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Ex Vivo Lung Perfusion: Current Achievements and Future Directions

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Abbreviations

A_{2A}R, Adenosine_{2A} Receptor

DAMP, Damage Associated Molecular Pattern

DCD, Donor after Circulatory Death

EVLP, Ex Vivo Lung Perfusion

IRI, Ischemia Reperfusion injury

OCS, Organ Care System

OGG1, 8-oxoguanine DNA glycosylase-1

PARP- poly (ADP-ribose) polymerase inhibitors

PGD, Primary Graft Dysfunction

ROS, Reactive Oxygen Species

TPN, Total Paraenteral Nutrition

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Abstract

There is a severe shortage in the availability of donor organs for lung transplantation. Novel strategies are needed to optimize utilization of available organs to address the growing global needs. Ex vivo lung perfusion (EVLP) has emerged as a powerful tool for the assessment, rehabilitation, and optimization of donor lungs prior to transplantation. In this review we discuss the history of EVLP, current evidence on its use for standard and extended criteria donors and consider the exciting future opportunities that this technology provides for lung transplantation.

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INTRODUCTION

Chronic lung disease is the 4th leading cause of death in the United States.¹ Lung transplant offers the potential for increased quality of life and lifespan for individuals with end stage lung disease. Although lung transplant volume has increased worldwide,² the demand for lung transplant donors exceeds supply^{3,4} with up to one-third of potential transplant candidates dying on the waiting list.⁵ This disparity relates to concerns over the quality of organs and the narrow window of time between organ procurement and transplant. Lung allografts are particularly susceptible to ischemia-reperfusion injury (IRI), which manifests clinically as primary graft dysfunction (PGD) and occurs in up to 25% of lung transplants.⁶ PGD increases morbidity and mortality in the short-term and is a risk factor for the development of chronic lung allograft dysfunction, which occurs in up to half the recipients at 5 years.⁷ Chronic lung allograft dysfunction remains the Achilles' heel of lung transplantation, preventing it from reaching its full potential

Innovations to expand the donor organ pool focus on 3 areas: use of extended criteria donors, improved preservation methods to extend time between procurement and transplant, and therapies to mitigate ischemia-reperfusion injury. Ex vivo lung perfusion (EVLP) has the potential to impact all these domains.

The purpose of this review is to briefly summarize the current EVLP technology, evidence to support its use and potential future directions at bench and bedside.

TRENDS IN THE USE OF EVLP

The concept of normothermic ex vivo perfusion of the lung to perform dynamic assessments of function was proposed in animal models as early as 1970,⁸ and was used successfully in 1978 by Hardesty and Griffith to extend preservation time for combined heart and lung transplants.⁹

Limitations in the technology of the time and the need for manual ventilation of the donor lungs may have curtailed the widespread adoption of this technique. In 2001 Stig Steen and the transplant team from Lund, Sweden reported the first successful in human lung transplant using EVLP on a lung from a donor after circulatory death (DCD).¹⁰ Worldwide there has been an increase in lung transplants performed as a result of EVLP- led by the Toronto group. They reported an associated increase in transplant volume of 70%,¹¹ without significant changes in the donor pool. Implementation in the United States has been slower. Between April 2015 and June 2018 EVLP was used on 3% (447/14 269) of all lung transplants. A third of these came from DCD donors and over half were transplanted.¹² Data are not available on rates of PGD in these patients, but the overall 1-year survival is 86.5% compared with 89.2% among those who do not undergo EVLP. Factors that limit the use of EVLP include delays in reimbursement,¹³ low transplant volume, or poor familiarity with the EVLP setup. To counter this, the use of 'EVLP Centers' has been proposed.^{14,15} This paradigm- with EVLP performed by specialized teams at remote locations from the donor and recipient centers - is being evaluated in 8 states in the USA as part of the multicenter prospective phase 2 PERFUSIX trial. Designated centers assess donor lung viability while on EVLP and potential candidacy for transplant can be assessed based on physiological parameters, with subsequent cold storage and transport to recipient center if approved.

CURRENT EVLP SYSTEMS AND CLINICAL DATA

There are 4 commercially available EVLP systems available worldwide- Organ Care System by Transmedics; XVIVO Perfusion System (XPS) and LS by XVIVO; Vivoline LS1 by Vivoline Medical; and Lung Assist by Organ Assist. The Organ Care System and XPS were FDA approved for use in the United states in 2018 and 2019. The main difference between these

systems is that they are either mobile- with the organ being placed immediately on EVLP after procurement; or fixed- with the organ preserved in ice and transported to a specialist center for normothermic perfusion.

Mobile systems

The Organ Care System by Transmedics is a portable normothermic perfusion system and has been studied in 2 prospective trials -INSPIRE¹⁶ and EXPAND¹⁷ (table 1.). INSPIRE recruited standard donor lungs for bilateral lung transplant and showed a 50% lower cumulative incidence of PGD3 compared with control, and similar short and long-term survival. In contrast the EXPAND trial recruited DCD donors over the age of 55 with $PaO_2/FiO_2 \leq 300$ and expected ischemic time greater than 6 hours. It demonstrated 98% 30-day survival but a PGD3 rate of 44% at 72 hours.

Fixed systems

The XVIVO perfusion systems are fixed normothermic platforms. Trials involving static systems (table 1) recruited extended criteria donors that were initially deemed unsuitable for transplant. The HELP trial¹⁸ was the first prospective clinical trial of EVLP and showed an incidence of PGD 2/3 at 72 hours of 15% compared to 30% in non-EVLP group with no significant difference in survival. The Vienna group¹⁹ showed comparable results in their single-center analysis, with no significant differences in ICU stay, hospital stay or 30 day mortality. NOVEL was the first prospective multicenter trial in North America to study the use of EVLP in extended criteria donors.²⁰ Among 17 enrolled centers there was a conversion rate of 50.9% for grafts initially deemed unsuitable for transplant. When compared with standard criteria donor transplants performed during the same period that were not on EVLP there was no difference in PGD3 at 72 hours, survival or lung function at 30 day follow up. The DEVELOP trial in the United Kingdom

transplanted 33% of lungs initially deemed unsuitable,²¹ but the requirement for extracorporeal membrane oxygenation support was significantly higher in the EVLP group (38%) compared with non-EVLP group (3%), leading to the trial being terminated early.

FUTURE DIRECTIONS IN THE USE OF EVLP

EVLP appears to be safe and has the potential to increase utilization of organs. It also shows promise as a tool to study lung transplant physiology, test novel therapies, and optimize donor lungs prior to transplant (Table 2). There are several advantages to the EVLP platform- 1) medications and therapies can be used without concern for impact on/from other physiological systems; 2) the effect of treatment on the lung can be monitored directly in real time; and 3) it is easier to translate large animal model results to human lungs ex vivo than in situ.

Prevention of IRI

IRI manifests clinically as primary graft dysfunction (PGD)- a syndrome of reduced PaO₂:FiO₂, infiltrates on chest x-ray and the finding of diffuse alveolar damage on lung biopsy. It is noted in up to 30% of transplanted lungs and is independently associated with a higher risk of mortality.²² A series of secondary messenger cascades during procurement result in downstream formation of reactive oxygen species (ROS), endothelial dysfunction leading to increased vascular permeability, and the production of inflammatory cytokines.²³ Attempts to minimize the impact of IRI on graft function have been targeted at the innate immune system and reduction of oxidative stress.²³ In addition to this the EVLP platform may itself reduce proinflammatory cytokines and damage associated molecular pattern (DAMP) molecules, which are induced by ischemia. This may reduce inflammatory/immune cell infiltration and activation during reperfusion. The pathways involved in IRI have been modelled to study potential therapeutic strategies at a cellular level- using genomic, proteomic and stem cell therapy- and at a

macroscopic level by modifying physiological parameters such as flow, position and ventilation of the lung on the EVLP circuit.

Genomic and proteomic

Advances in next generation sequencing allow characterization of the molecular profile of lungs undergoing EVLP. Ischemia at the time of procurement results in upregulation of genes for IL-1 β and TNF- α .²⁴ A shotgun LC-MS proteomic platform comparison between lungs in a murine transplant model at baseline, after cold ischemia and on EVLP shows a rebalancing of the proteome after EVLP toward the baseline condition of native lungs.²⁵ Proteins related to vesicle-mediated transport; myosin and actin-related proteins; and those involved in redox reactions appear to be downregulated after EVLP.²⁵ A similar attenuation of inflammatory gene expression is seen when modelling human lungs rejected for transplant on an EVLP circuit – with a reduction in circulating leukocyte cell-specific gene expression.²⁶ Mitochondrial DNA damage during ischemia-reperfusion injury can be repaired by using a fusion protein targeted at DNA repair 8-oxoguanine DNA glycosylase-1 (OGG1). Inclusion of mitochondrial OGG1 in perfusate is associated with reduction in lung edema and mitochondrial DNA fragments (an indicator of oxidative damage) in a murine donor after circulatory death (DCD) model.²⁷ Intrabronchial administration of adenoviral vectors in a porcine EVLP model have been used for targeted IL-10 expression, resulting in better gas exchange, fewer histological signs of inflammation and lower levels of IFN - γ in mediastinal lymph nodes.²⁸

Immune and stem cell therapy

EVLP on its own has been found to be associated with a reduction in the severity of inflammatory response of donor lungs. The altered immune profile includes lower allorecognition, infiltration and priming of recipient T cells.²⁹ In a murine model of lung

transplant, adding a leukocyte filter to the EVLP circuit is associated with trapping of pyroptotic leukocytes and reduced expression of IL-6.³⁰ In human lung transplants a reduction in IL-1 β levels and downregulation of intracellular adhesion molecule-1 is associated with increased inpatient survival.³¹

Stem cells may play a role in immune modulation. The intrabronchial administration of multipotent adult progenitor stem cells mixed in an albumin/plasmalyte solution has been shown to reduce the expression of TNF- α , IL-1 β and IFN- γ in the BAL samples of porcine EVLP models; although there was no significant difference in physiological parameters compared with controls.³² A similar anti-inflammatory effect is also seen when using mesenchymal stem cells in the form of extracellular vesicles, delivered into perfusate, resulting in downregulation of IL-17, HMGB1 and TNF- α production.³³ Administration of mesenchymal stem cells via perfusate rather than the airway may be associated with reduction in pulmonary edema and reduced expression of apoptotic markers.³⁴ Aside from their role in minimizing inflammatory response to ischemia reperfusion, mesenchymal stem cells may be used in the bioengineering of decellularized lung scaffolds to create chimeric grafts with recipient-derived airway epithelium and antigen expression.³⁵

Pharmacological interventions

Multiple pharmacological agents have been tested in animal EVLP models. The agents are added to the perfusate, administered as gas, or in a nebulized solution via the ventilator. Adenosine agonists have consistently shown promise in animal models and are currently being studied in human clinical trials.³⁶ The proposed mechanism of action is through interaction with multiple G protein-coupled receptors in the lung and activation of the Adenosine_{2A}Receptor (A_{2A}AR) on immune cells resulting in a pleiotropic downregulation of the inflammatory response.^{37,38} The

administration of a selective A_{2A}R agonist to perfusate during EVLP in a murine DCD model is associated with increased lung compliance; decreased pulmonary artery pressures; and reduced levels of CXCL1, CCL2, TNF- α , and neutrophil counts when compared with EVLP alone.^{39,40} Interestingly A_{2B}receptor agonists induce similar improvements in pulmonary compliance and reduced neutrophil infiltration but do not improve oxygenation or pulmonary edema.⁴¹ Other promising agents include poly(ADP-ribose) polymerase (PARP) inhibitors, which prevent the downstream effects of PARP (apoptosis and impaired cellular metabolism) to reduce expression of IL-6, IL-10, and reduce pulmonary edema⁴²; ascorbic acid, which not only provides an antioxidant effect but also improves the efficiency of mitochondrial activity⁴³; E3 ligase-associated protein inhibitors⁴⁴ which reduce NF- κ B transcription products and inflammatory damage; and α 1-Anti-trypsin which is associated with improved pulmonary compliance, partial pressures of oxygen, reduced pulmonary artery pressures, reduced pulmonary edema, and reduced markers of apoptosis and inflammation.⁴⁵

Of the inhaled agents, β -adrenoreceptor agonists are readily available, cost-effective and appear to consistently improve graft function during EVLP. β -adrenoreceptors are distributed throughout multiple cell types in the airways and pulmonary vasculature. They play a role in endothelial barrier function. In a canine DCD model using the Toronto EVLP protocol, β -adrenoreceptor agonists demonstrated significantly higher partial pressure of oxygen and higher compliance at every recorded time point up to 4 hours.^{46,47} Other potential inhaled agents include sevoflurane, which is associated with reduction in neutrophil chemoattractant factors, TNF- α and lower pulmonary edema⁴⁸; and hydrogen gas, which increases expression of free radical scavengers such as super oxide dismutase and heme-oxygenase.⁴⁹

System modifications

Modifications to EVLP protocols can be tested in animal models and easily translated to humans. Simple changes to type of perfusate used, perfusion parameters and position of the graft during EVLP can all have implications on graft function once transplanted.

There does not appear to be a significant difference in aerodynamics or graft viability when comparing cellular with acellular perfusates in porcine EVLP models^{50,51} although 1 study did show that 3 lungs in the acellular group had to have EVLP terminated prematurely due to severe lung edema.⁵² In a human model using lungs that were rejected for transplant, cellular perfusate (Steen solution and 2 units of packed red blood cells) in a high-flow (defined as 70ml/kg/min) Vivoline system was associated with lower transplant suitability by physiological parameters but lower pulmonary edema when compared with a low flow acellular XVIVO system.⁵³ Another group however has shown that target flow rates of 20% rather than 40% of cardiac output is associated with lower pulmonary edema and IL-1 β expression.⁵⁴ Others have experimented with use of a self-made perfusate,⁵⁵ rather than commercially available Steen solution, which appears to be safe in a porcine model. Using whole blood in EVLP perfusate has been shown to extend donor organ viability time for up to 24 hours.⁵⁶

Negative pressure ventilation may be associated with less inflammatory cytokine generation, reduction in bullae generation and less pulmonary edema than positive pressure ventilation in human and porcine models.⁵⁷ Conversely airway pressure release ventilation is associated with better oxygenation, compliance and lower pulmonary edema than standard positive pressure ventilation.⁵⁸ Prone positioning compared with supine is associated with lower IL-1 β levels, lower pulmonary edema and better oxygenation.^{59,60}

Lung monitoring and rehabilitation

EVLP platforms provide a way to gain insights into the function of a marginal donor organ prior to transplantation. The typical physiological parameters used to assess lung function and suitability for transplant included pulmonary vascular pressures, partial pressures of oxygen in perfusate, lung edema (as measured with wet-to-dry weight ratio), and PaO₂ -FiO₂ ratios. A range of novel strategies have been proposed to supplement these standard measures in the assessment of lung injury on EVLP and prediction of PGD. Microdialysis can be used to directly monitor interstitial fluid composition using a thin probe that is inserted into the lung parenchyma. Interstitial metabolites such as glutamate, lactate, glucose and pyruvate can predict suitability for transplant and PGD ≥ 2 with greater than 80% sensitivity and specificity.⁶¹ Other sophisticated methods of monitoring have been used in porcine models – functional pulmonary MRI,⁶² laser spectrometry⁶³ and ultrasound⁶⁴ - but do not appear to provide a significant advantage over conventional physiological parameters.

Aside from targeting the biological pathways that cause PGD there has also been interest in rescue of lungs that would otherwise be discarded due to overt lung injury. The most common injury-rescue model involves instillation of pepsin and hydrochloric acid to a porcine airway, simulating injury due to aspiration of gastric contents. There has been some promise in the use of intrabronchial surfactant lavage to mitigate lung injury.^{65,66} Other potential rescue agents for include methyl-prednisone,⁶⁷ lung nutrition with TPN⁶⁸ and α -1-antitrypsin.⁴⁵ Thrombolytics may play a role in the use of donor lungs containing arterial thrombus⁶⁹ but alteplase does not appear to produce any improvement in physiological parameters in a porcine EVLP model when compared with standard perfusate.⁷⁰

Finally, EVLP can be used to monitor and treat donor lung infections. Antibiotic and antifungal therapies can be used at high doses, with minimal complication, and significant reduction in microbial loads.⁷¹⁻⁷³ It may also be possible to use sterilizing techniques, to minimize transmission of viral blood-borne infections such as Hepatitis C, from donor to recipient. In vitro and Porcine EVLP models have shown a significant reduction in viral load, infectivity and minimal associated toxicity with the used of light-based photodynamic therapy on perfusate.⁷⁴ This may provide opportunities to further expand the donor pool.

IMPLICATIONS OF EVLP FOR THE FUTURE OF TRANSPLANT SURGERY

A recent review by the Toronto group has highlighted the potential of EVLP to facilitate “semi-elective” lung transplant surgery- they showed that EVLP can extend graft survival time up to 26 hours in large animal models.⁷⁵ This would provide multiple potential benefits in terms of patient convenience, scheduling of cases and to allow time for prospective crossmatch in sensitized recipients. In addition EVLP offers options for the treatment of pulmonary neoplasms using chemotherapeutics at doses that would not be tolerated systemically due to toxicity.⁷⁶

CONCLUSION

EVLP is a platform to evaluate donor graft function and potentially optimize the performance of lungs prior to transplantation. Factors that have limited the widespread use of EVLP include lack of familiarity with the setup, adherence to convention, and concerns about the use of extended criteria donor lungs. Results from ongoing trials may serve to normalize centralized evaluation of extended criteria lungs at EVLP centers, expanding a limited donor pool and improving the safety and efficacy of lung transplant practices.

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Table 1. Summary of prospective clinical trials for Ex Vivo Lung Perfusion

Trial	Number of patients	Number of centers	System	Design	Key points
Standard criteria donors					
INSPIRE ¹⁶	EVLP-151 Control- 169	12	OCS	RCT	PGD3 at 72 hours (EVLP vs control) - 17.7% vs 29.7% (p=0.015); 30d mortality- 4.3% vs. 0%; 1yr mortality –10.6% vs. 11.9%.
Extended criteria donors					
HELP ¹⁸	EVLP-23, Control- 116	1	XVIVO	Nonrandomized trial	PGD3 at 72 hrs. (EVLP vs. Control)- 15% vs 30% (p=0.11); Median ICU stay (days) – 4 vs.4; median hospital stay (days)- 23 vs 27 (p=0.39), 30d Mortality- 10% vs. 5% (p=0.33)
Vienna ¹⁹	EVLP -39, Control- 41	1	XVIVO	Nonrandomized trial	PGD3 at 72 hours- 0% in both groups; Median ICU stay (days) – 5.5 vs.6 (p=0.6); median hospital stay (days)- 20 vs 20.5 (p=0.8), 30d mortality- 0% vs.4.2% (p=0.6).

Novel ²⁰	EVLP- 110 control - 116	6	XVIVO	Nonrandomized trial	PGD3 at 72 hrs (EVLP vs. Control)-8.9% vs 9.5% (p=0.12); Median ICU stay (days) -9.9 vs 9.8 ; median hospital stay (days)- 23.9 vs 28.5; 1yr mortality -6.8% vs. 3.5% (p=0.84)
Expand ¹⁷	EVLP-79	11	OCS	Observational cohort	Incidence of PGD3 at 72hrs- 6.4%. 30d mortality- 1%, 1 yr mortality – 7%.
Develop ²¹	EVLP-102, Control- 306	5	Vivoline	Observational cohort	Higher frequency of ECMO in EVLP vs control- 39% vs 3%, trial stopped early.

Table 2. Summary of lab-based studies for investigation and treatment of ischemia reperfusion injury on Ex Vivo Lung Perfusion

	Agent/mechanism/modality	Target	Model	Experimental Groups (N)	Key points
<i>Pharmacologic</i>					
Wang 2020 ⁴²	3-aminobenzamide	Poly(adp-ribose) polymerase (PARP)	Murine	EVLP+PARP inhibitor (16); EVLP (15); Control (14)	EVLP+ PARP inhibitor has higher compliance; lower pulmonary edema; and lower LDH, IL-6, and IL-10.
Lin 2018 ⁴⁵	α 1-antitrypsin (A1AT)	-	Porcine	EVLP (6);EVLP+A1AT (6)	Reduced pulmonary artery pressure, pulmonary vascular resistance, pulmonary edema, apoptosis and IL-1 α & IL-8.
Wang 2018 ⁴⁸	Sevoflurane	-	Murine	EVLP+ Sevoflurane (6); EVLP (6); Baseline (3)	Reduced LDH, 3-nitrotyrosine, protein carbonyl, TNF- α , pulmonary edema. Increased oxygenation capacity and static pulmonary compliance.

Weathington 2018 ⁴⁴	BC1215 with Lipopolysaccharide stress test (LPS)	ubiquitin E3 ligase protein-FBXO3	Human	EVLP+LPS+BC1215 (3); EVLP+ LPS(4)	BC1215 is associated with reduced NF- κ B associated transcripts and inflammatory cytokines. Improved oxygenation
Hijiya 2017 ⁴⁷	Inhaled β 2 agonist	β 2 adrenoreceptor	Canine	EVLP (5); EVLP+ β 2 agonist (5)	Lower PVR, higher CAMP and ATP in β 2 group
Huerter 2016 ⁴⁰	Selective A2BR blocker- ATL802	adenosine A2B receptor (A2BR)	Murine	EVLP (9); EVLP+ATL802 (9)	ATL802 associated with reduced cytokine production, neutrophil infiltration, vascular permeability, and edema. Improved pulmonary compliance.
Haam 2015 ⁴⁹	Inhaled hydrogen	Oxidative pathways	Porcine	EVLP(5); EVLP+hydrogen(5)	Lower levels of IL-1 β , IL-6, IL-8, TNF- α in the EVLP +hydrogen group
Shaghghi 2015 ⁴³	Ascorbic acid	-	Murine	EVLP + Ascorbic acid (5);EVLP (5)	Total metabolite was signal larger in the ascorbate cohort and oxidative phosphorylation pathway was more active compared to control, suggesting improved mitochondrial function

Stone 2015 ³⁹	Adenosine 2A receptor agonist	Adenosine 2 A receptor (A2AR)	Murine	Cold static preservation (12); EVLP (12);EVLP+A2AR agonist (12)	Lung edema, cytokines IL-1, IL-8, IL-6, and IL-17) and neutrophil counts significantly lower in EVLP+A2AR compared with EVLP or cold static preservation.
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Gene therapy/diagnostics

Tan 2020 ²⁷	Fusion protein (FP) targeted at 8-oxoguanine DNA glycosylase	Mitochondrial DNA	Murine	Native(11) ; Native+FP(13)); DCD(7); DCD+FP (10); DCD+FPKO (8)	Higher fraction of intact mitochondrial DNA in DCD+FP group compared with DCD and knockout mice-associated with lower pulmonary edema and lower mitochondrial DAMPs.
Lonati 2018 ²⁴	Transcription factor analysis in Frozen lung biopsy specimens	-	Murine	Native (5) Pre-EVLP (5) Post-EVLP (5)	EVLP modulates NF-kB,signal transducer and activator of transcription 3, ERK1/2, p38, Akt, and stress-activated protein kinase/JNK signaling pathways . Increased transcription of genes related to inflammation and its regulation,

					apoptosis/survival, heat shock, and oxidative stress response
Rofia 2018 ²⁵	Nano LC-MS spectrometry in frozen lung biopsy	-	Murine	Native(5); Pre-EVLP (5); Post-EVLP (5)	Increased expression of super oxide dismutase-1 associated with reduced free radical damage on EVLP
Yeung 2018 ²⁶	Gene expression profiles in frozen lung biopsy specimens	-	Human	n=10	EVLP associated with increased gene expression for endothelial markers of inflammation. Decreased expression of leucocyte-specific genes.
Machuca 2017 ²⁸	Adenoviral vector(AV) for human IL10 gene delivery	Nuclear transcription machinery	Porcine	Cold ischemia (4); AV control (4); EVLP control (5); EVLP+AV (5)	Lower acute rejection score in EVLP+AV compared with EVLP; better gas exchange at 7 days. No significant difference in Il-1 β , IL6, IL8, or IFN γ
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Stem cell and immune therapy					
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Lonati 2019 ³³	Mesenchymal stem cell derived extracellular vesicles (EV) via perfusate	-	Murine	EVLP+EV (5); EVLP+ saline (5)	EVLP+EV had lower vascular resistance, higher perfusate nitric oxide metabolites, higher pulmonary ATP

Andreasson 2017 ³¹	-	Cytokine levels and neutrophil adhesion	Human	N= 42	Increased IL1 β and TNF α associated with inpatient mortality
Martens 2017 ³²	Multipotent adult progenitor cells (MAPC) via airway	-	Porcine	EVLP+MAPC (6); EVLP+albumin (6)	No difference in physiological parameters between groups. Lower BAL neutrophils, TNF- α , IL-1 β and IFN- γ in MAPC group.
Noda 2017 ³⁰	Leukofiltration (LF) ; caspase-1-inhibitor (INH)	Donor leukocytes, Caspase-1	Murine	EVLP (5);EVLP+LF (5); EVLP+ release of filtered ; cytokines (5); EVLP+INH (5)	Leukofiltration and caspase inhibitor associated with lower levels of IL6,TNF α and IL1 β ; 26% percent of filtered cells found to be pyroptotic.
Stone 2016 ²⁹	EVLP compared with standard lung transplant	Donor leukocytes	Porcine	EVLP (6); Control (6)	EVLP associated with reduced donor leucocyte transfer, allorecognition, T-cell priming and T-cell infiltration of recipient.