.12.2	Sample Collection, Storage, and Shipping	43
6.13	Safety Reporting	43
6.13.1	Definitions.....	43
6.13.2	Performing Adverse Events Assessments or Recording of Adverse Events	47
6.13.3	Timing of Adverse Event Collection	47
6.13.4	Severity	47
6.13.5	Relationship	48
6.13.6	Expectedness.....	48
6.13.7	Reporting Procedures.....	48
6.13.7.1	Reporting Serious Adverse Events	48
6.13.7.2	Reporting Procedure for Serious Adverse Events	49
6.13.8	Initial Safety Review.....	49
6.13.9	Halting Rules	50
6.14	Removal of Subjects from the Study	50
6.15	Premature Termination or Suspension of the Study	50
6.16	Appropriateness of Measurements.....	51
7	Study Activities.....	52
7.1	Screening Visit (Days -365 to -1 [-24 hours]).....	52
7.2	Intervention	52
7.2.1	Lung Assessment, Recipient Baseline, and Lung Transplant (Day 0)	52
7.2.1.1	Donor Lung Screening (Day 0, within 24 hours prior to transplant).....	52
7.2.1.2	Recipient Screening (Day 0, within 24 hours before transplant).....	52

7.2.1.3	Donor Lung EVLP Evaluation (at completion)	53
7.2.1.4	Lung Transplantation (Day 0)	53
7.3	Post-intervention Period; Analysis Phase (Day 1 to 1 Year Post-Transplant)	53
7.3.1	Visit 1 Procedures (24 Hours post-transplant)	53
7.3.2	Visit 2 Procedures (48 Hours post-transplant)	54
7.3.3	Visit 3 Procedures (72 Hours post-transplant)	54
7.3.4	Visit 4 Procedures (Day 30 post-transplant)	54
7.3.5	Visit 5 Procedures (Day 90 post-transplant)	54
7.3.6	Visit 6 Procedures (Month 6 post-transplant)	55
7.3.7	Visit 7 Procedures/ Final Visit (1 year post-transplant)	55
7.4	Post-intervention Period; Long-term Follow-up Phase (1 to 5 Years Post-Transplant)	55
7.5	Early Termination Procedures	56
7.6	Unscheduled Visits	56
8	Quality Control and Assurance	57
8.1	Sponsor's Quality Process	57
8.2	Training for Study Interventionalists	57
8.2.1	Transplant Center Research Personnel	57
8.2.2	Lung Recovery Surgeons	58
8.2.3	Lung Transplant Surgeons	58
8.2.4	EVLP Specialists	58
9	Planned Statistical Methods	60
9.1	General Considerations	60
9.2	Determination of Sample Size	60
9.3	Analysis Populations	61
9.4	Demographics and Baseline Characteristics	61
9.5	Primary Endpoint(s)	61
9.5.1	Primary Graft Dysfunction Grade 3 at 72 hours Post-transplant	61
9.5.2	30-Day Mortality	62
9.6	Secondary Endpoint(s)	62
9.7	Exploratory Endpoints	62
9.8	Missing Data	63
9.9	Other Safety Assessments	64
9.9.1	Adverse Events	64
9.9.2	Physical Examination and Vital Signs	64
9.9.3	Laboratory Data	64
9.10	Interim Analysis	64
10	Administrative Considerations	65
10.1	Investigators and Study Administrative Structure	65
10.2	Institutional Review Board (IRB) or Independent Ethics Committee (IEC) Approval	65
10.3	Ethical Conduct of the Study	65
10.4	Authorization and Consent	66
10.4.1	Donor Next of Kin Authorization	66
10.4.2	Subject (Recipient) Consent	66

10.5	Subject Confidentiality	67
10.6	Study Monitoring	67
10.6.1	Access to Centers	68
10.6.2	Summary of Monitoring Procedures & Responsibilities	68
10.6.3	Routine Monitoring	68
10.7	Case Report Forms and Study Records	69
10.8	Independent Medical Monitor	70
10.9	Protocol Violations/Deviations	70
10.10	Access to Source Documentation	71
10.11	Data Generation and Analysis	72
10.11.1	Data Management Responsibilities	72
10.11.2	Data Capture Methods	72
10.11.2.1	Donor Data	72
10.11.2.2	EVLP Data	72
10.11.2.3	Subject (Recipient Data)	72
10.12	Retention of Data	73
10.12.1	Retention of Study Documents	73
10.12.2	Future Use of Stored Specimens and Other Identifiable Data	73
10.13	Financial Disclosure	73
10.14	Publication and Disclosure Policy	73
10.15	Study Closure	74
11	Reference List	75
	Non-Fluid Path Hardware	82
	Single-Use Fluid Path Disposables	83

LIST OF IN-TEXT TABLES

Table 1.	Single-use Device Storage and Stability Conditions	38
Table 2.	Primary Graft Dysfunction Grading	41
Table 3.	Schedule of Events Within 1 Year Post-intervention	77
Table 4.	Schedule of Events: Long-term Follow-up	78
Table 5.	Composition and Properties of STEEN Solution.....	86
Table 6.	Medications Added to STEEN Solution during Prime	87

LIST OF IN-TEXT FIGURES

Figure 1.	2012 US Donor Lung Utilization.....	19
Figure 2.	Lung Retrieval Process to Determine Acceptance or Rejection for Transplant	23
Figure 3.	System Components of the Toronto EVLP System.....	33
Figure 4.	Schematic of Perfusion Circuit	85

LIST OF APPENDICES

Appendix 1	Schedule of Events.....	77
Appendix 2	Key Study Personnel Contacts.....	79
Appendix 3	Risk and Risk Mitigation of EVLP.....	81
Appendix 4	System Components of the Toronto EVLP System.....	82
Appendix 5	Sponsor Signatures.....	88
Appendix 6	Investigator's Signature	89

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

ABG	Arterial Blood Gas
ADL	Activities of daily living
AE	Adverse Event/Adverse Effect
ACIN	AOPO Credentials Information Network
ADL	Activities of daily living
BOS	Bronchiolitis Obliterans Syndrome
CI	Confidence interval
CIT	Cold ischemic time
CFR	Code of Federal Regulations
COP	Colloid-osmotic pressure
CRF	Case Report Form
CRO	Contract Research Organization
Cstat	Static compliance
CMX	Case Management Exchange
DBD	Donation after Brain Death
DCD	Donation after Circulatory (Cardiac) Death
DLT	Double-lung Transplant
ECLS	Extracorporeal life support
ECMO	Extracorporeal Membrane Oxygenation
EVLP	Ex Vivo Lung Perfusion
FDA	Food and Drug Administration
FEV ₁	Forced expiratory volume in one second
FiO ₂	Fraction of inspired oxygen
FPFV	First patient, first visit
GCP	Good Clinical Practice
GERD	Gastroesophageal Reflux Disease
HDE	Humanitarian Device Exemption
HIV	Human immunodeficiency virus
HSA	Human serum albumin
IBW	Ideal Body Weight
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors

ICU	Intensive Care Unit
ID	Identification
IDE	Investigational Device Exemption
IRB	Institutional Review Board
ISHLT	International Society for Heart and Lung Transplantation
LAP	Left atrial pressure
LASDDG	Lung Allocation Score Disease Diagnosis Group
LAS	Lung Allocation Score
LOCF	Last observation carried forward
LOS	Length of stay
LPLV	Last patient, last visit
mAwP	Mean airway pressure
MedDRA [®]	Medical Dictionary for Regulatory Activities
Min	Minutes
N	Number (typically refers to subjects)
OPO	Organ Procurement Organization
OPTN	Organ Procurement and Transplantation Network
PAF	Pulmonary arterial flow
PaO ₂	Partial pressure of oxygen in arterial blood
PAP	Pulmonary arterial pressure
PawP	Peak airway pressure
PCO ₂	Carbon dioxide partial pressure
platAwP	Plateau airway pressure
PO ₂	Oxygen partial pressure
PvO ₂	Partial pressure of oxygen in venous blood
PVR	Pulmonary Vascular Resistance
PGD	Primary Graft Dysfunction
PGD3	Primary Graft Dysfunction Grade 3
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event/Serious Adverse Effect
SAP	Statistical Analysis Plan
SCD	Standard Criteria Donor
SCP	Stöckert Centrifugal Pump
SCPC	Stöckert Centrifugal Pump Console

SD	Standard deviation
SLT	Single-lung Transplant
SOC	System Organ Class
SOP	Standard Operating Procedure
SRTR	Scientific Registry of Transplant Recipients
T72	72 hours post-transplant
TES	Toronto EVLP System
TGH	Toronto General Hospital
TPT	Total Preservation Time
UADE	Unanticipated Adverse Device Effect
UNK	Unknown
UNOS	United Network for Organ Sharing
US	United States
WIT	Warm ischemic time

1 INTRODUCTION

1.1 Lung Transplantation and Ex Vivo Lung Perfusion Background

Lung transplantation is often the only available therapy for patients suffering end-stage lung disease, and the number of patients who can appropriately benefit by lung transplantation far exceeds the donor lungs available. Importantly, the mismatch between demand for transplantable lungs and the supply of available organs is substantially higher than other solid organs, with the lungs being used for transplantation among only 20% of brain dead donors who are used for transplanting other solid organs.[[Munshi 2013](#); [SRTR 2011](#)] The described reason for declining the majority of the available donor lungs by transplant programs has been listed as being due to “Poor Organ Function” and “No Recipient Found.”[[SRTR 2011](#)]

It has been suggested that over 40% of the lungs rejected by the United States (US) lung transplant programs could have been transplanted if better information was available at the time of procurement, or additional time was available to meet the complex logistical demands of procurement and transplantation.[[Ware 2002](#)] Introduction of technologies, such as *Ex vivo* Lung Perfusion (EVLP), might facilitate more in-depth assessment of lung condition, thereby enabling transplant programs to use lungs for transplant that are currently discarded.

Evidence linking the pathophysiology of brain death to early organ dysfunction after transplantation continues to accumulate and seems especially relevant when considering donor lungs. Lung injury begins soon after a catastrophic event associated with massive brain injury with a massive sympathetic outflow, then an associated release of inflammatory cytokines. These mediators of inflammation then remain within the donor lung(s), ultimately being released into the recipient circulation after transplantation, potentially reflecting an important etiology of early graft dysfunction.[[Fisher 2001](#)]

Also, cerebral edema leading to brain stem herniation effects irreversible cessation of brain function (e.g., death). This is associated with complete loss of autonomic tone with massive vasodilation and hypotension. This is generally treated by administration of large volumes of fluids to maintain adequate circulation and end organ perfusion.[[Power 1995](#); [Hayek 1990](#); [Tropmann 2008](#)] Together with neurogenic pulmonary edema, another consequence of brain death, the overall clinical picture can obscure assessment of pulmonary function.[[Avlonitis 2007](#)]

Steen et al., described an *ex vivo* perfusion technique in 2001, seeking to better evaluate lungs from non-heart beating donors (Donation after Circulatory Death [DCD]).[[Steen 2001](#)]. This method demonstrated the proof-of-concept evidence that donor lungs could be assessed *ex vivo*, then successfully transplanted, initially into large animals, and later into humans.

Keshavjee’s group in Toronto extended those observations, applying a modified, normothermic (37°C) EVLP technique, using an acellular, albumin-based solution (STEEN Solution™), lower perfusion flow rates (40% of normal cardiac output), and a protective ventilation strategy to permit longer perfusion with excellent physiologic function after transplantation.[[Cypel 2008a](#); [Cypel 2008b](#); [Cypel 2009](#)] The method, called the Toronto EVLP System (TES) was demonstrated as technically feasible and safe, permitting perfusion of donor lungs for up to

6 hours (hrs) in clinical practice (and 18 hrs in the research setting). By permitting a longer preservation period, TES extended the time available for assessment of donor lungs under near-physiologic conditions.

1.1.1 Toronto Clinical Experience: Toronto EVLP System

Human studies were started during 2008 in Toronto with the Human *Ex Vivo* Lung Perfusion (HELP) trial. HELP was a prospective, non-randomized, single-center study of outcomes of lungs not meeting conventional lung donor criteria, receiving 4 hrs of extended preservation and assessment using EVLP. The EVLP treated lungs were compared to all other lungs transplanted (Control) during the same time period. In addition to the 4 hrs of extended preservation time, EVLP-treated lungs received an obligatory second cold ischemic period during transport after completion of *ex vivo* assessment.

The first three HELP recipients received bilateral lung transplants from a standard criteria donor (SCD) with one lung preserved using conventional hypothermic cold storage, and the second lung using one hour of TES. After no adverse consequences were identified in this initial experience, declined lungs from 23 donors were then assessed using the TES. Among those 20 lung transplants (86% utilization), 11 originated from brain-dead donors, and the remaining 9, from DCD cases. Selection criteria were not used to determine which recipient would receive the EVLP or conventional donor lung. Rather, selection was determined based on an algorithm where available lungs were assessed to establish if they met the standard for transplantation using conventional donor lung criteria. Donor lungs deemed not appropriate for transplantation using 'conventional' criteria were placed on EVLP then made available to potential recipients who previously provided informed consent for this study.

Upon trial completion, Health Canada permitted ongoing access through compassionate use, resulting in 39 additional EVLP transplants (total of 61 EVLP transplants prior to final marketing approval). STEEN Solution™ received full marketing approval by Health Canada on 06 Nov 2012 as a stand-alone device (i.e., could be used with any off-the-shelf perfusion equipment).

The initial study results suggested that transplantation of donor lungs that were physiologically stable during 4 hrs of EVLP, resulted in similar outcomes to those obtained with conventionally procured and hypothermically-treated lungs.[[Cypel 2011a](#)]

By protocol design, EVLP-treated lungs underwent a longer period of total preservation, including an initial cold ischemic time (CIT-1), a period of normothermic EVLP assessment, and a final round of CIT (CIT-2).[[Cypel 2011a](#)]

Ethical concerns limited the study design in that it was not possible to randomize the initially rejected donor lungs to 'EVLP' or 'no EVLP' study groups to determine post-transplantation success. However, review was possible from a clinical perspective as to whether the post-transplant outcomes were satisfactory for both groups. After 4 hrs of EVLP, the donor lung was evaluated to determine a number of parameters including the difference in pulmonary venous and arterial oxygen partial pressure (PO₂) (delta PO₂ = pulmonary vein O₂ - pulmonary artery O₂), pulmonary vascular resistance (PVR), peak airway pressure (PAwP), and lung

compliance. Both the EVLP and conventional donor lungs demonstrated similar outcomes after transplantation during years 1 through 5 post-transplant.[[Cypel 2012](#); [Tikkanen 2012](#)]

In the first six years after the HELP study began, the overall lung utilization rate in Toronto increased to 36% [S [Keshavjee, personal communication](#)] without significant changes to lung transplant volumes in surrounding regions.[[Tiwari 2014](#)] Therefore, it seems reasonable to infer that EVLP played a relevant role in the observed lung transplant volume increase. Long-term outcomes for the lung recipients in the HELP (and compassionate extension) trial, suggest that the lungs transplanted after EVLP had similar long-term outcomes compared to conventionally procured lungs.[[Cypel 2012](#)]

Taken together, more than 225 donor lung sets have been evaluated by the Toronto group with a consistent increase in the lung utilization rate approximating 80%.[C [Jaynes, personal communication](#)]. Overall, one-, three-, and five-year outcomes after lung transplantation have been similar to results obtained among lung recipients who received standard lung transplants without using EVLP.[[Cypel 2011a](#); [Cypel 2011b](#); [Cypel 2012](#); [Tikkanen 2012](#); [Munshi 2013](#); [Cypel 2013](#)]

1.1.2 Sponsor Clinical Experience: Toronto EVLP System

In 2015, Lung Bioengineering Inc (the Sponsor) merged with PERFUSIX USA, the previous Sponsor of this study, and Study PXUS 14-001 continues under the new sponsorship. The Sponsor's predecessor company, PERFUSIX USA, had considerable experience with the TES, in that its employees and consultants consisted of team members who developed the technology, validated the protocols through the HELP clinical trial,[[Cypel 2011a](#); [Cypel 2012](#); [Tikkanen 2012](#)] and designed the single-use disposable products used in the EVLP procedure.

Additionally, a separate Canadian corporation, PERFUSIX Canada, has been responsible for running all of the EVLP lung procedures in Ontario, Canada, as a service for Toronto General Hospital (TGH) since April 2013.

1.1.3 Report of Prior Investigations

A full report of prior investigations detailing the above and other EVLP studies will be provided to the Study Center Investigators and submitted to each reviewing IRB. One copy shall be filed in the Investigator Study File.

1.2 Potential Risks and Benefits

For many patients who choose evaluation and listing for lung transplantation, the inherent risks are outweighed by the benefit that transplantation is the only life prolonging therapy for end-stage lung disease. Factors underlying the exaggerated imbalance between lung donor supply and recipient mismatch compared with other solid organs have been reviewed earlier. EVLP-specific potential risks and benefits are presented below.

1.2.1 Potential Risks

The specific additional risks associated with EVLP beyond those encountered in lung transplant generally include extending ischemia beyond the initial cold ischemic period, increased potential for contamination due to additional handling and manipulation of the lungs, and potential lung damage that might result from the perfusion process and associated procedures, such as cannulation, bronchoscopy, and repackaging, among others. Specific risks and mitigation strategies are addressed in [Appendix 3](#).

1.2.2 Potential Benefits

The potential benefits of extended evaluation provided by EVLP include:

- Evaluation under fixed/ideal hemodynamics outside of the confounding influence of the pathophysiology resulting from brain death and accompanying somatic injury,
- Extended time enabling more careful examination/evaluation of donor organs.

Assessment of lung and recipient risk profiles is based largely on the individual experience of the transplanting surgeon, which varies program-to-program and becomes even more important when lungs from unconventional or DCD donors are assessed. *Ex vivo* assessment at the Sponsor's dedicated EVLP facility can therefore add a level of consistency (and therefore safety) in lung assessment, removing inherent variability between centers and surgeons. The additional expertise provided by Sponsor's specifically trained EVLP Specialists and Expert Medical Consultants can help the transplant surgeon make the most accurate judgment when evaluating the risk profile of an EVLP lung as compared to the risk profile of a potential recipient.

1.2.2.1 Increased Donor Lung Utilization

Strategies which increase the use of lungs from currently available donors provide the best opportunity to meaningfully increase the availability of lungs for end stage patients currently waiting for transplantation. In 2012, there were 8144 eligible donors recorded in the US and a total of 1634 lung transplants, resulting in a donor utilization rate of 20.1% [SRTR 2012]. EVLP is anticipated to increase the number of donor lungs used for transplantation by both enabling additional evaluation and increasing preservation time. Based on the experience at TGH, the lung utilization rate from deceased donors has increased to 36% following the introduction of EVLP in the form of the TES (cf. section 1.1.1). In the event that these results were extended to the US, an additional 1298 lung transplants might be expected to result from the introduction of EVLP (for a total of 2932 transplants). Importantly, to achieve a similar increase in lung transplants by increasing the donor pool, 6458 additional donors would have to be identified, a 79% increase over the current pool. Despite targeted campaigns to increase public awareness of the importance of organ donation, the annual number of donors has remained static. Thus, the low likelihood of significantly increasing the number of lungs available to patients with end-stage lung disease through currently available approaches argues that novel technologies to increase donor lung utilization such as the TES are needed.

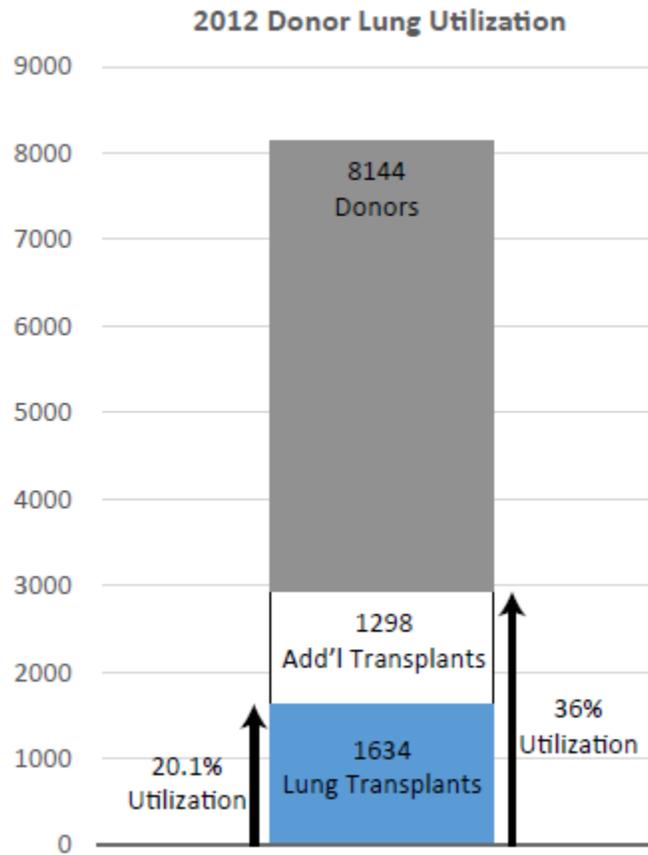


Figure 1. 2012 US Donor Lung Utilization

1.2.2.2 Shorter Recipient Waiting Times

Because EVLP may result in making more lungs available for transplantation, an additional potential benefit to lung candidates listed at a center participating in this study could be a shortened waiting time for a suitable donor (not typically evaluated by the center prior to EVLP) to become available.

2 STUDY OBJECTIVES

This traditional feasibility study will generate preliminary safety and effectiveness information, evaluating the TES at a central facility for extended donor lung preservation. This information may be used to plan an appropriate future pivotal study, as part of efforts to inform further development.

2.1 Primary Objective(s)

The primary objective of this study is to evaluate the short-term safety of subjects receiving a lung transplant, where the lung(s) was perfused by the Sponsor using the TES. Assessment of Primary Graft Dysfunction (PGD) Grade 3 at 72 hrs post-transplant (T72) and 30-day mortality will represent the outcome measures, and the primary endpoints are the following two measures:

- The proportion of recipients with PGD Grade 3 (PGD3) assessed at T72
- 30-day mortality post-transplant.

The above information will also be collected on patients enrolled in a contemporaneous control group to provide context for EVLP results and to inform control measures for future research.

2.2 Secondary Objective(s)

The secondary objectives of this study are to evaluate the short-term safety of subjects receiving a lung transplant where the lung(s) has been perfused via the TES performed by the Sponsor by assessment of the following:

- PGD Score (Grades 0-3) measured at 0, 24, 48 and 72 hours post-transplant
- Time to first extubation
- Intensive care unit (ICU) length of stay (LOS), measured as total number of days post-transplant in the ICU until first hospital discharge, inclusive of ICU readmissions during that hospitalization
- Hospital LOS measured as total number of days in the hospital prior to discharge post-transplant
- Total preservation time (TPT), defined as the elapsed time between first allograft lung removal from cold storage time prior to transplant in the recipient and the donor aortic cross clamp time at procurement
- Assessment of the overall safety of TES lung transplants during subject's participation in the study, as reported through adverse events (AEs).

The above information will also be collected on patients enrolled in a contemporaneous control group to provide context for EVLP results and to inform control measures for future research.

2.3 Other Objective(s)

Additional objectives of this study include evaluation of intermediate and longer-term safety and preliminary efficacy in subjects receiving a lung transplant where the lung(s) was perfused using the TES performed by the Sponsor. Assessments will include the following:

- Initial ICU partial pressure of oxygen in arterial blood (PaO₂): Evaluated as first PaO₂ measured after ICU admission.
- Forced Expiratory Volume in one second (FEV₁) evaluated at time of discharge, 30 days, 90 days, 6 months and 1 year post-transplant. FEV₁ is measured as a percentage of predicted normal value.
- Oxygen requirement at rest evaluated at time of discharge, 30 days, 90 days, 6 months and 1 year post-transplant. Oxygen required at rest is measured in liters per minute (L/min).
- Subject survival evaluated at 90 days, 6 months, and 1 year post-transplant. Measured as living or dead. If the subject expires within first year post-transplant, the date of death and primary cause of death will be collected.
- Graft survival evaluated at 30 days, 90 days, 6 months, and 1 year post-transplant. Measured as functioning or failed. If the graft fails within first year post-transplant, the date of graft failure will be collected.
- Primary cause of graft failure evaluated at 30 days, 90 days, 6 months, and 1 year post-transplant on all failed grafts.
- Bronchiolitis Obliterans Syndrome (BOS) Grade evaluated at 90 days, 6 months, and 1 year post-transplant.
- Number of re-hospitalizations after transplant evaluated at 6 months and 1 year post-transplant. Measured as total number of hospitalizations since initial discharge.
- Physical capacity evaluated at 90 days, 6 months, and 1 year post-transplant.
- Working for income after transplant evaluated at 90 days, 6 months, and 1 year post-transplant.

The above information will also be collected on patients enrolled in a contemporaneous control group to provide context for EVLP results and to inform control measures for future research.

3 INVESTIGATIONAL PLAN

3.1 Overall Study Design and Plan

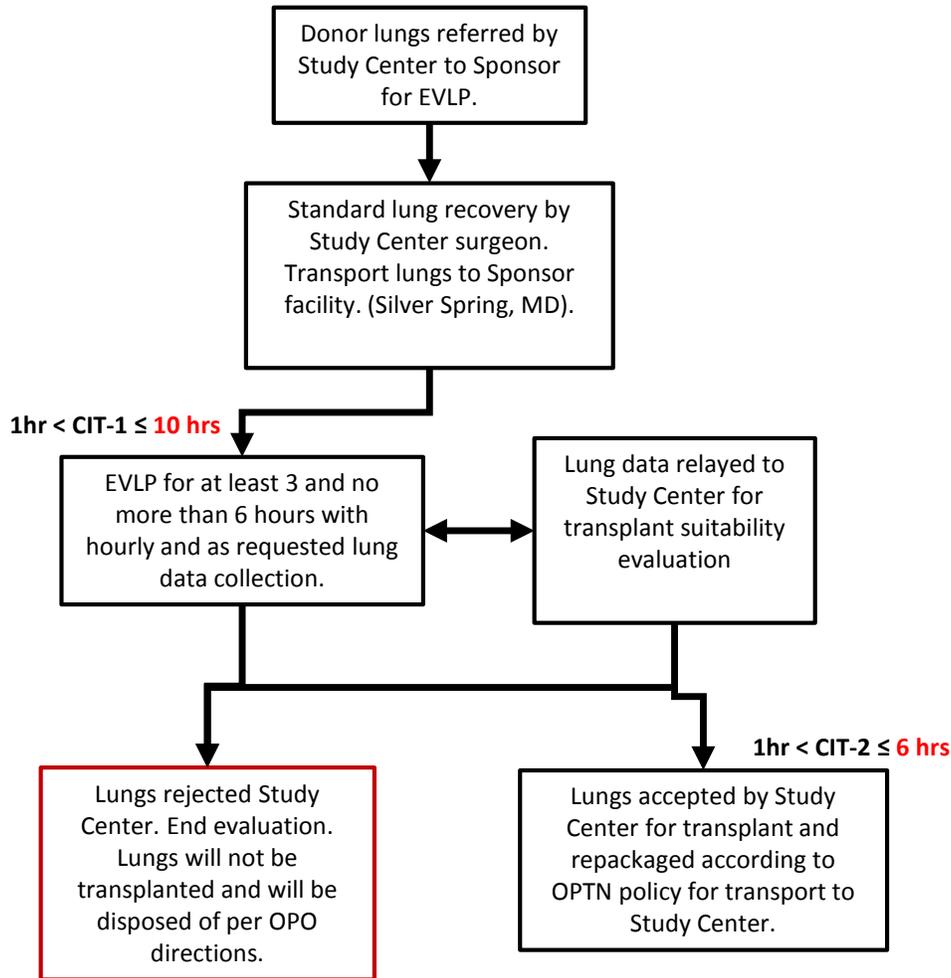
This is an unblinded, non-randomized, traditional feasibility study to evaluate the safety of subjects undergoing lung transplant using lungs after EVLP, performed exclusively by the Sponsor at the Sponsor's facility using the TES. The study will include male and female adult lung transplant recipients, requiring a SLT or DLT who are at least 18 years of age, and who are not receiving mechanical ventilation, Extracorporeal Membrane Oxygenation (ECMO) or other Extracorporeal Life Support (ECLS) at the time of the initial lung offer. Subjects who have signed informed consent ([Section 10.4](#)), match to a donor lung offer through the standard Organ Procurement and Transplantation Network (OPTN) allocation system, and meet the other eligibility criteria ([Section 4](#)), including matching with and undergoing a transplant with a Donor Lung, may participate. Patients who consent for this study (PXUS 14-001) but receive a conventional (i.e., non-EVLP) lung transplant will be considered for a contemporaneous control group matched to the EVLP treatment group. The control group will be selected to best match the EVLP group with respect to the following criteria: SLT/ DLT, and Lung Allocation Score Disease Diagnosis Group (LASDDG). LASDDG is assigned according to OPTN Policy 10.1.F.i. into one of four groups:

- Group A: Obstructive Lung Disease
- Group B: Pulmonary Vascular disease
- Group C: Cystic Fibrosis or Immunodeficient Disorders
- Group D: Restrictive Lung Disease

The goal is to provide a control group with similar characteristics and risk as the experimental group receiving EVLP.

A sample size of 132 subjects, receiving either SLT or DLT, is planned for this study, where 66 subjects have received donor lung(s) after EVLP (EVLP Group) and 66 subjects have received a conventional (i.e., non-EVLP) lung transplant and qualify for a contemporaneous control group (Control Group). Further, no more than two-thirds of the subjects enrolled (44 subjects per group) will have received a SLT or DLT.

Acceptance or rejection of donor lungs for EVLP will follow the process outlined in [Figure 2](#) and detailed in [Section 3.1.1](#).



Note: The red-bordered box indicates a procedural termination point.

Figure 2. Lung Retrieval Process to Determine Acceptance or Rejection for Transplant

Once the donor lung is transplanted following EVLP (see Section 5.2.3), the eligible recipient, who has provided written informed consent, is enrolled into the study. Patients who consent for the current EVLP study (PXUS 14-001) but receive a conventional (i.e., non-EVLP) lung transplant will be considered for a contemporaneous control group matched to the EVLP treatment group. This matching will take place on a patient-by-patient basis and only after an EVLP subject has been enrolled at that Study Center. Investigators and their team will be notified by the Sponsor on a real-time basis of the specific matching criteria required for a control subject as EVLP subjects are enrolled (see Section 5.3). In order to be considered for eligibility, the control subject must “match” a priori to an EVLP subject who has already been enrolled at that Study Center based on the following criteria:

- SLT versus DLT
- LASDDG

The post-intervention phase of the study will then be split into two phases: 1) the Analysis Phase, which includes follow-up within one year post-intervention and 2) Long-term Follow-up, which will capture outcome data annually from 1-year post-intervention until 5-years post-intervention. Although the study will continue after one year, the database will be locked for analyses of endpoints after the last subject has his/her study visit at 1 Year. The Long-term Follow-up data will be summarized when the study has completed in support of long-term evaluation.

Analysis Phase

Following transplantation, subjects will be admitted to the ICU, and the initial PaO₂ and PGD Score will be recorded. The subject's PGD Score will be recorded every 24 hrs for the next 72 hrs. The ICU discharge date will be documented. Study visits will occur 30 days (±5), 90 days (±10), 6 months (±2 weeks), and 1 year (±4 weeks) post-transplant. Study assessments will be performed as outlined in the Schedule of Events ([Appendix 1](#), Table 3).

Long-term Follow-up

Although the database will be locked and analyses of endpoints performed after the last subject has his/her study visit at 1 Year, the Sponsor will continue to collect specified outcome parameters that are required to be reported by transplant centers to OPTN/ United Network for Organ Sharing (UNOS). Collection will be annually for 5-years post-intervention; outcome collection is outlined in the Schedule of Events ([Appendix 1](#), Table 4).

3.1.1 Lung Collection and EVLP Procedure

3.1.1.1 Lung Referral

All donor lungs are allocated to transplant centers according to OPTN Policy 10. Once an acute care hospital identifies a potential organ donor, at or near death, the regional OPO is contacted to coordinate the organ donation. The OPO enters specific donor demographics into a single national data base of all persons needing an organ transplant to generate a match run list of patients for each organ to be donated from deceased individuals. In match run order, the OPO contacts a transplant center to offer the matched organ to a specific patient, and provides details of the donor's medical history and other relevant information. The transplant center has one hour to accept the lung for the matched patient, otherwise the lung offer continues in match run sequence order for additional patients until a center accepts the lung(s), the match run list is exhausted or there are timing issues which may cause the OPO to suspend subsequent lung offers. The lung acceptance by a center is based on a number of factors, but primarily depends on the transplant center's staff decision that a specific donor lung is suitable for the intended recipient. If the transplant center accepts, the lung is shipped to the transplant center for a standard of care transplant.

If the transplant center is also a Study Center in this trial, they then have the ability to enroll a consented patient into this study. The lung(s) is sent to the Study Center as normal, and if the designated recipient is also consented for this study, they may be eligible for enrollment in the control group. This eligibility is based on meeting the overall study inclusion criteria, not

meeting any exclusion criteria, and matching to an already enrolled EVLP recipient at that Study Center based on the criteria found in Section 5.3.

If the referred organ is not suitable for standard of care transplantation, and meets the inclusion criteria for the EVLP group in this study, and does not meet any of the exclusion criteria, the Study Center may refer the lung(s) to the LB-1 facility for EVLP. This decision is based on the protocol criteria as well as the medical expertise of the Study Center staff and the details of the particular case. The Sponsor does not make recommendations for referring an organ to the LB-1 facility for perfusion. The Sponsor will, however, verify that the inclusion/exclusion criteria for referred lungs are followed by the Study Center prior to accepting lungs for EVLP. The verification process will happen when the OPO contacts the Sponsor facility to notify them that an organ has been referred for EVLP by a Study Center, and to discuss lung transportation logistics and other case details. If it is determined that an error has been made by the Study Center in determining study eligibility, the lung(s) will not be sent to the Sponsor for EVLP.

3.1.1.2 Lung Retrieval

Upon retrieval, donor lungs will be packaged and transported to the Sponsor's EVLP facility in Silver Spring, MD, according to standard procedures, following OPTN Policy 16. Upon arrival at the Sponsor's facility, lungs will be placed onto the TES by a specifically-trained *Ex Vivo* Lung Specialist. Prior to starting perfusion, the duration of CIT-1 will be confirmed to be less than or equal to 10 hrs.

The EVLP procedure will be performed by *Ex Vivo* Lung Specialists under remote video supervision by one of Sponsor's Expert Medical Consultants: Drs. Keshavjee, Cypel, or Waddell. The Sponsor will perfuse the lung using the TES for up to 6 hrs, collecting and relaying lung function assessment data hourly, or as requested by the Study Center transplant surgeon. Additionally, the Study Center surgeon will have access to remote monitoring capabilities at the Sponsor EVLP Center for evaluating lung function data and monitoring the procedure through a dedicated audio/video link.

Perfusion Data Parameters

EVLP begins once lung cannulation and extracorporeal perfusion circuit priming is complete. The EVLP procedure shall run for 3 to 6 hrs. At any time between 3 and 6 hrs of perfusion, the EVLP Specialist, in consultation with the Study Center transplant physician, and in collaboration with an Expert Medical Consultant, can stop the procedure, at which time the lung will be packaged for the Study Center or the procedure will be stopped without transplantation. The following perfusion parameters will be collected for internal monitoring every 10 min during the initial 1-hr warm-up phase, then hourly thereafter:

- Pulmonary arterial flow (PAF), L/min
- Pulmonary arterial pressure (PAP), mmHg
- Left atrial pressure (LAP), mmHg

PVR will be calculated using the following formula, beginning at the first hour time point, then hourly for the duration of the EVLP:

$$\text{PVR (dynes x sec x cm}^{-5}\text{)} = [\text{PAP-LAP}] \times 80 / \text{PAF}$$

and measured with a LAP set to 5 and at end of ventilator expiration.

Ventilation Data Parameters

Ventilation shall begin when the lung temperature reaches 32°C measured from the temperature probe connected to the heat exchanger. Then, before each hourly oxygen challenge, the ventilator should be set at a tidal volume of 10 mL/kg (based on donor ideal body weight [IBW]) for 5 min at a fraction of inspired oxygen (FiO₂) of 100% (1.0) prior to obtaining perfusate gas samples. After the perfusate gas sample has been collected, the ventilator tidal volume is set back to the maintenance settings of 7 mL/kg and FiO₂ of 21% (0.21).

The following ventilation parameters will be measured and recorded hourly at the end of each oxygen challenge:

- Mean, peak and plateau airway pressure (mAwP, PAwP, platAwP), cm H₂O
- Static and dynamic compliance (mL/cm H₂O)
- Perfusate gas analysis at lung inflow and outflow: PO₂, partial pressure of carbon dioxide (PCO₂), pH, glucose, and lactose.

Other Data Parameters

Bronchoscopy: Bronchoscopy is performed with visual assessment observations recorded, including a description of secretions, at the one-, three-, and if necessary, five-hour marks of the EVLP procedure. A bronchoscopy may be performed following initial ventilation to clear the airways as indicated. Additional bronchoscopies can be performed upon request by the Study Center physician or the Expert Medical Consultant. All bronchoscopic evaluations will be recorded digitally and transmitted to the Study Center Investigator for review.

X-ray: A digital radiograph of the lung is taken after the first, third, and fifth (if necessary) hour marks to detect edema, consolidations, or other abnormalities; and the resulting image will be transmitted digitally to the Study Center Investigator and Expert Medical Consultant for review.

3.1.1.3 Transfer of EVLP Data to the Study Center

All lungs referred to and accepted by the Sponsor for EVLP must have been previously allocated by an OPO to a Study Center recipient in compliance with OPTN Policy 10.

Throughout the EVLP procedure, donor lung parameters are communicated by the EVLP Specialist for transplant suitability and evaluation by the Study Center Investigator, who will make the final determination of EVLP lung transplant suitability, based on the same set of donor

lung and recipient risk profiles used to evaluate and accept organs during *in vivo* donor assessment.

3.1.1.4 Acceptance of EVLP Lung for Transplant

The EVLP procedure must run between 3 and 6 hrs to qualify the lungs for inclusion into the study. The Expert Medical Consultants, working with the Study Center surgeon/team will monitor and evaluate the quality of the perfusion procedure data remotely. Together, they will serve as a collaborative team regarding the EVLP procedure, including the timing of the EVLP termination. The Expert Medical Consultant(s) will answer questions, provide guidance, and as requested offer perspectives based upon their considerable experience with EVLP and lung transplantation. However, final decisions reside with the Study Center surgeon/team. The Expert Medical Consultants will not issue a recommendation regarding transplanting or not transplanting the lung(s). Specifically, if the donor lung meets EVLP study inclusion criteria (Section 5.2), the Study Center Investigator/team will make the final determination of transplant suitability per OPTN Policy 5.7. Study Center Investigators will provide feedback on the utility of the data parameters assessed for determining transplant suitability via CRF. These data will be used solely to inform future study design.

Upon acceptance of an EVLP donor lung by the Study Center, the single lung or lung block is cooled according to TES methodology to 10°C. Thereafter, perfusion and ventilation are stopped.

This point marks the start of CIT-2. The lung(s) is prepared for hypothermic storage by flushing with 500 mL cold (4°C) STEEN Solution, maintaining lung inflation (trachea is clamped). Accepted lung(s) will be repackaged by the Sponsor according to OPTN Policy 16, stored at 4°C in low potassium dextran cold storage solution and transported to the Study Center using methods to minimize CIT-2.

Total preservation time (TPT) for the first lung transplanted from donor lung retrieval to end of CIT-2 must not be greater than a combined 22 hours and individual phase limits as follows:

$$(\leq 10 \text{ hours CIT-1}) + (3 - 6 \text{ hours EVLP}) + (\leq 6 \text{ hours CIT-2}) \leq 22 \text{ hours}$$

Total preservation time (TPT) for the second lung transplanted in DLT procedures from donor lung retrieval to end of CIT-2 must not be greater than a combined 26 hours and individual phase limits as follows:

$$(\leq 10 \text{ hours CIT-1}) + (3 - 6 \text{ hours EVLP}) + (\leq 10 \text{ hours CIT-2}) \leq 26 \text{ hours}$$

EVLP lungs that exceed 10 hrs of CIT-1, or are perfused for less than 3 hrs or more than 6 hrs will be discarded by the Sponsor according to the responsible OPO's directions.

First lung CIT-2 is the elapsed time between the end of the EVLP procedure and removal of the first lung from cold storage, marking the beginning of the implantation phase of the transplant procedure. This CIT-2 may not exceed 6 hrs. Prior to shipping EVLP lungs to the Study Center, an estimated time of arrival at the Study Center will be calculated by the EVLP Specialist in

consultation with the Study Center to ensure the criterion for CIT-2 will be met. Therefore, if the anticipated first lung CIT-2 will exceed 6 hrs due to logistical considerations (projected flight time and/or surgical staff schedule, etc.), the lungs shall not be transported to the Study Center. Similarly, if after leaving the EVLP facility, prolonged transport time or patient logistical or other issues compromise the ability to ensure actual first lung CIT-2 is less than 6 hours, the transplant shall not proceed.

The implantation phase of the transplant procedure begins upon removal of the first lung from cold storage, marking the end of CIT-2. The warm ischemic time (WIT) period then begins, which lasts until the lung is reperfused in the recipient. This time period is expected to be no longer than the typical WIT at each Study Center for standard lung transplants.

For DLT procedures, the second lung must remain in cold storage in low potassium dextran solution while the first lung implantation is being performed. The time of removal of the second lung from cold storage after the first lung is implanted and the second lung WIT should be consistent with historical surgical experience for sequential DLT at each Study Center. However, if the time between the start of CIT-2 and removal of the second lung from cold storage exceeds 10 hrs, a protocol deviation shall be documented on a CRF and shall be reported to the Sponsor. The reason for the delay and an assessment of the second lung's condition prior to transplant shall be recorded in the CRF. These subjects will continue enrollment in the study and will be followed in the EVLP Group.

3.1.1.5 Request for Reallocation of Lungs After EVLP

In the event the Study Center initially accepting, declines the lungs during or after completion of the EVLP assessment, where the lungs meet the inclusion criteria, the EVLP Specialist must contact the coordinating OPO (the "host" OPO) and provide the following information:

- OPTN/UNOS donor identification number (ID#);
- Name of the transplant Study Center and surgeon declining lungs after EVLP;
- Reason the lungs were declined;
- Names of all other Study Centers participating in the clinical trial;
- Current status of the lung(s) (i.e., still on EVLP, packaged on ice, in transit); and
- Contact information for the EVLP Specialist on call for the specific case.

If the lungs meet the trial acceptance criteria (see Section 5.2.3), the Sponsor personnel will recommend the host OPO resume lung allocation according to their internal policies and procedures, reminding the OPO of the Study Centers participating in Study PXUS 14-001, and stressing that the EVLP procedure is investigational, and potential recipients must have signed the EVLP trial informed consent prior to participating in any study-related assessments or procedures.

In the event the host OPO is able to reallocate the lungs to another participating Study Center, the host OPO will contact the EVLP Specialist, who will then contact the interested physician (Study Center Investigator) to facilitate Case Management Exchange (CMX) access and

information exchange about the case. The Investigator at the reallocated Study Center will assume the role of the transplant center for the EVLP case and will accept or decline the lungs upon review of the EVLP assessment data and in consultation with the Expert Medical Consultant on call.

In the event the host OPO cannot reallocate the lungs to a participating Study Center, the EVLP Specialist will discuss with the host OPO the option of contacting TGH to determine if there is a need at that facility. The decision to reallocate to TGH will rest solely with the host OPO and will be in accordance with their internal policies and procedures. The reallocation will be documented in CMX and on the EVLP facility's CRF. In the event the lungs cannot be reallocated, they will be discarded according to the Sponsor's procedures for handling biomedical waste.

3.2 Rationale for Study Design and Control Group

As described in [Section 1](#), EVLP is a novel technology that allows extended assessment of lungs whose suitability for transplantation is initially uncertain. The extended donor lung preservation time may also enable better logistical coordination of the recipient and donor hospital transplant teams.

This study is an unblinded, non-randomized, traditional feasibility multi-center study to evaluate the safety of subjects undergoing lung transplant after EVLP, as performed by the Sponsor using the TES. This study was built on the single-center experience including >100 EVLP-treated lung transplants at TGH, where similar survival to the contemporaneous (non-EVLP) lung transplant recipients was demonstrated. [[Cypel 2011a](#); [Cypel 2011b](#); [Cypel 2012](#); [Tikkanen 2012](#); [Munshi 2013](#); [Cypel 2013](#)] Importantly, the contribution of EVLP was associated with a 36% increase in total lungs transplanted at TGH in 2013, [[S Keshavjee, personal communication](#)] without negatively impacting transplant volumes at surrounding hospitals [[Tiwari 2014](#)], supporting the notion that the adoption of EVLP technology resulted in transplantation of lungs that would have otherwise been discarded. [[S Keshavjee, personal communication](#); [Munshi 2013](#)]

This study proposal seeks to evaluate the feasibility of reproducing the TES at a central US facility where the donor lungs require an additional travel leg (after concluding the EVLP procedure) compared to standard lung transplantation. This second period of cold preservation will be evaluated by assessing safety measures of subjects with lungs transplanted following EVLP and comparing with subjects who have undergone a traditional lung transplant (i.e., contemporaneous control group). The primary safety endpoints of PGD3 at T72 and 30-day mortality have been selected. PGD3 has been shown to be a good predictor of transplanted organ function, as it has a significant impact on short- and long-term recipient survival in DLT [[Suzuki 2013](#)], and 30-day mortality has been included as part of the primary endpoint, given the lesser degree of certainty of the prognostic significance of PGD3 in SLT (see also Section 6.16).

Randomization is not possible in this study, as lung transplant is considered a life-saving procedure, and donors must match in size and blood type to potential recipients, so withholding a potentially-matched lung transplant is not ethical. Patients not receiving an EVLP lung will be

considered for enrollment in a contemporaneous non-EVLP control group. The control group will be selected to best match the EVLP group with respect to the following criteria:

- SLT versus DLT
- LASDDG

In order to be considered for eligibility, the control patient must “match” a priori to an EVLP subject who has already been enrolled. Because the ratio of standard lung transplant to EVLP transplant is expected to be approximately 4:1, it is feasible to obtain a control-EVLP match within the anticipated duration of the study.

At the request of the Food and Drug Administration (FDA), long-term post-intervention follow-up has been included to assess outcome parameters within the 5 years after either standard or EVLP lung transplant.

3.3 Study Duration and Dates

Data will be collected from the organ donor, EVLP procedure (if applicable), and each subject from the time of lung transplantation and up to one year post-transplant for the Analysis Phase. In addition, outcome data will be collected annually up to 5 years post-transplant.

The study duration for the Analysis Phase (from first patient, first visit [FPFV] to last patient, last visit [LPLV] prior to database lock) is expected to be 3 years. The estimated time to complete enrollment is approximately 24 months.

4 STUDY POPULATION SELECTION

4.1 Study Population

The study will be open to all eligible male and female patients, aged 18 years or older, with end-stage lung disease listed for lung transplantation at a participating Study Center. These participants may not be receiving mechanical ventilation, ECMO or ECLS, at the time of initial lung offer prior to EVLP.

Study Centers will offer the opportunity to participate in the study to its own listed recipients during time of listing for transplant or during periodically scheduled patient visits. It is expected that enrollment and recruitment activities between male and female recipients will approximate a similar gender mix compared to the Study Center's general lung waitlist.

A sample size of 132 subjects, receiving either SLT or DLT, is planned for this study, where 66 subjects receive donor lung(s) after EVLP (EVLP Group) and 66 subjects are enrolled in a contemporaneous control group (Control Group). No more than two-thirds of the subjects enrolled (44 subjects per group) will have received a SLT or DLT.

4.2 Inclusion Criteria

Each patient must meet the following criteria to be enrolled in this study:

1. Male or female patients.
2. All patients, 18 years of age or older.
3. Patient already on or added to the active waiting list for a single or bilateral lung transplant.
4. Patient or patient's representative provides informed consent for participation in the study prior to participating in any study-related assessments or procedures.
5. Patient or patient's representative reconfirms informed consent for the study on the day of lung transplant if initial consent is > 24 hrs.
6. Patient matches with and undergoes a transplant with a donor lung.

4.3 Exclusion Criteria

Patients who meet any of the following criteria will be excluded from the study:

1. Patients listed for same-side lung re-transplantation.
2. Patients listed for multiple-organ transplantation including lung and any other organ.
3. Patients listed for live donor lobar transplant.
4. Patients positive for human immunodeficiency virus (HIV), active Hepatitis B or C, or *Burkholderia cepacia* infection.

5. Patients in the ICU at the time of the initial lung offer requiring ventilation, ECMO or ECLS. [Note: The decision to place the recipient on ECMO or other ECLS for post-transplant prophylaxis at time of or immediately prior to transplant is not considered an exclusion]
6. Patient receives a conventional (non-EVLP) lung transplant but does not match to an EVLP subject based on the criteria for control matching outlined in Section 3.1.

4.4 Strategies for Recruitment and Retention

This study will be offered to eligible end-stage lung recipients at Study Centers. Recipients will not be compensated for participation, but it is possible that a subject could receive an EVLP-treated lung transplant sooner than they would if they were waiting for a standard lung transplant. EVLP lungs have also been more extensively physiologically assessed than a conventionally procured lung.

Following lung transplant, subjects require routine follow-up visits for immunosuppression monitoring and infection control regimens. For this reason, it is unlikely that subjects will be lost to follow-up unless he/she withdraws consent to participate in the study or changes the healthcare facility where he/she receives follow-up care.

5 STUDY INTERVENTION

5.1 Description of Intervention

The TES and has been used by the Toronto group for more than 225 clinical EVLP procedures (including at least 176 completed lung transplants). Nearly all of the hardware and disposables used in the TES are 510(k)-cleared by the Food and Drug Administration (FDA) for human clinical use, indicating appropriate and safe manufacturing procedures. The exception to this is the organ chamber and the lung cannulas, which are both made from medical-grade materials and are currently being used in clinical EVLP under the Humanitarian Device Exemption (HDE) #H120003. There are two classifications of system components: Non-Fluid Path Hardware and Single-Use Fluid Path Disposables. A schematic that represents each classification component in relation to the other is shown in Figure 3.

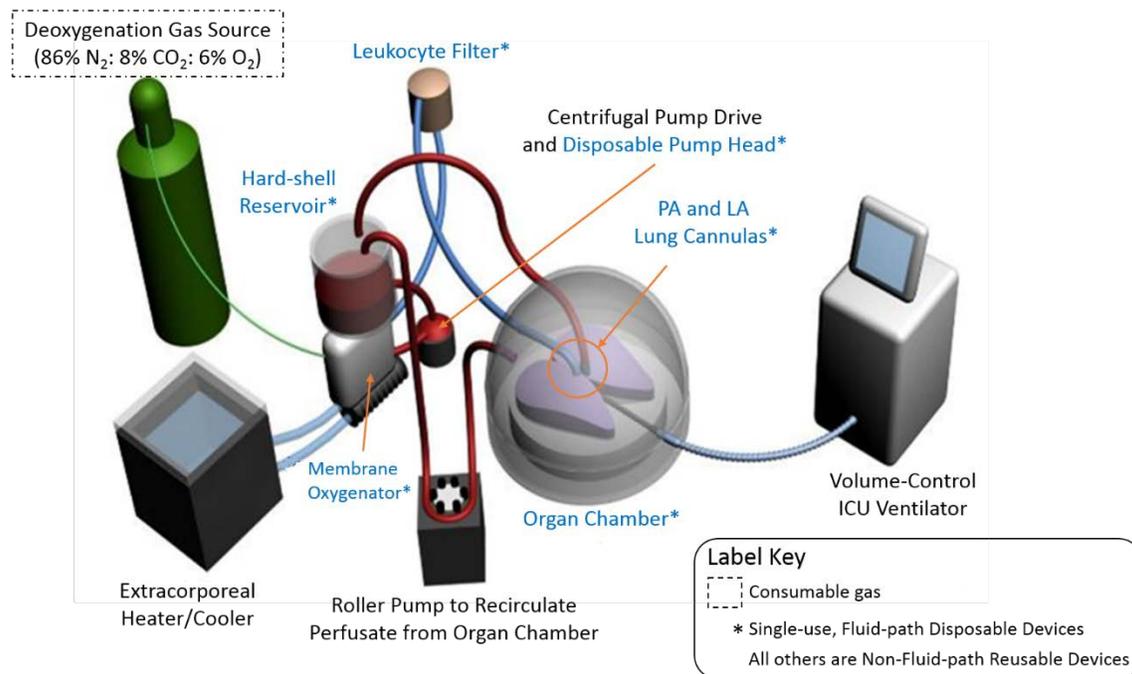


Figure 3. System Components of the Toronto EVLP System

The Toronto *Ex Vivo* Lung Perfusion System (“Toronto EVLP System” or TES) comprises a combination of FDA-approved, off-the-shelf hospital equipment, including: a centrifugal bypass pump, heater/cooler, ICU ventilator, and a disposable bypass perfusion circuit. In addition, the following non-FDA-approved single-use disposables are necessary: disposable organ chamber, disposable lung cannulas, and the perfusate STEEN Solution.

The proposed TES indication is for extending preservation and assessment of donor lungs using normothermic lung perfusion and ventilation in a controlled environment outside the body (*ex vivo*). The device is intended to be used to perfuse lungs for male and female first-time, SLT or DLT recipients, aged 18 years or older with end-stage lung disease, not requiring ECMO, ECLS, or ventilation at the time of initial organ referral prior to EVLP.

The TES is intended to be used exclusively by the Sponsor in a Sponsor-controlled facility established exclusively for the purpose of perfusing and ventilating lungs *ex vivo*. The TES is NOT intended to be sold to any other entity nor used by an entity other than the Sponsor.

Detailed descriptions of all system components currently in use can be found in [Appendix 4](#).

5.2 Lung Transplantation

Donor lungs are evaluated to assess if they meet the donor lung inclusion criteria (Pre-EVLP; Section 5.2.1). If the donor lungs meet criteria, they proceed through EVLP. After EVLP, the donor lungs are again evaluated for transplant suitability and must meet the donor lung inclusion criteria (Post-EVLP; Section 5.2.3.1) in order to be transplanted into a subject who meets the recipient eligibility criteria.

5.2.1 Donor Lung Inclusion Criteria (Pre-EVLP)

Lungs are eligible for use in this study when an OPO documents that without EVLP, the lungs would not otherwise be used for transplantation. Data will be collected on the match run, including the recipients' position on the list, the number of refusals prior to and after a study center's acceptance, and the reason(s) for refusal. The OPO will also document the decision to either discontinue further standard allocation, or expedite allocation directly to a Study Center for EVLP if circumstances so dictate either approach to make the organ available for further evaluation and potential transplant via EVLP.

Examples of such circumstances include:

- Match run exhausted: Attempts to place the lungs via the match run sequence have yielded refusal responses to standard (non-EVLP) offers, and the OPO decides that the remaining allocation time supports the decision for EVLP;
- Logistical challenges: The OPO does not have time to perform a lung-specific match run; for example, if the overall organ donation process is being accelerated due to donor deterioration, limited OR availability, donor family request, procurement teams for other organs with recipient urgency, other competing issues requiring donor surgery to be scheduled prior to lung placement, or allowing another procurement team to recover the lungs for an accepting center;
- Donation after circulatory death (DCD): Refusal rates by transplant centers is high in these cases because evaluation of the donor lung function is limited, and donor expiry after withdrawal of care is unpredictable, often not occurring within the time limits established by the transplant centers or donor hospitals. Because of these factors, many transplant centers do not entertain DCD offers at all, meaning that they would not normally be used for transplant;
- OR decline: An unexpected suitability issue is identified at time of procurement resulting in the decision that the previously designated recipient is no longer appropriate; frequently this severely limits the available time for the OPO to reallocate the lungs; or

1. Recipient availability: After procurement has started, the original intended recipient is unable to receive the transplant, again, creating a critical timing challenge for the OPO to allocate the lungs to another recipient.

5.2.2 Donor Lung Exclusion Criteria (Pre-EVLP)

The donor lung is excluded from EVLP if at least one of the following criteria have been met:

- The donor lung has confirmed pneumonia and/or persistent purulent secretions identified via bronchoscopy prior to donor lung excision.
- Any purulent secretions not considered persistent identified in the donor lung prior to excision that do not clear by the hour 3 assessment of EVLP.
- The donor lung has confirmed evidence of aspiration.
- The donor lung has significant mechanical lung injury or trauma.
- The donor has HIV or an active infectious disease such as Hepatitis B or C.
- The CIT, starting at donor aortic cross clamp/initial flush (CIT-1), required to transport the lung from the donor site to start of the EVLP procedure at the Sponsor's facility was > 10 hrs.

5.2.3 Donor Lung Criteria Post-EVLP Assessment

Throughout each EVLP procedure, the donor lungs are evaluated for transplant suitability by the Study Center Investigator (i.e., transplant surgeon), in consultation with the Expert Medical Consultants. The Study Center Investigator will make the final determination of EVLP lung transplant suitability based on the same set of donor lung and recipient risk profiles used to evaluate and accept organs during standard *in vivo* donor assessment.

In addition to the real-time review of the EVLP procedure by at least two lung transplant physicians, the Sponsor will conduct post-EVLP quality reviews of case data to ensure policies and procedures are adhered to.

5.2.3.1 Donor Lung Inclusion Criteria (Post-EVLP)

The donor lung must meet each of the following conditions post-EVLP for transplant suitability consideration by the Study Center Investigator:

- Partial pressure of oxygen in venous blood (PvO_2)/ $FiO_2 \geq 350$ mmHg at final EVLP evaluation time period
- < 15% increase from baseline value (defined as the first hour collection point) to final value of PVR,
- < 15% increase from baseline value to final value of PAwP,
- < 15% decrease from baseline value to final value of static lung compliance (Cstat),
- TPT does not exceed the following:

- the CIT-1 time from cross clamp in the donor to the start of EVLP > 1 hr and \leq 10 hrs,
 - the EVLP time > 3 hrs and \leq 6 hrs,
 - the estimated CIT-2 from EVLP cool down to the start of the implantation phase of the transplant procedure (time of removal of first lung from cold storage) must be \leq 6 hrs,
 - the estimated CIT-2 from EVLP cool down to the time of removal of the second lung from cold storage must be \leq 10 hrs,
- Study Center Investigator deems lung function suitable for intended subject.

5.3 Method of Assigning Patients to Intervention

A patient who is a potential candidate for this study may be approached by his/her treating physician regarding the patient's interest and desire for study information. This may take place during a Screening Visit up to one year prior to transplant, or during the Baseline visit on the day of transplant. Refer to Section 10.4 for informed consent procedures.

All patients who have provided informed consent to participate in this study will be screened and eligibility will be assessed according to applicable Inclusion (Section 4.2) and Exclusion Criteria (Section 4.3). Each Study Center will maintain a Screening Log where the applicable patient information will be captured, including their LASDDG and whether he/she is listed for SLT or DLT. Screening logs will be provided to the Sponsor on a regular basis so that patient matching criteria can be tracked. This will allow identification of potential control subjects as EVLP subjects become active in the study upon transplantation.

When lungs are matched from donor to recipient, the Study Center will make the determination if the lung qualifies for EVLP or not (Section 5.2). Based on the outcome of that decision and then review of criteria of that lung, either a standard transplant takes place or the lung undergoes EVLP for transplantation.

After at least one EVLP subject has been enrolled at a Study Center, that site's Investigators and their team will be notified that a Control Group slot is available for their Study Center based on the control matching criteria (SLT/ DLT and LASDDG). Study Centers will be notified again once that Control Group slot is filled at their site pending the next EVLP transplant. In this way, each Study Center will provide a matching Control Group subject for each EVLP Group subject enrolled at their site.

Identification of potential control subjects will be done prospectively. Retrospective matching of control patients after standard lung transplantation is prohibited. Study enrollment commences upon lung transplantation, and enrollment will be tracked separately at each participating center by the clinical research organization (CRO), using site-specific identification and coding assigned per the CRF Instructions. Recipient data provided to the Sponsor or the CRO will include Subject ID#, but will not include patient-identifying information (name, social security #, etc.).

Each participating lung transplant center will maintain a screening and study participant log, recording whether the subject received an EVLP-treated or non-EVLP-treated lung transplant, as

well as SLT or DLT and LASDDG, with subsequent screening identification numbers, dates of informed consent, and transplant dates.

In order that no more than two-thirds of the subjects enrolled (44 subjects per group) will have received either a SLT or DLT, Study Centers will be kept informed regarding the numbers and types of transplants that have taken place and if any one type of transplant (single or double) will be limited to enrollment as the proportion reaches two-thirds in the overall study.

Furthermore, to ensure the contemporaneous enrollment of Control Group subjects, Study Centers may not enroll more than three EVLP subjects prior to enrolling at least one matching control subject. In this way, at no time should a site have more than three enrolled EVLP subjects without matched control subjects. The Sponsor will notify any Study Center that reaches this three EVLP patient threshold to remind them that a control patient must be enrolled next.

5.4 Blinding

This study cannot be blinded to the intervention (standard lung transplant vs. EVLP).

5.5 Restrictions

There are no protocol-specific restrictions with respect to fluid, food, prior or concurrent medications, or subject activity during this study.

5.6 Intervention and Study Procedure Compliance

Once a subject receives a lung transplant, he/she is subject to routine follow-up visits due to life-long requirements for immunosuppression monitoring and infection control regimens. For this reason, it is unlikely that subjects will be lost to follow-up unless he/she withdraws consent to participate in the study or changes the healthcare facility where he/she receives follow-up care.

Refer to Section 10.9 regarding deviations from the study protocol.

5.7 Packaging and Labeling

Investigational devices will contain the following label according to 21 Code of Federal Regulations (CFR) 812.5(a):

“CAUTION-Investigational Device Limited by Federal (or US) Law to Investigational Use”

5.8 Storage and Accountability

The TES is comprised of perfusion cart hardware (centrifugal pump, heater/cooler, pressure monitor, ICU ventilator), fluid path and non-fluid path disposables (see [Appendix 4](#)). Additionally, STEEN Solution is the acellular perfusate used during the EVLP procedure.

All EVLP-related investigational device hardware and disposables will be located and used exclusively at the Sponsor's EVLP site in Silver Spring, MD. Devices from this study will never be sent to individual transplant study sites. A device disposition log shall be maintained by the Sponsor to track investigational product information including, but not limited to, product lot/serial/batch #, date of product expiration, manufacturer, date of use, date of disposal of unused product, and the staff initials.

5.8.1 STEEN Solution

The Sponsor will maintain an inventory log listing the quantity received, lot/batch number, and expiration date. The STEEN Solution will be stored in a secure location at the recommended temperature between +4-8°C.

Disposal of any opened STEEN Solution bottles from each procedure and any leftover solution will be performed according to the Sponsor's standard operating procedures. The solution used during the EVLP procedure contains human serum albumin (HAS) and may contain some human red blood cells, so it will be disposed of accordingly.

5.8.2 EVLP Disposables

The Sponsor will be responsible for the timely ordering and accurate dispensing of the TES disposables. The TES disposables will be shipped and stored at room temperature in a secure location at the Sponsor's EVLP site. All disposables will be labeled with the lot number and expiration date and included in the inventory control log. Used disposables are to be discarded in accordance with the Sponsor's policy. No TES disposables will be shipped from the Sponsor's facility to a Study Center.

5.9 Investigational Product Retention at Study Center

The Sponsor will be responsible for the accurate storage, inventory, and dispensing of the STEEN Solution and the TES disposables used exclusively at its own site. The TES disposables include (1) Sterile Organ Chamber, (2) Sterile Lung Cannulas, and (3) Sterile Disposable Lung Circuit.

Required single-use device storage and stability conditions are shown in Table 1.

Table 1. Single-use Device Storage and Stability Conditions

Device	Storage Condition	Device Shelf Life
XVIVO Organ Chamber	Room temperature, store in dry location	4 years
XVIVO Lung Cannula Set	Room temperature, store in dry location	4 years
STEEN Solution	Store refrigerated between 4-8°C protected from light	2 years
Disposable perfusion circuit	Room temperature, store in dry location	2 years

6 STUDY PROCEDURES

All study procedures post-transplant for subjects enrolled in this study will follow the Study Center's standard of care.

6.1 Donor and Donor Lung Information

Data from the donor and donor lung that goes through EVLP will be captured by the EVLP Specialist on a donor/donor lung-specific CRF (termed the EVLP CRF).

During the EVLP lung evaluation, perfusate blood gas will be collected by the EVLP Specialist hourly to assess PCO₂, PO₂ and pH both distally and proximally to the lung to assess the lung's capacity to effectively exchange gas (see Section 5.2.3 and Section 6.12).

The Sponsor will collect the following donor information once a donor lung becomes available for EVLP:

- Date of Birth
- Gender
- Ethnicity
- Race
- Weight
- Height
- Blood group
- Date and Time of Death
- Cause of Death
- Mechanism of Death
- Circumstances of Death
- Donor type (donation after brain death [DBD]/DCD)
- Donor next of kin consent for research
- Hospital information
- PaO₂:FiO₂

For Control Subjects, donor information will not be readily available to the Sponsor; therefore, the donor information listed above must be obtained by the Investigator (or designee) and forwarded to the Sponsor for data entry.

6.2 Informed Consent

The informed consent process is described in Section 10.4.

6.3 Medical History

Medical history should be obtained by the Study Center for all patients as standard of practice. Subject (recipient) medical history of diseases or conditions ongoing in the year prior to the Screening Visit will be recorded. Medical history will be reviewed with the subject at the

Baseline (Day 0) visit, within 24 hrs prior to transplant, to include any updates since the Screening Visit.

6.4 Concomitant Medications

Concomitant medications will be reviewed with the subject at Baseline (Day 0; within 24 hrs of transplant), 30 days post-transplant, and 6 months post-transplant. Eligibility to participate in the study will not be prohibited based on medication alone. Concomitant medications will also be captured for the 2-week period prior to the onset of a serious adverse event (SAE).

6.5 Physical Examination

Physical examination(s) should be performed by the Study Center for all patients as a standard of practice prior to listing patient for transplantation.

A physical examination should be performed at the Baseline Visit. Symptom-directed physical examinations will be performed again at Day 30 and Month 6 post-transplant. Any values judged by the Investigator to be clinically significant abnormal changes from Baseline (before study intervention) should also be recorded on the Adverse Event CRF as an AE.

Specific tests to assess lung capacity will also be obtained and recorded on the CRF. Upon admission to the ICU, the subject's initial PaO₂ is to be recorded.

FEV₁, measured as a percentage of predicted normal value, and oxygen required at rest are to be recorded at the time of hospital discharge (if prior to 30 days post-transplant), Day 30, Day 90, Month 6, and Year 1 post-transplant, and premature discontinuation (if applicable).

In addition, after completion of the Analysis Phase, FEV₁ and oxygen at rest will be collected annually by the Sponsor for the Long-term Follow-up Phase (Year 2, 3, 4, and 5).

6.6 Vital Signs

Assessments of vital signs are to be collected at each scheduled study visit. The following vital sign parameters should be recorded: height (baseline only), weight, heart rate, blood pressure, respiration rate, and oxygen saturation.

Heart rate, blood pressure, respiration rate, and oxygen saturation will be measured after the subject has been seated at rest for at least 5 minutes. Systemic blood pressures may be obtained by cuff, and systemic arterial oxygen saturation may be obtained by pulse oximetry.

6.7 Primary Graft Dysfunction Score

The subject's PGD Score is to be determined by the Investigator at Baseline (Day 0) after transplantation and upon admission to the ICU, as well as 24, 48, and 72 hrs post-transplant. [Christie 2005]

PGD Score will be as graded using the parameters in Table 2, where the P/F ratio is PaO₂/FiO₂.

Table 2. Primary Graft Dysfunction Grading

Grade	P/F Ratio	Chest X-ray
0	>300	Normal
1	>300	Diffuse allograft infiltrates
2	200-300	Diffuse allograft infiltrates
3	<200	Diffuse allograft infiltrates

If the subject is on ECMO at any time point, the PGD grade is automatically assessed as “3”.

If the subject has been extubated prior to assessment, the presence or absence of radiographic infiltrates consistent with pulmonary edema will be reviewed and graded as follows for the PGD Grade:

- If subject is extubated AND the chest x-ray is “Absent” of infiltrates, then the PGD Score is 0.
- If subject is extubated AND the chest x-ray is “Present” of infiltrates, then the PGD Score is 1.

Graft survival will be evaluated by the Investigator (or his/her designee) at Day 30, Day 90, Month 6, Year 1 post-transplant, and premature termination (if applicable). Graft survival will be measured as functioning or failed. If the graft fails within first year post-transplant, the date of graft failure will be collected. The primary cause of graft failure will also be captured as one of the following:

- Primary Non-Function
- Acute Rejection
- Chronic Rejection
- or Other: (if Other, Specify)

After completion of the Analysis Phase, Graft Survival will be evaluated and collected annually by the Sponsor for the Long-term Follow-up Phase (Year 2, 3, 4, and 5).

6.8 Survival

Subject survival, measured as living or dead, will be recorded Day 30, Day 90, Month 6, and Year 1 post-transplant. If the subject expires within first year post-transplant, the date of death and primary cause of death will be collected.

After completion of the Analysis Phase, subject survival will be collected annually by the Sponsor for the Long-term Follow-up Phase (Year 2, 3, 4, and 5).

6.9 Bronchiolitis Obliterans Syndrome Grade

During the Analysis Phase, evaluation of BOS will be conducted by the Investigator (or his/her designee) at Day 90, Month 6, Year 1 post-transplant, and premature termination (if applicable). Assessment will be noted as one of the following:

- NO BOS
- Yes, Grade 0-p
- Yes, Grade 1
- Yes, Grade 2
- Yes, Grade 3
- Yes Grade Unknown (UNK)
- Unknown

After completion of the Analysis Phase, BOS Grade will be collected annually by the Sponsor for the Long-term Follow-up Phase (Year 2, 3, 4, and 5).

6.10 Physical Capacity

During the Analysis Phase, evaluation of physical capacity will be conducted by the Investigator (or his/her designee) at Day 90, Month 6, Year 1 post-transplant, and premature termination (if applicable). Assessment will be noted as one of the following:

- No Limitations
- Limited Mobility
- Wheelchair bound or more limited
- Hospitalized
- UNK

After completion of the Analysis Phase, Physical Capacity will be collected annually by the Sponsor for the Long-term Follow-up Phase (Year 2, 3, 4, and 5).

6.11 Work Status

During the Analysis Phase, evaluation of work status will be conducted by the Investigator (or his/her designee) at Day 90, Month 6, Year 1 post-transplant, and premature termination (if applicable). Assessment will be noted as one of the following:

- Working Full-time
- Working Part-time due to Demands of Treatment
- Working Part-time due to Disability
- Working Part-time due to Insurance Conflict
- Working Part-time due to Inability to Find Full-time Work
- Working Part-time due to Subject Choice
- Working Part-time Reason UNK
- Working Part-time vs. Full-time UNK

After completion of the Analysis Phase, Work Status will be collected annually by the Sponsor for the Long-term Follow-up Phase (Year 2, 3, 4, and 5).

6.12 Clinical Laboratory Tests

6.12.1 Laboratory Parameters

During the EVLP lung evaluation, perfusate blood gas will be collected by the EVLP Specialist hourly to assess PCO₂, PO₂ and pH both distally and proximally to the lung to assess the lung's capacity to effectively exchange gas (see [Appendix 4](#), ABG Analyzer). The Sponsor's facility will test perfusate blood gas samples using a Siemens RapidPoint 500 ABG analyzer.

Subject (recipient post-transplant) arterial blood gases (ABGs) must be determined by the Investigator, in order to calculate PGD grade (Section 6.7), at Baseline (Day 0, within 24 hrs of transplant), and 24, 48, and 72 hrs post-transplant. Laboratory results for tests performed as part of PGD scoring for this study and will be recorded on the Primary Graft Dysfunction CRF with the date and the time the assessment was performed.

6.12.2 Sample Collection, Storage, and Shipping

The Sponsor will collect pre- and post-EVLP donor lung biopsies for future analysis or quality assurance (QA), and/or if requested/ required by transplanting center. Additionally, perfusate samples will be collected from the EVLP perfusion circuit for QA and future analysis. Samples will be identified by donor ID number. Consent for collecting and storing donor lung samples pre-transplantation for future research purposes will be obtained from the donor's Legal Next of Kin (Section 10.4.1).

6.13 Safety Reporting

This study is designed to evaluate the safety of extending the preservation and assessment time of donor lungs using the TES as administered by the Sponsor in a dedicated and centralized facility. Subjects enrolled in this study suffer from end-stage lung failure where the only available treatment is lung transplantation.

6.13.1 Definitions

Adverse Events

An adverse event (AE) is any untoward medical occurrence, unintended disease or injury, or any untoward clinical signs (including abnormal laboratory findings) in a clinical investigation subject which does not necessarily have a causal relationship to the investigational medical device. All AEs are assessed by the Study Centers for severity, causality, outcome, seriousness, and if unanticipated, documented as such (see below).

Serious Adverse Event (SAE)

A serious adverse event (SAE) is defined by federal regulation as any AE that results in any of the following outcomes: death, life-threatening AE, hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect.

Important medical events that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Unanticipated Adverse Device Effect (UADE)

Any serious adverse device effect on health or safety or any life-threatening problem or death caused by or associated with a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

Anticipated Adverse Events not Related to EVLP

Anticipated Adverse Events not related to EVLP are events inherent to the lung transplant surgical procedure and immunosuppressant medications that are expected to occur throughout the initial post-hospital stay in all subjects.[Lyu 2009] These events do not require reporting and are not considered AEs as defined or described elsewhere in this protocol. For the purposes of this protocol, Anticipated Adverse Events not related to EVLP are:

- Surgical
 - Anesthesia related nausea/vomiting
 - Surgical incision, thoracotomy, incision-related pain and bleeding
 - Moderate bruising at surgical site
 - Chest tube placement, chest tube pain
 - Surgical lines, including arterial, Swan-Ganz catheter, triple lumen catheter
 - Ventilator use during and after surgery
 - Back pain related to operating room table or referred pain
- Gastrointestinal
 - Nausea/vomiting
 - Constipation
 - Diarrhea
 - Gastroparesis
 - Hematemesis
 - Melena
 - C. difficile colitis
 - Gastritis
 - Esophagitis
 - Gastric/duodenal ulcer
 - Decreased appetite
 - Gastroesophageal reflux disease
 - Ileus

- Partial intestinal obstruction
- Malnutrition/anorexia
- Systemic fever/pain/malaise/weakness
- Respiratory
 - Wheezing
 - Cough
 - Pleurisy
 - Pleural effusion
 - Pneumothorax
 - Bronchospasm
 - Diaphragmatic paralysis
 - Intra-pulmonary shunt
 - Caustic lesions
 - Bleeding secondary to biopsy
- Cardiovascular observations
 - Phlebitis
 - Arrhythmias
 - Hypertension
 - Cold extremities
 - Pericarditis
 - Tamponade
 - Myocardial ischemia
 - Heart failure
 - Pericardial effusion
 - Peripheral edema
 - Hyper/hypotension
 - Hyperlipidemia
 - Deep vein thrombosis
- Neurological
 - Hallucinations
 - Confusion
 - Headaches
 - Insomnia
 - Psychosis
 - Neuropathy
 - Anxiety
 - Depression
 - Tremor
 - Visual disturbances
- Head/Eyes/Ears/Nose/Throat:
 - Sinusitis
 - Sore throat
 - Hoarseness
 - Paralyzed vocal cord
 - Cough

- Epistaxis
- Thrush
- Herpes
- Renal/Genitourinary
 - Urinary tract infection
 - Urinary retention
 - Dysuria
 - Hematuria
 - Renal failure
 - Azotemia
 - Dehydration
 - Electrolyte imbalance
- Hematological
 - Anemia
 - Leukopenia
 - Thrombocytopenia
 - Pancytopenia
 - Leukocytosis
 - Coagulopathy
- Musculoskeletal
 - Myopathy
 - Tendonitis
 - Deconditioning
 - Weakness
 - Osteoporosis
 - Fracture
 - Pain
 - Osteonecrosis
- Endocrinological
 - Osteopenia
 - Diabetes
 - Decreased testosterone
 - Vitamin D deficiency
 - Hyper/hypothyroidism
 - Adrenal insufficiency
 - Cushingoid changes

Events Related to Study Endpoints

Any adverse events that are also defined within the primary and secondary endpoints do not require reporting as AEs since these are reported within the CRF. For example, PGD within the first 72 hrs or subject death does not require separate AE reporting from the Study Center since these events are captured via CRF. However, if a subject death was the outcome of a fatal SAE, then that SAE should be captured on the CRF. Additionally, if an AE defined as a primary or secondary endpoint meets the UADE criteria, expedited reporting is required.

6.13.2 Performing Adverse Events Assessments or Recording of Adverse Events

The Study Center staff will record and monitor all AEs throughout the Analysis Phase of the study (1-year post-intervention), from the time of transplanted lung reperfusion until the end of the subject's study participation. At all study visits, reports of AEs will be elicited by a verbal probe ("How are you feeling?"). Subjects will be encouraged to contact the Study Center to report an AE at any time. Appropriate measures, including medical intervention and/or procedures, will be instituted if clinically indicated at the discretion of the Investigator.

All AEs occurring during the study must be documented, and any AEs occurring during the first 90 days post-transplant must be reported in the CRFs for AEs. For each AE, the Investigator will evaluate the severity and seriousness, as well as the relationship of the AE to the device and procedure. Information relating to the AE, such as onset and cessation date and times, seriousness, severity, relationship to device/procedure, action taken, and outcome will also be documented in the subject's source documentation for assessment. Where possible, AEs should be recorded using standard medical terminology. If several signs or symptoms are clearly related to a medically defined diagnosis or syndrome, the diagnosis or syndrome should be recorded, not the individual signs and symptoms.

6.13.3 Timing of Adverse Event Collection

All AEs are collected from the time of transplanted lung reperfusion through subject participation duration (1 year post-operative). Reporting procedures are described in Section 6.13.7.

Subjects with AEs that are ongoing at the subject's last Study Center visit must be followed until resolution or for 30 days after the subject's last Study Center visit, whichever comes first. Adverse events that are recorded during the 7 days following the subject's last Study Center visit will be followed until resolution or for up to the 30 days after the subject's last Study Center visit, whichever comes first. All AEs that are ongoing after follow-up for 30 days' time will be recorded as ongoing. The Investigator is expected to provide or arrange appropriate supportive care for any subject with ongoing AE(s).

Serious adverse events (SAEs) should be followed until they resolve or the event or their sequelae stabilize. Supplemental measurements and/or evaluations may be necessary to investigate fully the nature and/or causality of an AE or SAE. Such supplementary assessments may include additional laboratory tests, diagnostic procedures, or consultation with other healthcare professionals. The subject's source documents and CRFs for AEs should be updated with any new or additional information as appropriate.

If the subject reports or the Investigator learns of any new SAE that occurs up to 30 days after the subject's last clinical site visit, the clinical site personnel will ensure that these data are reported on the AE CRF.

6.13.4 Severity

The Investigator will use the following scale to grade the severity of any AEs:

- Mild: no intervention required; no impact on activities of daily living (ADL)
- Moderate: minimal, local, or non-invasive intervention indicated; moderate impact on ADL
- Severe: significant symptoms requiring invasive intervention; subject seeks medical attention, needs major assistance with ADL

6.13.5 Relationship

The relationship of an AE to EVLP procedure and lung transplant must be assessed by the Investigator using the following guidelines:

- **Related:** The event is associated with the use of the investigational device or with procedures beyond a reasonable doubt
- **Possibly Related:** The relationship with the use of the investigational device or procedure is weak but cannot be ruled out completely; alternative causes are also possible.
- **Unlikely:** The relationship with the use of the investigational device or procedure seems not relevant and/or the event can be reasonably explained by another cause, but additional information may be obtained.
- **Not related:** There is no temporal relationship between the investigational device or procedure and the event onset, an alternate etiology has been established.

6.13.6 Expectedness

The Sponsor, Independent Medical Monitor, and the Study Principal Investigator will be responsible for determining whether an SAE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the intervention. Anticipated adverse events not related to EVLP as listed in Section 6.13.1 are for purposes of this study considered to be “expected” events and do not require AE reporting.

6.13.7 Reporting Procedures

The Sponsor will report all AE’s occurring during the first 90 days post-transplant, and all SAEs occurring during the one-year Analysis Phase of the study to the FDA in the annual Investigational Device Exemption (IDE) report. Each reported AE will include date of onset, actions taken, severity, seriousness of the event, relationship of the event to the EVLP procedure or lung transplant, and the outcome of the event.

6.13.7.1 Reporting Serious Adverse Events

All SAEs, including subject deaths, are reported to the CRO within one working day after awareness via the Adverse Event CRF. The Sponsor shall review all SAEs to assess if related or anticipated. Any event that meets the serious criteria, is unanticipated, and related to the device system is reviewed by the Sponsor Independent Medical Monitor and subject to expedited reporting to FDA and participating IRBs in accordance with the Unanticipated Adverse Device Effect requirements (21 CFR 812.150). The Sponsor or Sponsor’s representative will report any

results of an evaluation of UADE to the FDA, Institutional Review Board (IRB), and all Investigators within 10 business days following receipt of notice of the event(s).

All other SAEs for the Analysis Phase of the study will be reported to FDA in the annual IDE report.

In addition to the above, it is the responsibility of the Study Investigator to abide by the AE reporting requirements stipulated by the IRB.

6.13.7.2 Reporting Procedure for Serious Adverse Events

Any AE meeting the specified SAE criteria will be submitted on an AE CRF to the CRO. This report may be sent by fax or email. Once submitted, the CRO will send a confirmation email to the Investigator within one business day. The Investigator should contact the CRO if this confirmation is not received. This process applies to both initial and follow-up SAE reports.

SAE Reporting Contact Information:

Safety Fax Line (US): 1-800-673-7523

Product Safety Email: ctisafety@ctifacts.com

General questions about SAE reporting can be directed to the Safety Help Line (available 8:00AM – 5:00PM Eastern Time): **1-800-755-0742**

The Study Investigator will complete an AE CRF with fax cover sheet found in the study manual and submit via fax or email within the following timelines:

- All SAEs, including deaths, regardless of relationship, will be reported by fax or email within 24 hrs of site awareness.
- All SAEs will be followed until resolution or stabilization.

6.13.8 Initial Safety Review

The Sponsor will temporarily pause the study after the twentieth (#20) subject has been enrolled to allow time for the FDA to review safety data on the first 10 EVLP subjects and any control subjects enrolled who have reached 90-days post-transplant.

Data from the first 10 subjects through their 90-day visit will be submitted to FDA as an IDE Supplement for review. Submitted data will include graft survival, aggregate PGD scores, FEV₁, oxygen required at rest, and BOS Grade. The study will be on hold during this review with the exception of Subjects 11 through 20 who are “ongoing”; these subjects will continue to come to the Study Center as specified in the protocol. Upon satisfactory review, the FDA will permit the continuation of the study to the full subject enrollment. If the FDA has concerns, the Sponsor will address these in a satisfactory manner.

6.13.9 Halting Rules

The Sponsor shall implement an immediate enrollment pause if there is any of the following:

- a third subject death within the first 30 days post- transplant;
- if the PGD3 rate at T72 $\geq 50\%$ in the first 10 subjects; or
- the Medical Monitor shall review all UADEs and deaths in the study and will make a determination if an unreasonable risk to subjects exist. If an unreasonable risk has been determined based on the opinion of the Medical Monitor, the trial, or the parts that cause the risk, shall be stopped until an alternate solution can be devised by the Sponsor and accepted by each IRB and FDA.

6.14 Removal of Subjects from the Study

Subjects may withdraw voluntarily from the study or the Investigator may terminate a subject's participation. Withdrawing from the study will not affect subject's status/ability to receive a standard lung transplant.

Subjects are free to withdraw from participation in the study at any time upon request.

The Investigator may withdraw a subject from the study for any reason, including but not limited to, the following:

- Any clinical AE, laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject.
- The subject meets an exclusion criterion (either newly-developed or not previously recognized) that precludes further study participation.
- A protocol violation occurs.
- The Sponsor or Investigator terminates the study.

It is unlikely that a subject will discontinue study participation after receiving a lung transplant, as a lifetime of required follow-up, immunosuppression monitoring, and infection control is required after transplantation to minimize the chance of graft failure and/or death. Every effort will be made to undertake protocol-specified safety follow-up procedures to capture AEs, SAEs, and UADEs.

6.15 Premature Termination or Suspension of the Study

This study may be suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to Study Investigators, the IDE Sponsor, and all regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to subjects.
- Insufficient adherence to protocol requirements.
- Data that are not sufficiently complete and/or evaluable.
- Determination of futility.

6.16 Appropriateness of Measurements

PGD3 at T72 and 30-day mortality are the primary safety endpoints measured for subjects in this study (Section 2.1).

PGD3 has been shown in the literature to be a very good predictor of transplanted organ function as it has a significant impact on short- and long-term recipient survival including:

- PGD3 at T72 demonstrated a significantly higher risk of 30-day mortality, accounting for 50% of all-cause deaths compared to PGD0 at T72.
- PGD3 at T72 is associated with an increased 90-day mortality rate of 18% and an increased 1-year mortality rate of 23%.
- PGD3 at T72 has the strongest association with long-term mortality compared to PGD scores at other time points post-transplant even after 1 year.[[Suzuki 2013](#)]

Over 100 lung transplants using lungs after undergoing EVLP at TGH and showed similar 30-day, 1-year, and 3-year survival outcomes compared to the contemporaneous (non-EVLP) recipient populations.[[Cypel 2011a](#); [Cypel 2011b](#); [Cypel 2012](#); [Tikkanen 2012](#); [Munshi 2013](#); [Cypel 2013](#)] Similarly, in this study, short-term and long-term survival will be documented for all subjects.

While the prognostic value of PGD grading in DLT has gained clinical acceptance, the experience with PGD scores in SLT is more limited. In DLT, the measurement of oxygenation reflects the contribution of both transplanted lungs. In contrast, measured oxygenation reflects contributions from both a diseased, native lung and a transplanted graft when only a single lung is transplanted. The Lung Transplant Outcomes Group found the SLT itself was a risk factor for adverse PGD scores and was associated with worse long-term outcomes [[Diamond 2013](#)], although the reasons for this association are not understood. Thus, to address the lack of clinical clarity, the measurement of 30-day mortality is included as a co-primary endpoint, and is intended to account for the uncertainty provided by PGD scoring of SLT. Both co-primary endpoints will be considered to inform on prediction of long-term survival in SLT recipients. The contemporaneous control group will be used to provide context for interpreting these results.

7 STUDY ACTIVITIES

A review of the transplant candidate files, donor information, and lung evaluation is required to determine preliminary eligibility according to recipient and donor lung inclusion and exclusion criteria.

The Study Schedule of Events is tabulated in [Appendix 1](#) for both the Analysis Phase (Table 3) and the Long-term Follow-up Phase (Table 4).

7.1 Screening Visit (Days –365 to -1 [-24 hours])

A Screening Visit may occur any time from up to one year to the day of lung transplant. The following may be conducted during this visit:

- Obtain informed consent per Section 10.4.
- Determine patient eligibility according to applicable Inclusion (Section 4.2) and Exclusion Criteria (Section 4.3).

7.2 Intervention

7.2.1 Lung Assessment, Recipient Baseline, and Lung Transplant (Day 0)

7.2.1.1 Donor Lung Screening (Day 0, within 24 hours prior to transplant)

For EVLP lung donors, the Sponsor will perform the following:

- Collect donor information (see Section 6.1)
- Confirm donor lung eligibility according to the donor Inclusion (Section 5.2.1) and Exclusion criteria (Section 5.2.2) for EVLP Assessment.

For Control subjects, the Investigator will perform the following:

- Collect donor information (see Section 6.1)
- Forward donor information for entry into Sponsor CRF

7.2.1.2 Recipient Screening (Day 0, within 24 hours before transplant)

For both EVLP and Control subjects, the Investigator will perform the following:

- Consent or reconfirm consent in accordance with the IRB policy.
- Collect the following recipient information:
 - Medical history of diseases or conditions ongoing in the past year.
 - Date of Birth

- Gender
 - Ethnicity
 - Race
 - Weight
 - Height
 - LASDDG
 - LAS
 - Oxygen requirement status (L/min)
- Perform physical examination.
 - Confirm recipient eligibility in accordance with the recipient Inclusion (Section 4.2) and Exclusion Criteria (Section 4.3).

7.2.1.3 Donor Lung EVLP Evaluation (at completion)

The Sponsor will perform the following:

- Record preservation information (see Section 6.1): cross clamp time/ start of first cold preservation (CIT-1), volume, type, macroscopic lung evaluation.
- Confirm donor lung eligibility in accordance with the donor lung Inclusion (Section 5.2.3.1) for transplant suitability AFTER EVLP.
- Clinical parameters will be collected and used as part of the EVLP suitability evaluation that is performed by the Investigator.

7.2.1.4 Lung Transplantation (Day 0)

Provided all recipient and lung Inclusion and Exclusion Criteria have been met, the Investigator or his/her designee will record the following at the time of admission to the ICU after lung transplantation:

- Hospital admission date
- Transplant date and time, including removal from cold storage time for each lung
- ICU admission time and date
- Initial PaO₂ and FiO₂ upon ICU admission
- PGD score (0 hrs)

7.3 Post-intervention Period; Analysis Phase (Day 1 to 1 Year Post-Transplant)

7.3.1 Visit 1 Procedures (24 Hours post-transplant)

At 24-hrs post-transplant (\pm 4 hrs), the Investigator or his/her designee should perform the following:

- Record PGD Score (Section 6.7)

- Assess any AEs

7.3.2 Visit 2 Procedures (48 Hours post-transplant)

At 48-hrs post-transplant (\pm 8 hrs), the Investigator or his/her designee should perform the following:

- Record PGD Score (Section 6.7)
- Assess any AEs

7.3.3 Visit 3 Procedures (72 Hours post-transplant)

At 72-hrs post-transplant (\pm 12 hrs), the Investigator or his/her designee should perform the following:

- Record PGD Score (Section 6.7)
- Record extubation date/time (if applicable)
- Record ICU discharge time/date (if applicable)
- Assess AEs

7.3.4 Visit 4 Procedures (Day 30 post-transplant)

On Day 30 post-transplant (\pm 5 days), the Investigator or his/her designee should record the following:

- Subject and graft survival and if failed, cause of failure
- If extubated >72 hrs post-transplant, extubation time
- FEV₁ (Day 30 or upon hospital discharge, if before 30 days)
- Oxygen required at rest (Day 30 or upon hospital discharge, if before 30 days)
- Hospital discharge date and readmission date(s) (if applicable)
- Assess AEs

7.3.5 Visit 5 Procedures (Day 90 post-transplant)

On Day 90 post-transplant (\pm 10 days), the Investigator or his/her designee should record the following:

- Subject and graft survival and if failed, cause of failure
- FEV₁
- Oxygen required at rest
- BOS Grade
- Physical capacity

- Working for income status
- Hospital readmission date(s) (if applicable)
- Assess AEs

7.3.6 Visit 6 Procedures (Month 6 post-transplant)

At Month 6 post-transplant (± 2 weeks), the Investigator or his/her designee should record the following:

- Subject and graft survival and if failed, cause of failure
- FEV₁
- Oxygen required at rest
- BOS Grade
- Physical capacity
- Working for income status
- Hospital readmission date(s) (if applicable)
- Assess AEs

7.3.7 Visit 7 Procedures/ Final Visit (1 year post-transplant)

The final study visit for the Analysis Phase will occur one year post-transplant At Year 1 post-transplant (± 4 weeks), the Investigator or his/her designee should record the following:

- Subject and graft survival and if failed, cause of failure
- FEV₁
- Oxygen required at rest
- BOS Grade
- Physical capacity
- Working for income status
- Hospital readmission date/s (if applicable)
- Assess AEs

7.4 Post-intervention Period; Long-term Follow-up Phase (1 to 5 Years Post-Transplant)

Outcome data will be collected annually for 5 years post-intervention. However, lung transplantation requires follow-up care for the life of the subject. The hospital where the transplant was performed, or another transplant hospital if the subject moves, will continue providing standard of care follow-up services to the subject for the rest of subject's lifetime.

Visit 8 (Year 2), Visit 9 (Year 3), Visit 10 (Year 4), and Visit 11 (Year 5)

The following data will be collected annually for Year 2 through Year 5 (± 4 weeks) by the Study Center and reported to OPTN/UNOS database and retrieved by the Sponsor (see Section 10.11.2.3):

- Subject and graft survival and if failed, cause of failure
- FEV₁
- Oxygen required at rest
- BOS Grade
- Physical capacity
- Working for income status
- Hospital readmission date/s (if applicable)

7.5 Early Termination Procedures

As lung transplant requires life-long follow-up care from the transplant center, it is unlikely a subject will withdraw from this study post-transplant. Subjects may withdraw consent to participate prior to receiving the intervention (lung transplant) within this study without consequence to the subject or study. In the event that a patient withdraws consent after lung transplant, the following data will be collected by the Study Center either through OPTN and/or SRTR reports or via an early termination visit:

- Subject and graft survival and if failed, cause of failure
- FEV₁
- Oxygen required at rest
- BOS Grade
- Physical capacity
- Working for income status
- Hospital readmission date/s (if applicable)
- Assess AEs

7.6 Unscheduled Visits

Upon receiving any lung transplant, a subject may require a number of unscheduled visits for a variety of reasons, including infection and rejection. If an unscheduled visit is performed due to a documentable AE, then it will be tracked as such. After 30 days post-transplant, all hospital readmissions and root cause will be documented and tracked in the standard OPTN/UNOS database and made available to the Sponsor.

8 QUALITY CONTROL AND ASSURANCE

Quality Management is the overall process of establishing and ensuring the quality of processes, data, and documentation associated with clinical research activities. It encompasses both quality control (QC), and QA activities. All sites conducting research under the sponsorship of the Sponsor are required to have a plan in place for assuring the quality of the research being conducted.

Each site should have standard operating procedures (SOPs) and/or a quality management plan that describe:

- How data will be evaluated for compliance with the protocol and for accuracy in relation to source documents.
- The documents to be reviewed (e.g., CRFs, clinic notes), who is responsible, and the frequency for reviews.
- Who will be responsible for addressing QA issues (correcting procedures that are not in compliance with protocol) and QC issues (correcting errors in data entry).
- Staff training methods and how such training will be tracked.
- If applicable, calibration exercises conducted prior to and during the study to train examiners and maintain acceptable intra- and inter-examiner agreement.

8.1 Sponsor's Quality Process

The Sponsor will conduct the EVLP lung assessment procedure exclusively at the Sponsor's dedicated facility. All post-EVLP procedures will be reviewed by an in-house QA manager for compliance with the TES User Manual and clinical trial protocol, as well as for accuracy in relation to source documents.

In addition, the Sponsor will use an independent CRO to oversee its activities. Finally, Independent Medical Monitors will review all UADE to verify if they are related to the EVLP procedure, the lung transplantation, or other controllable/ uncontrollable event (Section 10.8).

8.2 Training for Study Interventionalists

Training will be completed prior to participation in the clinical trial as outlined in the following sections.

8.2.1 Transplant Center Research Personnel

Study Center site personnel will be trained on study activities, provided a review of Good Clinical Practice (GCP), obtaining informed consent, and the study protocol.

8.2.2 Lung Recovery Surgeons

Surgeons recovering lungs for this study will be experienced lung recovery surgeons listed in the AOPO Credentials Information Network (ACIN).

8.2.3 Lung Transplant Surgeons

All Study Center Investigators will be experienced practitioners of lung transplantation. Current licensure will be maintained in the study records for each participating surgeon.

8.2.4 EVLP Specialists

Ex Vivo Lung Specialists, specifically trained to perform EVLP by University Health Network in Toronto, Canada, will perform all EVLP procedures exclusively at the Sponsor's dedicated site in Silver Spring, MD. Each donor lung, meeting study Inclusion Criteria (Section 5.2) and offered to the Sponsor by a participating transplant center, will be perfused normothermally by the EVLS for up to 6 hours according to the procedures described in the TES User Manual.

All EVLP Specialists performing procedures at the Sponsor's site will have extensive training in the TES through a 6-month in-residence program at TGH, culminating in successful completion of the *Ex Vivo* Lung Specialist Certification and Privileging Exam administered through University Health Network, Toronto, Ontario. This training program is the first of its kind, and the most comprehensive for the newly emerging field of EVLP.

The didactic portion of the EVLS training includes lectures and self-guided reading of applicable medical literature in the following areas:

- a. Lung transplantation- history and overview
- b. Lung allograft donation and procurement
- c. Legislative framework
- d. Lung anatomy and physiology
- e. Perfusion concepts
- f. Ventilation strategies
- g. TES

The practical, hands-on experience includes observing a minimum of five research (animal) EVLP procedures and actively participating in at least 25 research EVLP procedures. Additionally, a minimum of five clinical (human) EVLP procedures will be observed and at least five more clinical cases will be performed with active participation, of which three must be transplanted at the end of the procedure.

The final exam includes written, oral and practical assessments while independently performing a 12-hour long research procedure using the TES to perfuse bilateral swine lungs while being evaluated by a preceptor for the following skills:

- a. Cannulation of the pulmonary artery and left atrium without leaks.

- b. Proper placement and securing of the endotracheal tube correctly in trachea.
- c. Able to discuss equipment used in the TES and rationale for why each piece was specifically chosen.
- d. Calibrate the ventilator, pump flow and pressure sensors independently.
- e. Effectively de-air the perfusion circuit without difficulty.
- f. Place lungs on the EVLP circuit independently without kinks in the cannulas.
- g. Performs retrograde flushing of the lungs prior to perfusion.
- h. Calculates the correct lung flow and ventilation targets.
- i. Warms up lung in correct sequence.
- j. Turns on deoxygenation gas mixture at the correct time and begins ventilation in the correct sequence.
- k. Makes independent changes to the ventilator as required.
- l. Can operate and retrieve data from the Stöckert Centrifugal Pump Console (SCPC) pump, heater/cooler, pressure and temperature monitor, ventilator and ABG machine (see [Appendix 4](#)).
- m. Can perform x-ray and bronchoscopy of the lung independently.
- n. Can maintain a lung on the TES for a minimum of 12 hours without decrease in function.
- o. Can cool down lung in correct sequence.
- p. Can remove lung and repackage independently according to OPTN/UNOS policy.

9 PLANNED STATISTICAL METHODS

9.1 General Considerations

This section describes the planned statistical analyses in general terms. A complete description of the methodology will be specified in a statistical analysis plan (SAP), which will be finalized prior to database lock. Any changes in the statistical methods described in this protocol that occur prior to database lock will be documented in the statistical analysis plan and will not require a protocol amendment.

Statistical analysis will be performed primarily on data collected during the Analysis Phase (1-year post-intervention). The database will be locked for analysis of endpoints after the last subject completes his/her study visit at Year 1, and all queries have been resolved. The Long-term Follow-up data will be summarized when the study has completed in support of long-term evaluation.

No statistical hypotheses will be tested in this study. For ease of presentation, contextual review and statistical programming, the descriptive statistics of the contemporaneous Control group will be presented with those of the EVLP group. Descriptive statistics will be presented as means, standard deviations (SD), medians, and ranges for the continuous variables and as frequencies and percentages for categorical variables. Tabulations will be used to summarize results from descriptive summaries and statistical analyses. A one-sided 95.0% CI will be provided, as appropriate, for the primary endpoints, and two sided 95.0% CI will be presented for all endpoints, as appropriate.

9.2 Determination of Sample Size

In order to provide a sufficient number of subjects for reliable estimation of the primary endpoints, sample size was calculated based on a primary endpoint of PGD3 at T72. Estimates of PGD3 at T72 were based on historical data from EVLP transplant studies where EVLP subjects had rates of PGD3 at T72 ranging from 0% to 15% and conventional transplant subjects ranging from 16.8% to 30% [XVIXO Perfusion Inc. 2014; Cypel 2012; Cypel 2011a; Machuca 2014; Aigner 2012; Diamond 2013]. Assuming a PGD3 at T72 of 10% and a one-sided 95.0% confidence interval (CI) for a single proportion using the large sample normal approximation adjusted for a finite population of size 2057 (number of lung transplants performed in the US in 2015 [OPTN 2015]), 66 subjects will be needed for the expected upper bound of the CI to not exceed a rate of 16% (nQuery Advisor 7.0, 2007). A total of 66 EVLP subjects and 66 Control subjects will be included in this study.

Rates of 30-day mortality in the EVLP transplant studies have been low, ranging from 3% to 4%. For a sample size of 66, the probability of observing at least one event will be approximately 87% to 93% if the true probability of 30-day mortality is 3% to 4% (nQuery Advisor 7.0, 2007). Subjects will be entered into the study as EVLP transplants occur; however, enrollment to either transplant subtype (single-lung, double-lung) will be limited to 44 subjects to provide a reasonable number of subjects of both subtypes for subgroup examinations.

A contemporaneous Control group will be entered into this study. This Control group will provide context of the EVLP results and estimation of Control group parameters for design of future studies. All analyses of the Control group will be descriptive and no formal statistical comparison will be made between the EVLP and Control groups. As such, the sample size for the Control group will be 66 subjects, and subjects will be matched to the EVLP Group for similarity. Because the ratio of standard lung transplant to EVLP transplant is estimated to be approximately 4:1, it is feasible to obtain a control-EVLP match within the anticipated duration of the study.

9.3 Analysis Populations

All subject included in the analyses to the extent data are available. Planned exceptions will be noted in the SAP.

Deviations from inclusion/exclusion criteria will be listed by subject.

9.4 Demographics and Baseline Characteristics

Demographic data and baseline characteristics for all subjects collected prior to transplant will be listed and summarized overall, by Transplant Group (EVLP or Control), as well as by transplant subtype (SLT or DLT) using descriptive statistics.

Medical history (medical conditions ending prior to EVLP transplant) will be coded using the WHO-drug dictionary, presented as a listing and summarized overall and by transplant subtype using the system organ class (SOC).

9.5 Primary Endpoint(s)

9.5.1 Primary Graft Dysfunction Grade 3 at 72 hours Post-transplant

The PGD3 Score at 72 hrs post-transplant (T72), as defined in Section 6.7, will be used as the basis for the primary safety endpoint for analysis. The primary endpoint (PGD3 T72) will be calculated from PGD T72 as follows: PGD T72 = {0, 1, & 2} will be coded to PGD3 T72 = 0 and PGD T72 = {3} will be coded to PGD3 T72 = 1.

If a subject dies prior to the T72 measurement, they will be coded to PGD3 T72 = 1. If the T72 value is missing (not collected, not reported, not properly documented), then PGD3 will be coded to PGD3 T72 = 1 for the primary safety analysis.

The primary analysis will be descriptive as the proportion of subjects with PGD3 T72 by Transplant Group (EVLP and Control). A one-sided 95% CI will be calculated around the proportion. A two-sided 95% CI will also be calculated.

There will be no inferential testing conducted.

9.5.2 30-Day Mortality

Subject survival, measured as living or dead, will be recorded for Day 30, Month 6, and Year 1 post-transplant (see Section 6.8). The primary endpoint of 30-Day mortality will be derived as follows: Living at Day 30 will be coded to 0, and Dead before or on Day 30 will be coded to 1. If the vital status of the subject is missing (not collected, not reported, not properly documented), then 30-Day Mortality will remain missing for the primary analysis.

The primary analysis will be descriptive as the proportion of subjects with 30-Day mortality, summarized by Transplant Group. A one-sided 95% CI will be calculated around the proportion. A two-sided 95% CI will also be calculated.

Other supportive endpoints may be provided to supplement the primary analysis, including, but not limited to, median number of days to death and primary cause of death.

Results may be displayed graphically. There will be no inferential testing conducted.

9.6 Secondary Endpoint(s)

Secondary endpoints included the following:

- PGD Score (Grades 0-3) measured at 0, 24, 48 and 72 hours post-transplant
- Time to first extubation
- ICU LOS, measured as total number of days post-transplant in the ICU until first hospital discharge, inclusive of ICU readmissions during that hospitalization
- Hospital LOS, measured as total number of days in the hospital prior to discharge post-transplant
- TPT defined as the elapsed time between first allograft lung removal from cold storage time prior to transplant in the recipient and the donor aortic cross clamp time at procurement
- Assessment of the overall safety of TES lung transplants during subject's participation in the study, as reported through AEs (refer to Section 9.9.1 below).

All secondary endpoints will be presented using descriptive statistics along with two sided 95% CIs by Transplant Group. Other supportive endpoints may be provided to supplement the secondary analyses.

Results may be displayed graphically. There will be no inferential testing conducted.

9.7 Exploratory Endpoints

Other safety endpoints that will be analyzed include:

- Initial ICU PaO₂: Evaluated as first PaO₂ measured after ICU admission.
- FEV₁: Evaluated at time of discharge, 30 days, 90 days, 6 months and 1 year post-transplant. FEV₁ is measured as a percentage of predicted normal value.

- Oxygen Requirement at Rest: Evaluated at time of discharge, 30 days, 90 days, 6 months and 1 year post-transplant.
- Subject Survival: Evaluated at 90 days, 6 months and 1 year post-transplant. Measured as living or dead. If the subject expires within first year post-transplant, the date of death and primary cause of death will be collected.
- Graft Survival: Evaluated at 30 days, 90 days, 6 months and 1 year post-transplant. Measured as functioning or failed. If the graft fails within first year post-transplant, the date of graft failure will be collected.
- Primary Cause of Graft Failure: Evaluated at 30 days, 90 days, 6 months and 1 year post-transplant on all failed grafts.
- Bronchiolitis Obliterans Syndrome (BOS) Grade: Evaluated at 90 days 6 months and 1 year post-transplant.
- Number of Re-hospitalizations after Transplant: Evaluated at 6 months and 1 year post-transplant. Measured as total number of hospitalizations since initial discharge.
- Physical Capacity: Evaluated at 90 days, 6 months and 1 year post-transplant.
- Working for Income after Transplant: Evaluated at 90 days, 6 months and 1 year post-transplant.

All exploratory endpoints will be presented using descriptive statistics along with two sided 95% CIs by Transplant Group. Other supportive endpoints may be included as needed.

Results may be displayed graphically. There will be no inferential testing conducted.

In addition, supplemental analyses comparing the EVLP group and the Control Group may be performed to provide context of results observed in this study (details will be described in the SAP).

9.8 Missing Data

The primary safety analysis is the proportion of subjects with a PDG3 at T72. PGD is measured in all lung transplant subjects at specific intervals following transplantation, including 24, 48, and 72 hrs. Protocol training will focus on the importance of obtaining PGD at T72 in every study subject.

If a subject dies prior to the T72 measurement, they will be counted as a “failure” and classified as PGD3 for the primary safety analysis. If the T72 value is missing (not collected, not reported, not properly documented), then PGD3 will be assumed (“failure”), and they will be classified as such for the primary safety analysis.

9.9 Other Safety Assessments

9.9.1 Adverse Events

Adverse events will be recorded throughout the study, but only those occurring within the first 90 days post-transplant will be reported to the Sponsor, unless they are considered SAEs. All SAEs will be recorded and reported to the Sponsor throughout the Analysis Phase. Adverse events and medical conditions will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) coding dictionary. Intervention-emergent AEs will be defined as those events, which are newly occurring or worsening from Baseline (Day 0). In all cases only intervention-emergent AEs will be summarized.

Intervention-emergent AEs will be summarized by Transplant Group as the number and percentage of subjects having any intervention-emergent AE, having an AE in each body system, and having each individual AE. Adverse events will further be categorized by severity, relationship to study intervention, and action taken. Other information collected will be listed, as appropriate.

9.9.2 Physical Examination and Vital Signs

Physical examinations will be performed as described in Section 6.5, and vital signs will be recorded as described in Section 6.6.

Vital sign data will be listed for each subject. Clinically significant values will be flagged.

Any clinically significant abnormality prior to enrollment is to be recorded as medical history, and tabulation of those events will occur within medical history summaries. Any clinically significant abnormal change from baseline post-transplant is to be recorded as an AE and tabulation of those events will occur within AE summaries.

9.9.3 Laboratory Data

Laboratory data will be collected as described in Section 6.12.

Laboratory data will be listed for each subject. Laboratory data will be summarized for overall and Transplant Group by presenting the proportions of subjects with clinically significant abnormalities; shift tables, baseline to most extreme post-baseline value, using normal ranges; summary statistics of raw data and change from baseline values (means, medians, standard deviations, ranges), as appropriate.

9.10 Interim Analysis

There are no formal interim analyses planned for this study. However, as described in Section 6.13.8, the Sponsor will temporarily pause the study at EVLP subject #20 to allow time for the FDA to review safety data on the first 10 subjects enrolled who reach 90 days post-transplant.

Data from the first 10 subjects through their 90-day visit will be submitted to FDA as an IDE Supplement for review. Submitted data will include subject and graft survival, aggregate PGD scores, FEV₁, oxygen required at rest, and BOS Grade.

10 ADMINISTRATIVE CONSIDERATIONS

10.1 Investigators and Study Administrative Structure

The term “Investigator” as used in this protocol and in the CRFs refers to the Principal Investigator at each study site, or another staff member listed as a subinvestigator on the study site delegation log. The site Principal Investigator is ultimately responsible for the conduct of all aspects of the study at that site. The site Principal Investigator is also responsible for ensuring that all site staff working on the study are appropriately trained and supervised; staff training and delegation of responsibilities should be documented in the files.

Prior to site activation, the Investigator must read, understand, and sign the Investigator Agreement in the protocol. The Investigator Agreement documents agreement to conduct the study according to the protocol, International Conference on Harmonization / Good Clinical Practice (ICH/GCP), and Code of Federal Regulations (CFR). Additional requirements must be met by the Investigator and institution, as described below.

10.2 Institutional Review Board (IRB) or Independent Ethics Committee (IEC) Approval

The protocol, informed consent form(s), recruitment materials, and all subject materials will be submitted to each participating study site’s local IRB for review and approval. In addition, the Sponsor will operate its EVLP site under the review of an independent third party IRB (Appendix 2).

Approval of both the protocol and the ICF must be obtained before any subject is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented in the study.

Each IRB must operate in compliance with 21 CFR 56, including being registered with the FDA. The IRB is to provide some form of documentation of compliance via a Statement of Compliance and/or a Federal Wide Assurance Number. If any member of the research team is a member of the IRB, written documentation is required that he/she did not vote or participate in the approval process

10.3 Ethical Conduct of the Study

The Investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, as drafted by the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6; 62 Federal Regulations 25691 (1997).

10.4 Authorization and Consent

10.4.1 Donor Next of Kin Authorization

Authorization for organ donation will be obtained by the federally-designated OPO in accordance with the requirements of the Uniform Anatomical Gift Act in the state of the donor hospital and in compliance with OPTN Policy 2. The OPO also must comply with its internal policy regarding donor (or donor family) authorization for additional lung assessment using EVLP.

10.4.2 Subject (Recipient) Consent

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout study participation. Subjects listed for single or bilateral lung transplantation with UNOS may be offered the option to participate in this study. A legal authorized representative may be used to consent for a subject that is unable to sign/ consent for themselves. The Study Center must adhere to IRB and State law requirements as to who is an acceptable Legal Authorized Representative. The Study Center will also obtain documentation that person signing consent is authorized to do so.

Extensive discussion of risks and possible benefits of study participation will be provided to subjects and their families. A consent form describing in detail the study procedures and risks will be given to the subject. Each participating Study Center will be provided with a model informed consent form (ICF). Each institution may revise or add information to comply with institution consent templates, but may not remove procedural or risk content from the model form. Consent forms will be IRB-approved, and the subject is required to read and review the document or have the document read to him or her. The Investigator or designee will explain the research study to the subject and answer any questions that may arise.

The subject will sign the ICF prior to any study-related assessments or procedures. Study-related assessments are defined as any study activities in which data are specifically collected from a potential subject that could later be used for analysis under this protocol. Pre-screening of potential subjects based on previously known medical histories or other information is not considered a study-related assessment. Study-related procedures are any medical procedures performed to screen or prepare a subject for lung transplantation under this protocol. Screening or other procedures performed for the purposes of general lung transplantation suitability or compatibility are not considered study-related procedures.

If consent is obtained the same day that the subject begins study-related assessments or procedures, the subject's medical record should document that consent was obtained prior to these activities. Subjects will be given the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The consent may be signed during an extended period of time prior to the transplant. This extended period timeframe shall be determined by the local IRB in reference to the timeframe before a subject is re-consenting versus reaffirming the original consent. The consent obtained prior to the day of transplant shall be reconfirmed at day of transplant by subject or subject's legal representative. This shall be documented in the source

document by the site staff and will not involve the resigning of the consent unless requested by the IRB. A subject may withdraw consent at any time throughout the course of the study.

A copy of the signed ICF will be given to subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their clinical care will not be adversely affected if they decline to participate in this study.

The consent process will be documented in the clinical or research record.

A subject is only considered enrolled in the study when he/she has signed the ICF and, at an approved Study Center, is transplanted with a donor lung.

10.5 Subject Confidentiality

Subject confidentiality is strictly held in trust by the Investigators, study staff, and the Sponsor and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to any study information relating to subjects.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the Sponsor.

The study monitor or other authorized representatives of the Sponsor may inspect all study documents and records required to be maintained by the Investigator, including but not limited to, medical records (office, clinic, or hospital) for the study subjects. The clinical study site will permit access to such records.

Donor records will be redacted for any identifying donor information and instead will only use a pre-assigned UNOS Donor ID# to connect donor to recipient. The Sponsor will use the UNOS ID# to request baseline donor, 6- and 12-month recipient post-transplant reports from OPTN/SRTR to maintain recipient privacy.

10.6 Study Monitoring

The study will be monitored according to the guidelines summarized below. The Sponsor may choose to perform random inspections throughout the study as an element of quality assurance. Investigators shall allow auditing of their clinical investigation procedures and data.

Data will be collected from EVLP lung transplant recipients and the non-EVLP lung transplant recipients included in the Control Cohort. These data will include basic lung transplant information along with primary and secondary endpoints as listed in this protocol.

The monitor(s) will visit the site soon after the first subject is enrolled to assist in any questions the site might have concerning the study or CRF completion. Monitoring visits will occur based on number of subjects enrolled at a Study Center, duration of the study, level of compliance or a significant change in compliance, or a change in Study Center staff.

It is the responsibility of the study Sponsor to ensure that proper monitoring of this investigation is conducted. Appropriately trained personnel, appointed by the Sponsor, will complete any monitoring that is performed. The monitoring will be the responsibility of Sponsor study personnel or as designated. Monitors will ensure that the investigation is conducted in accordance with:

- The signed Investigator's Agreement
- The study protocol
- Appropriate laws and regulations
- Any approval conditions imposed by reviewing IRB and/or regulatory agencies

10.6.1 Access to Centers

The Investigator is required to permit monitoring and study record access in order for the monitors to perform their responsibility. The Investigator(s), co-investigator(s), and other study staff must be available during onsite visits by the study monitor. The Sponsor may choose to perform random inspections throughout the study as an element of QA. Investigators shall allow auditing of their clinical investigation procedures.

10.6.2 Summary of Monitoring Procedures & Responsibilities

It is the responsibility of the study Sponsor to ensure that proper monitoring of the investigations is conducted and that IRB review and approval of the investigation is obtained. Monitoring visits will occur based on transplant volume at the center or at least annually. Trained personnel or delegates appointed by the study Sponsor will perform study monitoring in order to ensure that the investigation is conducted, recorded, and reported in accordance with: the aforementioned agreements, plans, and regulations. The Sponsor (or designee) must be allowed access to the subjects' files as per the informed consent at the investigator's site when so requested.

10.6.3 Routine Monitoring

The following factors will be taken into account when determining the frequency of the monitoring visits: subject accrual rate at each center, total number of subjects enrolled at each center, protocol violations, risk-based assessments for the primary and secondary endpoints and study protocol compliance at each center. Monitors will require direct access to subject medical records pertinent to the study (and study inclusion criteria), study management documents, regulatory documents and Subject Informed Consent documents, as well as other potential applicable records not listed here.

Monitors may ensure the Study Investigators maintain staff and facilities to conduct the clinical investigation safely and effectively. Monitors may conduct the following monitoring activities throughout the study:

- Verification that the current IRB-approved informed consent was signed and dated by each subject.

- Verification of documentation in the subject's record that informed consent was signed prior to participating in any study-related assessments or procedures and that a copy of the signed and dated consent form was provided to the subject.
- Source documentation verification by reviewing the CRFs against source documentation for accuracy and completeness of information.
- Verification that subjects and donor lung met study enrollment criteria.
- Confirmation that the study is being conducted according to the study protocol and applicable regulations.
- Verification that study deviations are documented and reported.
- Verification that the procedures for recording and reporting adverse events to the Sponsor are followed.
- Ensuring proper error correction.
- Verification of training documentation of all study personnel participating in study related activities.
- Reviewing all correspondence and regulatory documents, including confirmation of IRB-approved study protocol or amendments.
- Resolution of outstanding issues and completion of assigned tasks will be documented by the monitors.

10.7 Case Report Forms and Study Records

Case report forms (CRFs) are used to transmit the information collected in the performance of this study to the Sponsor (or its designee) and to governmental agencies. CRFs must be completed for each subject enrolled. This study may use paper or electronic CRFs. All paper CRFs must be legible and completed in black ball point ink. Data entries for paper CRFs may be corrected only by drawing a single line through the incorrect entry and writing in the correct entry. Incorrect entries are not to be obliterated by blacking out or use of correction fluid or erasers. All corrections must be initialed and dated. Systems for electronic CRFs will be properly designed, validated, and implemented prior to use.

A selected number of donor and subject variables are required data collected by the transplant center via the OPTN and UNOS. A data sharing agreement may be developed with the OPTN and/or UNOS for electronic transfer of those data elements directly to the Sponsor for each enrolled subject/donor.

CRF general guidelines will be used to complete all CRFs. Subjects will be given identification numbers so that their identity cannot be determined by the Sponsor and donor data will be identified for use by their UNOS ID. The research coordinator will keep a Master Subject List that will equate the identifiers to the actual donors and subjects that are a part of this study. This list will be kept in a secure location and will not be part of the Sponsor's regulatory documents. This information will only be made available to the Sponsor and/or their designee for routine verification of CRFs and also to the FDA if required for regulatory affairs.

The Investigator will review the CRFs for completeness and accuracy and sign and date the forms where indicated. Questions regarding missing or uninterpretable data will be communicated to the Principal Investigator/study coordinator for resolution and entered into a CRF correction form. The CRF correction form(s), after resolution of discrepancies, will be signed by the Principal Investigator and the revised data entered into a database. All records which support CRFs of this study or follow-up must be retained in the files of the Principal Investigator for a minimum of two years or until notification by the Sponsor, whichever is earlier.

10.8 Independent Medical Monitor

In addition to the Principal Investigator's responsibility for oversight, study oversight will be under the direction of Independent Medical Monitors. The first Independent Medical Monitor is Dr. Martin Zamora, lung transplant pulmonologist at the University of Colorado, who has direct experience taking care of post-EVLP lung recipients. He is also the author of the medical journal article Medical Complications of Lung Transplantation.[Lyu 2009] Dr. Zamora is considered an expert in this area and well qualified to make a determination of unreasonable risk in relation to lung transplantation and EVLP.

The second Independent Medical Monitor is Dr. Michael Mulligan, cardiothoracic surgeon at the University of Washington. Dr. Mulligan is clinically active with lung transplantation and lung volume reduction surgery.

The Independent Medical Monitors are independent of the study and will be available in real time to review and recommend appropriate action regarding AEs and other safety issues. Their contact information is located in [Appendix 2](#).

10.9 Protocol Violations/Deviations

A study deviation is defined as an event where the Study Investigator or site personnel did not conduct the study according to the study protocol, applicable laws or regulations or the Investigator agreement.

Prior approval by the Sponsor is expected in those situations in which the Investigator anticipates, contemplates, or makes a conscious decision to depart from procedures specified in the study protocol, except when necessary to protect the life or physical well-being of a subject in an emergency. Prior approval is not expected in situations where unforeseen circumstances are beyond the Investigator's control (e.g., inadvertent mistakes, equipment failure, subject unable to do required testing due to illness).

All study deviations will be recorded on a Protocol Deviations form, including a description of the deviation, justification for the deviation, and corrective action for the deviation. Study deviations may be discovered through a variety of sources, such as during the CRF review, telephone conversations, and site monitoring. Study deviations should be reported to the applicable IRB in accordance with IRB policies and/or local laws. When relevant, ethics committees, competent authorities or the appropriate regulatory bodies should be informed. The

Sponsor will review all deviations from the study protocol. Corrective action and preventative action will be performed after protocol deviations are discovered during monitoring visits.

Deviations will be reported to the CRO and the Sponsor regardless of whether medically justifiable, pre-approved by the Sponsor or taken to protect the subject in an emergency. Study deviations should be reported as soon as possible upon notification of the deviation. Investigators must notify the Sponsor and reviewing IRB within five working days. Examples of deviations from the study protocol may include:

- No informed consent prior to transplant within a study subject.
- Incorrect version of consent provided to subject.
- Enrollment of subject prior to IRB approval.
- Enrollment of subject during an IRB approval lapse.
- Subject did not meet inclusion/exclusion criteria.
- Required testing and/or measurements not performed.
- Source data permanently missing.
- Adverse event not reported by Investigator in the required regulatory time frame.
- Unauthorized physician performed study procedures (e.g. implanting physician was not an Investigator).

10.10 Access to Source Documentation

Each participating site will maintain appropriate medical and research records for this study, in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. Each site will permit authorized representatives of the Sponsor and regulatory agencies to examine (and when required by applicable law, to copy) research records for the purposes of QA reviews, audits, and evaluation of the study safety, progress and data validity.

Procedures and data collection will be documented utilizing site SOP or will be captured in site generated worksheets and data collection forms considered source documentation. Source documentation is defined as original documents, data, and records (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical trial).

Source document worksheets may be used to facilitate data collection. The center may create source worksheets or use ones provided by the Sponsor or Sponsor's representative. The worksheets may be used in conjunction with other center documentation. Source worksheets shall be provided for the EVLP data collection and post-EVLP assessments. During the study, it may be necessary to provide additional worksheets to the Study Center. This may occur without a formal process or written notification.

During the study, the use of the CRF as the original entry or source may occur if approved by the Sponsor. This agreement shall be documented in writing and filed with the protocol in the Investigator study file. The original data will be collected onto source documents (either the subject's chart or a separate research subject chart that is kept in the research department) by the research site coordinator. This information will then be transcribed to the CRFs provided by the Sponsor for use in this study.

10.11 Data Generation and Analysis

The Investigators are responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data (Section 10.10). The Investigators will maintain adequate case histories of study subjects, including accurate CRFs, (Section 10.7) and source documentation.

All donor identifying data will be redacted for the Sponsor and participating Investigators according to OPTN Policy 16 and only unique UNOS ID# shall be used to connect the donor to the recipient (subject).

10.11.1 Data Management Responsibilities

Data collection and accurate documentation are the responsibility of the study staff under the supervision of the Investigator. All source documents and laboratory reports must be reviewed by the study team and data entry staff who will ensure that they are accurate and complete. Unanticipated problems and AEs must be reviewed by the Investigator or designee.

10.11.2 Data Capture Methods

10.11.2.1 Donor Data

Donor lung data will be provided by the OPO and/or Study Centers through secure-access (read-only) online database ('DonorNet') and/or through a paper-based medical record that accompanies the lung, or copies of donor information forwarded via electronic mail or facsimile.

10.11.2.2 EVLP Data

EVLP procedure data will be recorded by the Sponsor during the clinical EVLP procedure. Data will be collected electronically in a secure online medical database (CMX).

10.11.2.3 Subject (Recipient Data)

Subject data for the Analysis Phase, post-lung transplantation, will be collected by participating study site using the Sponsor's CRF. Long-term follow-up data (Year 2 through Year 5) will be collected via the OPTN/UNOS database (TIEDI®) recipient follow-up forms.

10.12 Retention of Data

10.12.1 Retention of Study Documents

Study documents should be retained for a minimum of two years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least two years have elapsed since the formal discontinuation of clinical development of the investigational device. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the Sponsor, if applicable. It is the responsibility of the Sponsor to inform the Investigator when these documents no longer need to be retained.

10.12.2 Future Use of Stored Specimens and Other Identifiable Data

Donor lung biopsies and EVLP perfusate samples will be obtained and stored for potential future use. Donor lung biopsies and perfusate samples will be identified only by UNOS ID#.

Donor next of kin will provide authorization for research use of the donor lung tissue and samples taken during the EVLP process. Additionally, subject consent includes a statement that pre-transplant donor lung “biopsies (about the size of your small finger) may be taken using a surgical stapler,” and that “Pieces of lungs may also be removed with staplers from standard organ donors as well.”

10.13 Financial Disclosure

Per 21 CFR 54, all Investigators are required to complete a Financial Disclosure form prior to site activation, attesting to the fact that they are unaware of any financial conflicts of interest, or disclosing potential conflicts, including payments, proprietary interests and/or equity interests. Completed forms will be maintained in the Trial Master File.

10.14 Publication and Disclosure Policy

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a clinical trials registration policy as a condition for publication. The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or concurrent comparison or control groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Medical interventions include drugs, surgical procedures, devices, behavioral treatments, process-of-care changes, and the like. Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and AEs. The ICMJE policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. The Sponsor will register this study in ClinicalTrials.gov within 30 days of study start, so the research results may be considered for publication in ICMJE member journals. The ICMJE does not review specific studies to determine whether registration is necessary; instead, the committee recommends that researchers who have questions about the need to register err on the side of registration or consult the editorial office of the journal in which they wish to publish.

U.S. Public Law 110-85 (Food and Drug Administration Amendments Act of 2007 or FDAAA), Title VIII, Section 801 mandates that a "responsible party" (i.e., the Sponsor or designated Principal Investigator) register and report results of certain "applicable clinical trials:"

The Sponsor will develop subsequent publication policies prior to locking of the study data.

10.15 Study Closure

Study closure is defined as a specific date that is determined by study completion and/ or regulatory requirements have been satisfied per the study protocol and/or by decision of the Sponsor or FDA. Study closure visits will be conducted at all enrolling Study Centers in order to review record retention requirements with site personnel. A telephone contact may take the place of a study closure visit if appropriate (e.g., low subject enrollment, recent monitoring visit). Monitoring visits will be conducted by trained monitors and designees. A detailed Monitoring Plan will be developed identifying the frequency of monitoring and training requirements of the monitors.

11 REFERENCE LIST

Aigner, C., et al. Clinical ex vivo lung perfusion – Pushing the limits. *Am J Transplant* 2012; 12: 1839-1847.

Avlonitis, VS, et al. Early Hemodynamic Injury During Donor Brain Death Determines the Severity of Primary Graft Dysfunction after Lung Transplantation. *Am J Transplant* 2007;7, 83-90.

Christie, J.D., et al. Report of the ISHLT Working Group on Primary Lung Graft Dysfunction part II: definition. A consensus statement of the International Society for Heart and Lung Transplantation. *J Heart Lung Transplant* 2005;24, 1454-1459.

Chris Jaynes, CEO Perfusix Canada.

Cypel M, Yeung J, Hirayama S, Keshavjee SK et al. Technique for Prolonged Normothermic Ex Vivo Lung Perfusion. *J Heart Lung Transplant* 2008a;27(12):1319-1325.

Cypel M, Hirayama S, Rubacha M, Keshavjee SK et al. Ex-Vivo Normothermic Lung Perfusion (EVLP) Interrupts Ischemic Injury and Restores Cellular Metabolism. *J Heart Lung Transplant* 2008b;27(2S):S199-200.

Cypel M, Rubacha M, Yeung J, et. al. Normothermic Ex Vivo Perfusion Prevents Lung Injury Compared to Extended Cold Preservation for Transplantation. *Am J Transplantation* 2009;9:1-8.

Cypel M, Yeung J, Yeung JC, Keshavjee SK et al. Normothermic Ex Vivo Lung Perfusion in Clinical Lung Transplantation. *N Engl J Med* 2011a; 364:1431-40.

Cypel M, Sato M, Yeung, JC, Keshavjee SK et al. One Year Survival and Functional Status of Patients from the human Ex Vivo Lung Perfusion (HELP) Trial. *J Heart Lung Transplant* 2011b;30(4S):S8.

Cypel M, Yeung JC, Keshavjee SK et al. Experience with the first 50 ex vivo lung perfusions in clinical transplantation. *J Thorac Cardiovasc Surg* 2012; 144(5): 1200-07.

Cypel M, Aigner C, Keshavjee SK et al. Three Center Experience with Clinical Normothermic Ex Vivo Lung Perfusion. *J Heart Lung Transplant* 2013; 32(4S):S16.

Diamond JM, et al. Clinical risk factors for primary graft dysfunction after lung transplantation. *Am J Respir Crit Care Med*. 2013 Mar 1;187(5):527-34.

Fisher, A.J, et al. Elevated levels of interleukin-8 in donor lungs is associated with early graft failure after lung transplantation. *Am J Respir Crit Care Med* 2001;163:259-265.

Hayek D, Veremakis C, O'Brein JA. Time-dependent characteristics of hemodynamic instability in brain dead organ donors. *Crit Care Med* 1990;18:S204.

Lyu DM, Zamora MR. Medical Complications of Lung Transplantation. *Proc Am Thorac Soc* 2009;6:101-107.

Machuca, TN and Cypel M. Ex vivo lung perfusion. *J Thorac Dis* 2014;6(8):1054–1062.

Munshi L, Keshavjee SK, Cypel M. Donor management and lung preservation for lung transplantation. *Lancet (online)* 2013; 1:318-328.

Organ Procurement & Transplantation Network. National Data 2015. US Department of Health and Human Services. <https://optn.transplant.hrsa.gov/data/view-data-reports/national-data/#> [Accessed 16Mar2016].

Power BM, Van Heerden PV. The physiological changes associated with brain death: current concepts and implications for the treatment of the brain dead organ donor. *Anaesth Intens Care* 1995;23:26-36.

Scientific Registry of Transplant Recipients (SRTR) Database. 2012 Annual Data Report. http://srtr.transplant.hrsa.gov/annual_reports/2012/

Shaf Keshavjee, MD, Surgeon in Chief at University Health Network in Toronto, Canada, personal communication.

Steen S, et al. Transplantation of lungs from a non-heart-beating donor. *Lancet* 2001;357:825-829.

Suzuki Y, Cantu E, Christie JD. Primary Graft Dysfunction *Semin Respir Crit Care Med* 2013;34:305-19.

Tikkanen JM, Cypel M, Keshavjee SK et al. Long-Term Results of Transplantation of Normothermic Ex-Vivo Perfused Donor Lungs. *J Heart Lung Transplant* 2012; 32(4S):S146-7.

Tiwari JL, et al. Ex-vivo lung perfusion does not have a negative effect on transplant rates at centers without this technology in a single organ procurement organization. *J Heart Lung Transplant* 2014;33(4S):S269.

Tropmann C. Management of the organ donor. In *Intensive Care Medicine*, sixth edition Irwin RS and Rippe JM editors. Wolters Kluwer Health/Lippincott Williams & Wilkins. 2008:2091-2115.

Ware, L.B, et al. Assessment of lungs rejected for transplantation and implications for donor selection *Lancet* 2002;360:619-620.

XVIVO Perfusion Inc. Sponsor Executive Summary 2014. <http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/MedicalDevices/MedicalDevicesAdvisoryCommittee/Gastroenterology-UrologyDevicesPanel/UCM389396.pdf> Last accessed 03/05/16.

Appendix 1 Schedule of Events

Table 3. Schedule of Events Within 1 Year Post-intervention

Evaluation	Screening Days -365 to 0	Baseline; Day 0	Post-Intervention Period							Follow-up
			Visit 1 (ICU); 24hr ± 4	Visit 2 (ICU); 48hr ± 8	Visit 3 (ICU); 72hr ± 12	Visit 4; Day 30 ± 5	Visit 5; Day 90 ± 10	Visit 6; 6 mon ± 2wks	Visit 7; 1 year ± 4 wks	Premature Discont'n
Signed Informed Consent ^b	X	X	-	-	-	-	-	-	-	-
I/E Criteria	X	X ^a	-	-	-	-	-	-	-	-
Print conmeds source		X ^a	-	-	-	X	-	-	X	-
Donor data collection ^c (Sponsor responsibility)	-	X	-	-	-	-	-	-	-	-
EVLP data collection ^d (Sponsor responsibility)	-	X	-	-	-	-	-	-	-	-
Demographics and Baseline Disease Characteristics	X	X ^a	-	-	-	-	-	-	-	-
Medical History	X	X ^a	-	-	-	-	-	-	-	-
Study intervention (lung transplant)	-	X	-	-	-	-	-	-	-	-
Post-transplant Assessments (Study Investigator)										
Vital sign assessments	X	X	X	X	X	X	X	X	X	X
Physical Examination	-	X ^a	-	-	-	X	-	X	-	-
PGD Score (Section 6.7)	-	X	X	X	X	-	-	-	-	-
PaO ₂ and FiO ₂	-	X	-	-	-	-	-	-	-	-
Pulmonary function tests						X ^c	X	X	X	(X)
FEV ₁	-	-	-	-	-	X ^c	X	X	X	(X)
Oxygen at rest	-	-	-	-	-	X ^c	X	X	X	(X)
BOS Grade	-	-	-	-	-	-	X	X	X	(X)
ICU discharge	-	-	-	-	(X)	(X)	-	-	-	(X)
Extubation date/time					(X)	(X)				
Hospital discharge	-	-	-	-	-	(X)	-	-	-	(X)
Hospital readmission	-	-	-	-	-	(X)	(X)	(X)	(X)	(X)
Physical capacity	-	-	-	-	-	-	X	X	X	(X)
Work status	-	-	-	-	-	-	X	X	X	(X)
Adverse event assessment	-	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)

Graft Survival Assessment	-	-	-	-	-	X	X	X	X	(X)
Survival Assessment	-	-	-	-	-	X	X	X	X	(X)

Abbreviations: BOS=bronchiolitis obliterans syndrome; conmed=concomitant mediations; discont'n=discontinuation; FEV₁=forced expiratory volume in one minute; hrs=hours; ICU=intensive care unit; I/E=inclusion/exclusion; mon=month; PaO₂=partial pressure of oxygen in arterial blood; primary graft dysfunction; wks=weeks

Note(s): (x)=indicates “if applicable or as appropriate”

- ^a Data at Baseline are to be collected within 24 hrs prior to lung transplant.
- ^b Informed consent must be provided by the subject up to one year in advance and confirmed up to 24 hrs in advance of the lung transplant if required (Section 10.4.1).
- ^c Collection of donor demographics and suitability data. Confirm donor lung inclusion/exclusion criteria (Section 5.2.1 and Section 5.2.2).
- ^d Collect EVLP case data to determine transplant suitability and lung inclusion/exclusion criteria (Section 5.2.3).
- ^e At the time of discharge.

Table 4. Schedule of Events: Long-term Follow-up

Evaluation	Post-Intervention Period				Follow-up
	Visit 8; 2 year ± 4 wks	Visit 9; 3 year ± 4 wks	Visit 10; 4 year ± 4 wks	Visit 11; 5 year ± 4 wks	Premature Discont'n
FEV ₁	X	X	X	X	(X)
Oxygen at rest	X	X	X	X	(X)
BOS Grade	X	X	X	X	(X)
Physical capacity	X	X	X	X	(X)
Work status	X	X	X	X	(X)
Graft Survival Assessment	X	X	X	X	(X)
Survival Assessment	X	X	X	X	(X)

Abbreviations: BOS=bronchiolitis obliterans syndrome; discont'n=discontinuation; FEV₁=forced expiratory volume in one minute; PaO₂=partial pressure of oxygen in arterial blood; primary graft dysfunction; wks=weeks

Note(s): (x)=indicates “if applicable or as appropriate”

Appendix 2 Key Study Personnel Contacts

Expert Medical Consultants:	<p>Shaf Keshavjee, MD MSc FRCSC FACS Chief Scientific and Medical Officer, PERFUSIX, Inc. Surgeon in Chief, Sprott Department of Surgery Director, Toronto Lung Transplant Program Director, Latner Thoracic Research Laboratories 190 Elizabeth St., RFE 1-408, Toronto, ON, M5G 2C4 Phone: 416.340.3863 Fax: 416.340.3185 Email(s): shaf.keshavjee@perfusix.com shaf.keshavjee@uhn.ca</p> <p>Marcelo Cypel MD, MSc Director of Clinical & Research Programs, PERFUSIX, Inc. Canada Research Chair in Lung Transplantation (Tier 2) Thoracic Surgeon, University Health Network Assistant Professor of Surgery, Division of Thoracic Surgery 200 Elizabeth St. 9N969 Toronto ON M5G2C4 Canada Phone: 416.340.4800 ext 5156 Fax: 416.340.3478 Email(s): marcelo.cypel@perfusix.com marcelo.cypel@uhn.ca</p> <p>Thomas K Waddell, MD, MSc, PhD Director of Advanced Research, PERFUSIX, Inc. Chair, Division of Thoracic Surgery, University of Toronto Head, Division of Thoracic Surgery, University Health Network Senior Scientist, Toronto General Research Institute, UHN 200 Elizabeth Street, 9N-949 Toronto, ON M5G 2C4 Canada Phone: 416.340.3432 Fax: 416.340.4556 Email(s): tom.waddell@perfusix.com Tom.waddell@uhn.ca</p>
Independent Medical Monitors:	<p>Martin Zamora, MD Transplant Pulmonologist Medical Director, Lung Transplant Program University of Colorado Anschutz Medical Campus Research Bldg 2, 9th Floor 12700 E 19th Ave. Aurora, CO 80045</p>

	<p>Phone: 303.724.6822 Email: marty.zamora@ucdenver.edu</p> <p>Michael S. Mulligan, MD Cardiothoracic Surgeon Director, Lung Transplant Program Director, Advanced Lung Disease Surgery Program University of Washington Medical Services 1959 NE Pacific Street Seattle, WA 98195 Phone: 206.598.4477 Email: msmmd@u.washington.edu</p>
Clinical Research Organization:	<p>CTI Clinical Trial and Consulting Services 10123 Alliance Road Cincinnati, OH 45242 Phone: 513.598.9290</p>
Sponsor IRB	<p>Sterling IRB 6300 Powers Ferry Road Suite 600-351 Atlanta, GA 30339 Phone: 1.888.636.1062</p>

Appendix 3 Risk and Risk Mitigation of EVLP

Risk	Risk Mitigation	Document Reference
Lung exposed to potential infectious material	<ol style="list-style-type: none"> 1) Add broad spectrum antibiotic to circulating perfusate (Imipenem, 500mg) 2) Strict aseptic technique 3) Pre-sterilized, single-use disposables wherever possible 4) OR qualified procedure rooms with appropriate air handling and HEPA filtration 	<ol style="list-style-type: none"> 1) TES Instruction for Use manual 2) PX-1 Facility engineering drawings 3) Specialist Training Core Curriculum 4) Clinical Protocol
Hardware generates bad/incorrect data	<ol style="list-style-type: none"> 1) Use calibrated equipment 2) Toronto consulting surgeons and EVLP Specialists trained to identify incorrect data ranges for each parameter 	<ol style="list-style-type: none"> 1) TES Instruction for Use manual 2) Specialist Training Core Curriculum
Multiple donor lungs become switched/ mixed up at facility	<ol style="list-style-type: none"> 1) Only 1 procedure per room is allowed 2) Clearly identify all organ packaging and generated documentation using standard donor UNOS ID # 3) Repackage lungs according to OPTN/UNOS guidelines 	<ol style="list-style-type: none"> 1) OPTN Policy 16: Organ and Vessel Packaging, Labeling, Shipping, and Storage 2) Clinical Protocol
Over-ventilation of lung beyond safe volume	<ol style="list-style-type: none"> 1) Use a volume-controlled ventilator 2) Use strict warming and protective ventilation volume strategies 3) Monitor EVLP lung over time to observe function 	<ol style="list-style-type: none"> 1) TES Instruction for Use manual 2) Specialist Training Core Curriculum
Donor lung procured incorrectly damaging its anatomy	<ol style="list-style-type: none"> 1) Study Site surgeon will retrieve lung(s) whenever possible. 2) Lungs will be procured with the expectation that they will go back to the study site surgeon's center/recipient. 3) Toronto Procurement Protocol is available for review and implementation at donor sites. 	<ol style="list-style-type: none"> 1) Clinical Protocol
Donor lung lost during transportation/ logistical mishap, exceeding its safe CIT storage period	<ol style="list-style-type: none"> 1) This is a risk with or without EVLP. We will mitigate this risk by using the same transportation methods (medical charter company/ medical courier) used by OPOs currently to transport organs to transplant centers 	<ol style="list-style-type: none"> 1) Clinical Protocol

Abbreviations: CIT=cold ischemic time; HEPA= ; OPTN=Organ Procurement and Transplantation Network; UNOS ID=United Network for Organ Sharing Identification

Appendix 4 System Components of the Toronto EVLP System

A schematic of the TES was shown in Figure 3. There are two classifications of system components: Non-Fluid Path Hardware and Single-Use Fluid Path Disposables.

Non-Fluid Path Hardware

None of the following perfusion machine hardware used in the TES touches the fluid path. Therefore, each can be reused with minimal risk of contamination.

1. Centrifugal Pump

The benefit of using a centrifugal pump over a roller pump for *ex vivo* lung circulatory support is profound. Unlike a roller pump that pumps at a continuous pressure, a centrifugal pump will automatically back down its pressure when the internal resistance of the lungs increases. This is critical when warming up a hypothermically-preserved lung which is very cold (4°C) and thus, very stiff. A centrifugal pump will allow more flow and pressure to go through the lung only as its vascular resistance decreases during the warm-up phase. A roller pump cannot make these adaptations and can only pump a constant pressure through the lung, cold or warm, potentially over-pressurizing it and causing irreparable harm. The Stöckert Centrifugal Pump (“SCP”, FDA# K011838) and the Stöckert Centrifugal Pump Console (“SCPC”, FDA# K020571) are used by the Toronto Group for all EVLP procedures and will be the centrifugal pump used in the TES. The reason this pump was chosen over any other available pump is that the integrated flow sensor can be connected close to the pulmonary artery cannula, giving a much more accurate reading of flow rate at the point going into the lung.

2. Extracorporeal Heater/Cooler

The TES requires controlled, step-wise heating to warm up the lung to normothermic temperatures (37°C) from the wet ice storage and transportation temperatures. This allows the lung to be assessed at normal physiologic temperature and full metabolic activity. After the lung is determined to be transplantable, it is rapidly re-cooled down to 10°C, then placed back into a wet ice shipping container for delivery to the Study Center. Thus, the extracorporeal heater/cooler must be capable of delivering controlled liquid temperatures between 10 to 37°C. The Stöckert 3T (FDA# K052601) is a full-sized, compressor-cooled cardiac perfusion temperature-control device. It is capable of providing up to 23 L/min of flow into the heat exchanger and can deliver water temperatures from 0 to 50°C to maintain the temperature of an entire person during cardiac bypass. The 3T is used by the Toronto Group for all EVLP procedures and will be the heater/cooler used in the TES. The advantage that this device gives to the System is precise, digital control of temperatures and the ability to rapidly cool the assessed lung, minimizing warm ischemia.

3. Volume-Controlled ICU Ventilator

The TES requires an ICU-type ventilator to ventilate the lungs once they are at normal temperatures. The primary purpose of the ventilator is to allow physiologic respiration to occur within the lung tissue (O₂ in, CO₂ out) in order to measure the lung’s ability to exchange these

gasses. A volume-controlled ventilator is a critical component of the TES, because since there is no thoracic cavity to prevent excessive volumes from being applied to the lung, the ventilator itself must provide the protection. The Maquet Servo-i (FDA# K010925) is considered the gold-standard of volume-control ICU ventilators, allowing precise control of tidal volumes to minimize stretch injuries and pressures to protect against high airway pressures. The Servo-i is used by the Toronto Group for all EVLP procedures and will be the ventilator used in the TES.

4. ABG Analyzer

The ability of the lung to exchange CO₂ with oxygen can be measured using a traditional blood gas analyzer (known as “ABG analyzer”). The Siemens RapidPoint 500 ABG Analyzer (FDA# K113216 and calibration cartridge K122398) measures PO₂, PCO₂, pH, glucose, and lactose, among other analytes (note: this device is not directly connected to the TES, and therefore, is not shown in the above perfusion diagram). The Siemens RapidPoint 500 Analyzer analyzes samples of the STEEN Solution perfusate, measuring dissolved gas levels using a disposable sample cartridge that is replaced monthly according to manufacturer’s instructions. The Siemens RapidPoint is used by the Toronto Group for all EVLP procedures and will be the base analyzer for the TES.

Single-Use Fluid Path Disposables

All devices that come into direct contact with the fluid path will be single-use and pre-sterilized by the manufacturer.

1. Organ Chamber

The design of the organ container must ensure that the organ is protected in an aseptic environment and allowed to respire within the container, creating a slightly humid atmosphere to prevent the lung tissue from drying out. Additionally, there should be interfaces through the container to aseptically connect the lung to the perfusion circuit. Finally, as the lung starts to warm-up to normothermic temperatures, the bronchial arteries will start to perfuse and slowly trickle the perfusate out into the container, so it must be designed to collect the leaks aseptically and to connect back to the perfusion circuit to recirculate the captured perfusate. Currently, the XVIVO Organ Chamber™ is designed as a sterile, single-use container intended to be used as a temporary receptacle for isolated donor lungs in preparation for eventual transplantation into a recipient, and therefore, fits the requirement. If other Organ Chambers become available, they may be used if they meet these criteria.

2. Lung Cannula Set

There are two required cannulas to connect the lung to the perfusion circuit. The first attaches to the left atrial appendage (also called a ‘cuff’) at the point where the left atrium of the heart typically connects to the lung. The objective of this cannula is to connect the soft cuff of tissue to a device that tents (keeps open) the pulmonary veins, allowing the perfusate to flow out of the veins back into the perfusion circuit. In addition, there is a pressure cannula connected to the inside (lumen) of the cannula to collect accurate left atrial pressures to ensure a slight positive pressure (3-5 mmHg) in the atrium is maintained, preventing the veins from collapsing upon

themselves. The second required cannula is to connect the pulmonary artery to the perfusion circuit. In most cases, the pulmonary artery cannula can be a straight tube going into the pulmonary artery proximal to the point of bifurcation and therefore, perfuse both the left and right lungs simultaneously. However, in some cases when the heart is also procured for transplant, the pulmonary artery may not be long enough for a straight cannula and therefore, would need another flexible cannula (similar to the left atrium cannula) to be customized and anastomosed directly to the available pulmonary artery, ensuring a tight fit.

Currently, the XVIVO Lung Cannula Pack™ fits this requirement as a sterile, single-use pack intended to be used to connect isolated donor lungs to the disposable perfusion circuit for *ex-vivo* assessment. There are three cannulas included in the Pack: one cone-shaped left atrial cannula (green tape), one straight pulmonary artery cannula (yellow tape), and one cone-shaped cannula (white tape) to use either as a left atrial or as a pulmonary artery cannula back-up. If other Lung Cannula Sets become available, they may be used if they meet these criteria.

3. Disposable Perfusion Circuit

The Disposable Perfusion Circuit is a single-use, pre-sterilized, disposable extracorporeal perfusion circuit intended to connect to the perfusion hardware to facilitate perfusion of perfusate through isolated donor lungs during EVLP assessment. The components are provided by Maquet in a preassembled, sterilized surgical pack.

Component Overview

The Disposable Perfusion Circuit is made up of the fluid-path components. Any part that comes in direct contact with the fluid path is manufactured as single-use and packaged together as much as possible. In EVLP, the disposable circuit acts as the artificial circulatory system, interfacing with the centrifugal pump motor (via magnetic drive), a hollow-fiber oxygenation membrane that works in reverse to deoxygenate the perfusate by adding carbon dioxide and nitrogen and removing oxygen, a leukocyte filter to remove activated leukocytes, perfusion tubing, and a hard shell fluid reservoir to hold the excess perfusate. By running the oxygenator essentially in reverse, a mix of gases enters the lungs in a similar proportion as physiologic venous gas. This allows the lung itself to act as the ‘oxygenator’ in the circuit, and the resulting PO₂ coming out of the lung can be measured and trended over time as part of the overall lung evaluation strategy. These components are used in an identical assembled combination every day in nearly every hospital in the world to support extracorporeal circulation during cardiac surgery and ECMO procedures. In fact, tens of thousands of these procedures using identical perfusion disposables occur each year in the US alone.

Perfusion Circuit Function

The perfusion circuit delivers the normothermic, deoxygenated perfusate to the lungs, then returns oxygenated perfusate to the reservoir for sampling and recirculation. The steps are summarized as follows:

1. STEEN Solution perfusate is added into the disposable circuit through a standard aseptic interface on the hard shell reservoir.

2. The centrifugal pump head draws the Solution out through the reservoir and pumps it through the membrane oxygenator, which is connected to a venous gas mix (86% nitrogen, 8% carbon dioxide, 6% oxygen) where the dissolved oxygen in the circuit is displaced to a lower physiologic level prior to entering the lungs. This is also where perfusate is heated/cooled during the EVLP procedure via conduction by the extracorporeal heater/cooler.
3. The Solution passes through a leukocyte reduction filter that traps activated leukocytes, preventing them from circulating back through the lung and from being transplanted into the recipient.
4. The (non-disposable) flow sensor monitors flow rate (L/min) going into the lung via interface from the centrifugal pump control.
5. The deoxygenated Solution enters the lung through the pulmonary artery. The pulmonary artery Lung Cannula records pressure at the point of interface between the cannula and lung. This is the pulmonary artery pressure (PAP).
6. The oxygenated Solution exits the lung through the LA. The LA Lung Cannula records pressure at the point of interface between the cannula and left atrium. This is the left atrium pressure (LAP).
7. The Solution drains back into the hard shell reservoir where it can be sampled for testing of pH, glucose, lactose, PO_2 and PCO_2 to assess lung function.
8. Solution temperature is monitored exiting the lung at the reservoir and entering the lung at the oxygenator.
9. The cycle repeats until the determination is made to either transplant or turn down the lung.

A schematic of the perfusion circuit is shown in [Figure 4](#).

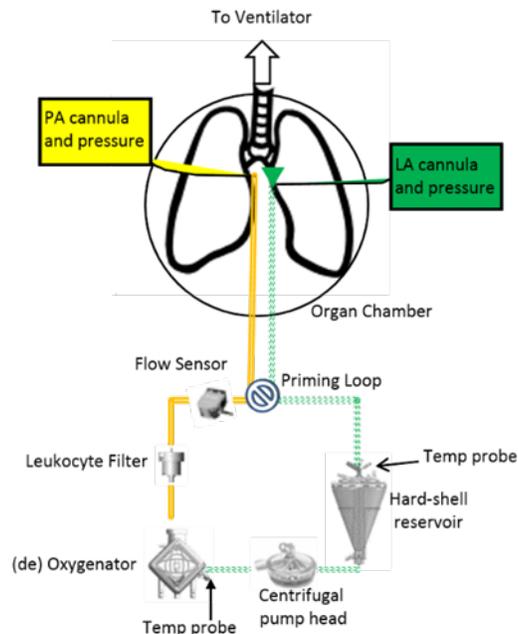


Figure 4. Schematic of Perfusion Circuit

Lung Perfusate

STEEN Solution is a clear, sterile, non-pyrogenic, non-toxic physiological salt solution containing HSA and dextran 40. This solution is a low potassium (extracellular) electrolyte solution with physiological colloid-osmotic pressure (COP) designed for use as a temporary continuous normothermic machine perfusion solution for assessment and extended preservation of isolated donor lungs.

The composition of STEEN Solution was designed to simulate the COP and crystalloid-osmotic properties of human plasma to permit safe, continuous *ex-vivo* lung perfusion at normothermia (37°C) for at least 12 hrs without the development of either tissue edema or dehydration. The COP and osmotic pressure of the solution were chosen to maintain optimal water balance between the cellular and the intra- and extra-vascular spaces. The optimal COP is provided by a proprietary combination of HSA (FDA-approved source) and dextran 40. The electrolyte composition is essentially extra-cellular (low potassium) with a phosphate buffer. The composition and physical properties of STEEN Solution are shown in Table 5.

Table 5. Composition and Properties of STEEN Solution

Composition	calcium chloride, dextran 40, phosphate glucose, HSA, magnesium chloride, potassium chloride, sodium bicarbonate, sodium chloride, sodium dihydrogen, and water
Solution Properties	
pH (20°C and ambient atmosphere)	7.4 ± 0.3
Osmolality (mOsm/kg)	295 ± 20

The primary mode of action for STEEN Solution is its ability to provide optimal COP, permitting stable fluid compartment homeostasis for sufficient time to allow meaningful functional evaluation of the lung at 37°C. STEEN Solution, therefore mimics the *in vivo* environment without the risk of edema formation in the absence of harmful pro-inflammatory cytokines and activated leucocytes associated with trauma, stress and brain death. This mode of action is primarily related to the water binding properties of albumin and its distributions across semi-permeable membranes between the intra- and extra-vascular spaces within the lung, and is not based on any metabolic or pharmacological action of either colloid. Additionally, it has been shown that red blood cells are not required for STEEN Solution to carry oxygen to the lung tissue as oxygen dissolves well in this Solution, and the lung is very efficient at removing oxygen from the Solution.[Cypel 2007] Acellular STEEN Solution has been used by the Toronto Group for all of their clinical EVLP procedures to date and will be the base perfusate used in the TES.

The disposable EVLP perfusion circuit is primed with 1.5 L of STEEN Solution prior to use. STEEN Solution is allowed to warm-up to room temperature during the priming procedure pre-perfusion. The medications (and dosages) are added to the 1.5 L STEEN Solution prime are shown in Table 6.

Table 6. Medications Added to STEEN Solution during Prime

Drug	Dosage	Reason added
Heparin	3000 IU	Prevent residual blood from clotting
Methylprednisolone (e.g., Solu-medrol)	500 mg	Steroid: to decrease inflammation
Imipenem (e.g., Primaxin)	500 mg	Broad-spectrum antibiotic to minimize bacterial infection risk

Appendix 5 Sponsor Signatures

Study Title: A Phase 2, Multicenter, Open-Label Study to Measure the Safety of Extending Preservation and Assessment Time of Donor Lungs Using Normothermic Ex Vivo Lung Perfusion and Ventilation (EVLP) as Administered by the Sponsor Using the Toronto EVLP System

Study Number: PXUS 14-001, v6.0

Final Date: 03 November 2017

This clinical study protocol was subject to critical review and has been approved by the Sponsor. The following personnel contributed to writing and/or approving this protocol:

Signed: _____ **Date:** _____
Marc I. Lorber, MD
Chief Medical Officer *DD Mon YYYY*

Signed: _____ **Date:** _____
Jordan Shin, MD, PhD
Medical Director *DD Mon YYYY*

Signed: _____ **Date:** _____
Michael Roberts, MA, RAC
Director, Regulatory Affairs *DD Mon YYYY*

Appendix 6 Investigator's Signature

Study Title: A Phase 2, Multicenter, Open-Label Study to Measure the Safety of Extending Preservation and Assessment Time of Donor Lungs Using Normothermic Ex Vivo Lung Perfusion and Ventilation (EVLP) as Administered by the Sponsor Using the Toronto EVLP System

Study Number: PXUS 14-001, v6.0

Final Date: 03 November 2017

The signature below indicates the approval of this protocol and its appendices, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Principal Investigator:

Signed: _____ Date: _____

Printed Name: _____

Title: _____

Institution: _____