Review

Doug A. Gouchoe, Zhentao Zhang, Jung-Lye Kim, Yong Gyu Lee, Bryan A. Whitson and Hua Zhu*

Improving lung allograft function in the early post-operative period through the inhibition of pyroptosis

https://doi.org/10.1515/mr-2023-0066
Received December 7, 2023; accepted May 4, 2024;
published online May 16, 2024

Abstract: Lung transplantation is the only definitive therapy for end-stage pulmonary disease. Less than 20 % of offered lungs are successfully transplanted due to a limited ischemic time window and poor donor lung quality manifested by pulmonary edema, hypoxia, or trauma. Therefore, poor donor organ recovery and utilization are significant barriers to wider implementation of the life-saving therapy of transplantation. While ischemia reperfusion injury (IRI) is often identified as the underlying molecular insult leading to immediate poor lung function in the post-operative period, this injury encompasses several pathways of cellular injury in addition to the recruitment of the innate immune system to the site of injury to propagate this inflammatory cascade. Pyroptosis is a central molecular inflammatory pathway that is the most significant contributor to injury in this early post-operative phase. Pyroptosis is another form of programmed cell death and is often associated with IRI. The mitigation of pyroptosis in the early post-operative period following lung transplantation is a potential novel way to prevent poor allograft function and improve outcomes for all recipients. Here we detail the pyroptotic pathway, its importance in lung transplantation, and several therapeutic modalities that can mitigate this harmful inflammatory pathway.

Keywords: lung transplantation; pyroptosis; ischemia reperfusion injury; IRI; *ex vivo* lung perfusion; EVLP

***Corresponding author: Hua Zhu**, PhD, Department of Surgery, Division of Cardiac Surgery, The Ohio State University Wexner Medical Center, Columbus, OH 43210, USA, E-mail: Hua.Zhu@osumc.edu
Doug A. Gouchoe, Jung-Lye Kim, Yong Gyu Lee and Bryan A. Whitson, Department of Surgery, Division of Cardiac Surgery, The Ohio State University Wexner Medical Center, Columbus, OH, USA; and COPPER Laboratory, The Ohio State University Wexner Medical Center, Columbus, OH, USA. https://orcid.org/0000-0002-3177-4637 (D.A. Gouchoe)
Zhentao Zhang, Department of Surgery, Division of Cardiac Surgery, The Ohio State University Wexner Medical Center, Columbus, OH, USA

Introduction

Lung transplantation is the only definitive therapy for end-stage pulmonary disease [1]. Currently, eight patients are added to the lung transplant waitlist daily, which is a 27.7 % increase since 2010 [2]. Present day, there are ~4,000 patients on the waitlist, however, the number of transplants performed annually has stagnated at ~2,000/year since 2010 [3, 4]. Therefore, while the waiting list of patients eligible for transplant grows daily, the ability for providers to find suitable donor allografts to perform lung transplants falls far short of the demand [1, 2, 5]. This is highlighted by the fact that less than 20 % of offered lungs are successfully transplanted due to a limited ischemic time window and poor donor lung quality manifested by pulmonary edema, hypoxia, or trauma [1, 6–10]. Therefore, poor donor organ quality and utilization are significant barriers to extend the life-saving therapy of transplantation.

Due to the nature of lung transplantation, the prospective allograft will suffer a period of ischemia when procured from the donor, and thereafter suffer a reperfusion injury when re-implanted in the recipient. This pattern of injury is known as ischemia reperfusion injury (IRI). At the cellular level, IRI leads destruction of the endothelial-epithelial barrier and loss of function, which subsequently allows the migration of immune cells into the alveolar space causing increased vascular permeability, which results in increased extravascular water/edema and, ultimately, respiratory failure [11]. Clinically, this contributes to decreased allograft quality and function, which manifests as primary graft dysfunction (PGD). Of those patients fortunate enough to receive a transplant, ~30 % will develop severe, grade 3 PGD [12]. IRI is often identified as the underlying molecular insult leading to PGD [13], and this injury encompasses several pathways of cellular injury in addition to the recruitment of the innate immune system to the site of injury to propagate this inflammatory cascade [14]. However, pyroptosis is a central molecular inflammatory pathway that is the most significant contributor to injury in this early post-operative phase [15]. Pyroptosis is another form of

programmed cell death and is more often associated with IRI than apoptosis [16]. IRI leads to the formation of damage associated molecular patterns (DAMPs), which are recognized by NOD-like receptors (NLRs) and activation results in the formation of inflammasomes. Inflammasomes recruit, and subsequently turn pro-caspase-1 into its active form, caspase-1, which serves two functions within the pyroptotic pathway: turn pro- IL-1β to IL-1β, and cleave Gasdermin-D (GSDMD) into its N-terminus (GSDMD-N) [16]. GSDMD-N interacts with cell-membrane lipids to form transmembrane pores which allow IL-1β, additional cytokines and signals to be released and promote further inflammation and cell death (Figure 1).

As pyroptosis can be a quick process, it leads to significant damage early within the peri-operative period and is a significant contributor to PGD and poor allograft function. The objective of this review is to provide: (1) a basic overview of the pyroptotic pathway; (2) the current understandings of pyroptosis as it relates to lung transplantation and pyroptotic mitigation, (3) future directions of novel therapies to improve pyroptotic-related injury.

Discovery of pyroptosis

First described by Zychlinsky et al. in 1992, pyroptosis was initially characterized in macrophages infected by bacteria [17]. However, at that time it was thought to be a subset of apoptosis due to its similarities such as caspase involvement, nuclear condensation and damage [18]. Finally in 2001, the term pyroptosis was coined by D'Souza et al. to describe inflammatory programmed cell death as opposed to

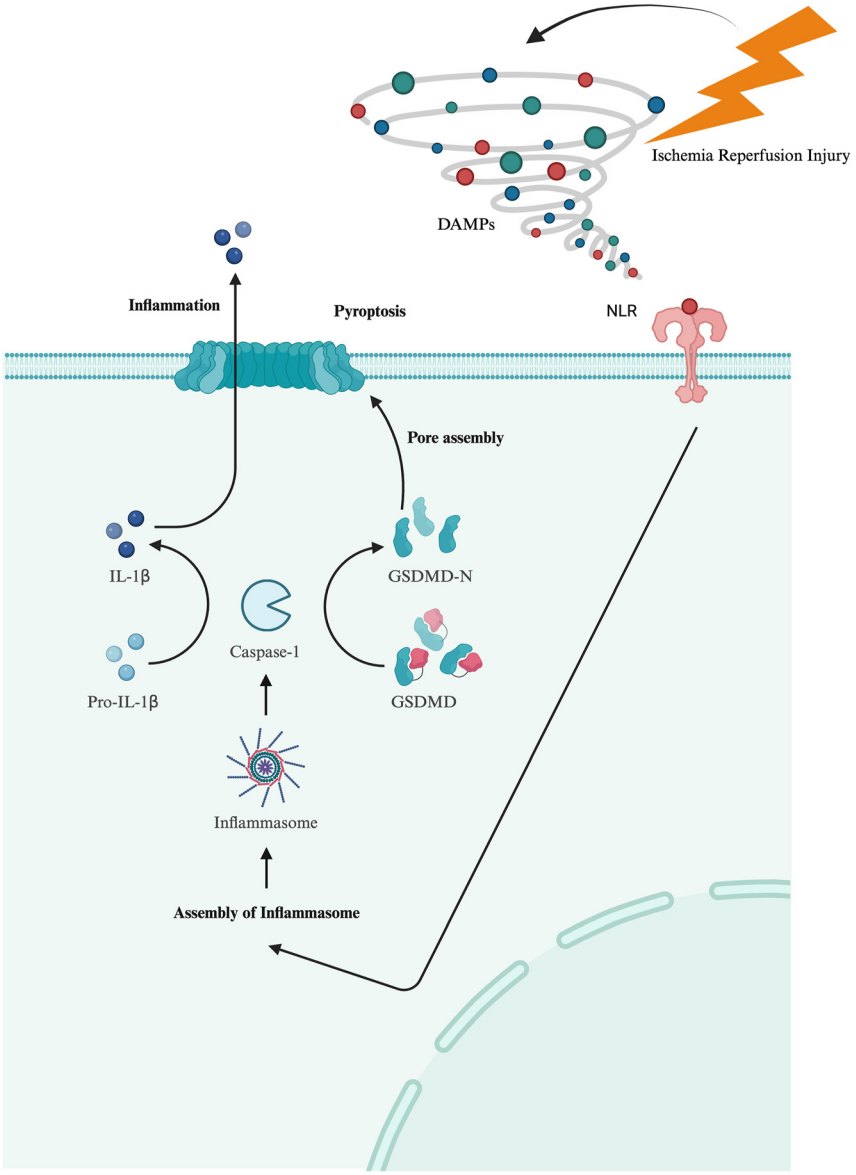

Figure 1: Basic overview of pyroptotic pathway. Ischemia reperfusion injury leads to the formation of damage associated molecular patterns (DAMPs), which are recognized by NOD-like receptors (NLRs) and activation results in the formation of inflammasomes. Inflammasomes recruit, and subsequently turn pro-caspase-1 into its active form, caspase-1, which serves two functions within the pyroptotic pathway: turn pro-IL-1β to IL-1β, and cleave Gasdermin-D (GSDMD) into its N-terminus (GSDMD-N). GSDMD-N interacts with cell-membrane lipids to form transmembrane pores which allows IL-1β, additional cytokines and signals to be released and promote further inflammation and cell death.

non-inflammatory cell death (apoptosis) [19]. As our understanding of the pyroptotic pathway progressed, inflammasome activation was discovered to activate caspases and subsequently lead to the transformation of pro-IL-1β to IL-1β [20]. While initially thought to be caspase-1 dependent, it was soon discovered that caspase-4/5/11 were also involved [21] in addition to caspase-3 [22] and caspase-8 [23]. Furthermore, it was also discovered that caspase activation eventually led to the cleavage of GSDMD and subsequent formation of pores within cell membranes resulting in membrane rupture [21].

Molecular mechanism of pyroptosis

Ultimately, pyroptosis is a form of programmed cell death like apoptosis or necroptosis.

Apoptosis and pyroptosis has some similar end results such as chromatin condensation, direct DNA damage and membrane blebbing [24–26]. However, the differences in the inflammatory cascade activation and associated local effects are different. Apoptosis is generally considered to be anti-inflammatory - akin to a controlled demolition, whereas pyroptosis is an inflammatory form of programmed cell death more like an 'explosion' with associated 'damage' effecting nearby cells [27]. Ultimately, pyroptosis differs from apoptosis in that cell death arises from plasma membrane disruption, organelle swelling and mitochondria dysfunction. The result of pyroptosis is that the GSDMD-N forms pores within the cell membrane. These pores serve two functions, to allow water molecules to enter the cell and cause swelling and rupture, and furthermore allow for the escape of IL-1β and IL-18 [28].

There are two main pathways in which pyroptosis are executed: either through the canonical or non-conical pathway. The non-conical pathway is mainly activated by lipopolysaccharide (LPS), and therefore will not be covered in this manuscript [29]. The canonical pathway is directly mediated by inflammasome assembly, which leads to GSDMD cleavage into GSDMD-N and of IL-1β and IL-18 release [30]. Inflammasomes are molecular complexes that are formed in response to injury, which could be from toxins, bacteria, viruses or ischemic injury [31]. The assembly of the inflammasome begins when pattern recognition receptors (PRRs) recognize pathogen-associated molecular patterns (PAMPs) and DAMPs (which are generated during IRI) [32]. Activation of PRRs leads to the assembly of procaspase-1, adapter apoptosis-associated speck-like protein containing a caspase recruitment domain (i.e., ASC) and NOD-like receptors (NLRs) to form the inflammasome [33]. Specific to IRI, the NLRs involved in inflammasome formation are mainly NLR family pyrin domain containing 3 (NLRP3). As its name suggests, NLRP3 consists of a leucine-rich repeat, a nucleotide-binding oligomerization domain, and pyrin domain (PYD) [34]. After inflammasome assembly, pro-caspase-1 is cleaved in to its active form, caspase-1 [35]. Caspase-1 serves two functions. First, caspase-1 cleaves the executor protein GSDMD at the Asp275 site to form the 31 kDa GSDMD-N [25], which subsequently interacts with the cell membrane to form pores leading to swelling and pyroptosis [36]. Secondarily, caspase-1 cleaves pro-IL-1β and pro-IL-18 to their active forms, IL-1β and IL-18. These inflammatory cytokines are additionally released through the pores formed by GSDMD-N, resulting in further pyroptosis and damage (Figure 2) [37, 38].

While there has been much research around the NLRP3/GSDMD axis regarding IRI, recently Hou et al. discovered that under hypoxic conditions, gasdermin-C (GSDMC) might play an important role as well. Under hypoxic conditions, the activation of p-Stat3 facilitates the movement of PD-L1 into the cell nucleus. Once inside the nucleus, PD-L1 and p-Stat3 collaborate to enhance the production of GSDMC. Furthermore, they found that caspase-8, which is activated by TNF-α coming from macrophages, is capable of cleaving GSDMC to produce an N-terminal (GSDMC-N) to again form pores within the plasma membrane and trigger pyroptosis [39].

Role of pyroptosis in lung transplantation

No cure exists for patients with end-stage lung disease and lung transplant is the only effective treatment strategy. The transplant waiting list grows each year though the number of transplants performed annually has stagnated at ~2,000/year since 2010 [5]. The lack of quality lung donors is limiting with only 17 % of donated lungs successfully being transplanted [40]. Of those patients fortunate enough to receive a transplant, ~30 % will develop severe PGD3, which detrimentally impacts patient outcomes and survival [11, 12]. An evolving strategy to address the critical unmet demand for viable lungs suitable for transplantation involves the utilization of extended criteria donors (ECD), notably through the implementation of donation after circulatory death (DCD) [8]. In the DCD process, a donor has care withdrawn and if the donor passes in an acceptable time (up to 120 min for the lung) [41], an emergent organ recovery is performed. Unfortunately, IRI, which affects all donor organs universally in transplantation, is especially pronounced in these DCD allografts. As one might expect, this

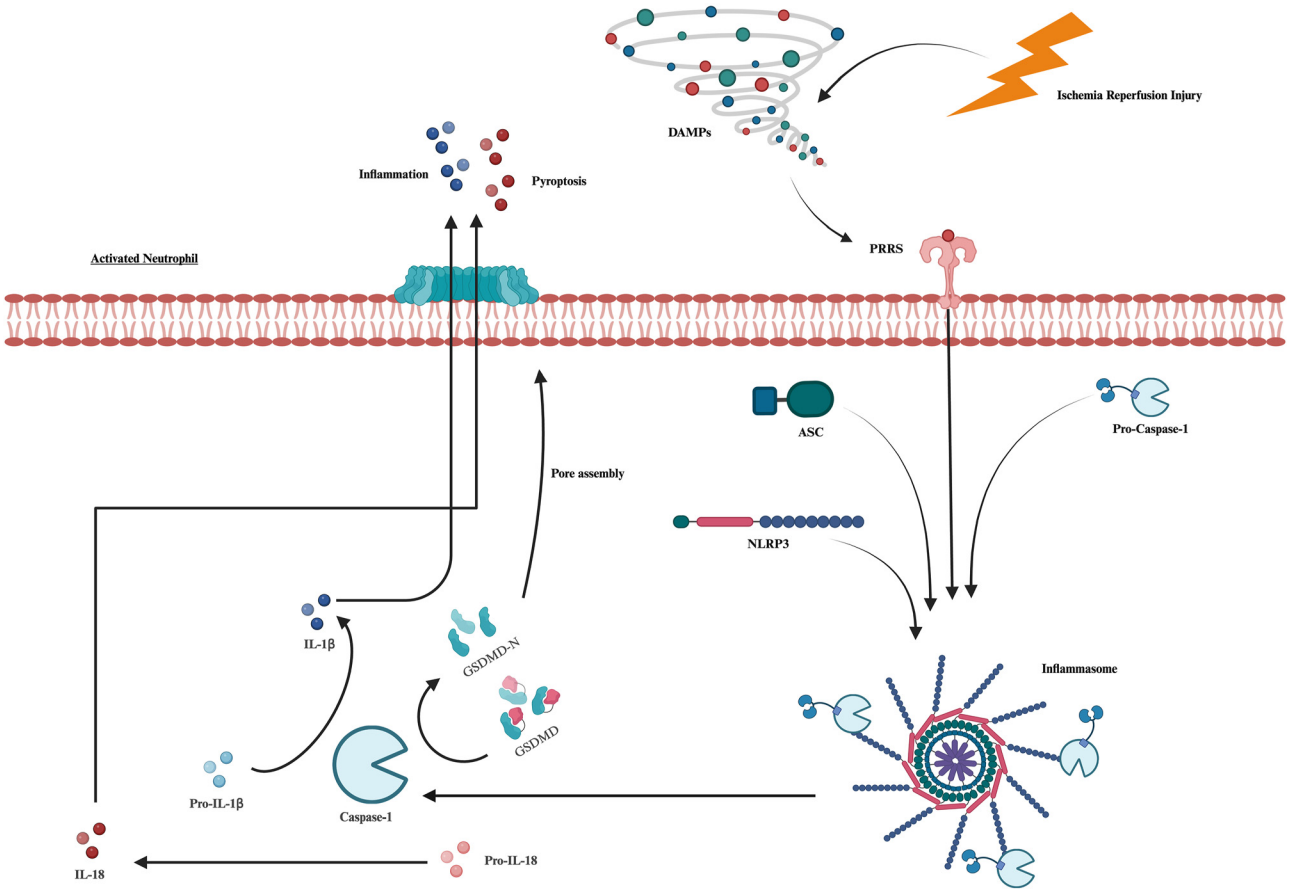

Figure 2: Canonical pathway for pyroptosis. The assembly of the inflammasome begins when pattern recognition receptors (PRRs) recognize damage-associated molecular patterns (DAMPs). Activation of PRRs leads to the assembly of pro-caspase-1, adapter apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) and NOD-like receptors family pyrin domain containing 3 (NLRP3) to form the inflammasome. After inflammasome assembly, pro-caspase-1 is cleaved into its active form, caspase-1. Caspase-1 cleaves: (1) the executor protein Gasdermin-D (GSDMD) to form its N-terminus (GSDMD-N), which subsequently interacts with the cell membrane to form pores leading to swelling and pyroptosis and; (2) cleaves pro-IL-1β and pro-IL-18 to their activate forms, IL-1β and IL-18.

uncontrolled death process and variable period of warm ischemia leads to a great deal of variability in donor quality which has the potential to manifest as poor recipient outcomes.

IRI drives the development of PGD whose hallmark is inflammation, leukocyte infiltration, and the activation of cellular immune responses that causes morbidity and mortality [13, 42]. This inflammation leads to cell death, as shown by cell-death related pathways being enriched during transplantation, and during machine perfusion prior to transplantation [43]. As discussed previously, pyroptosis, a lytic form of cell death, is a key pathway triggering inflammation [21, 25, 28, 30, 33, 35, 36, 38]. Pyroptosis is critical for inflammatory response to lung IRI [44, 45], and current studies have shown that IL-1β levels are significantly higher in lung allografts that are deemed unsuitable for transplantation [46]. Specific to lung IRI, monocytes have been shown to promote pyroptosis in endothelial cells in transplant relevant models of injury [45]. The endo-epithelial barrier is of extreme important to the overall integrity of the alveolar space, and pyroptosis mediated damage to the endothelium serves as significant injury that leads to poor graft function and eventually PGD. In addition to endothelial cells, GSDMD can be primarily found within neutrophils, macrophages, and monocytes [44, 45, 47] as well. Specifically important to the pathophysiology of PGD in lung transplantation is the presence of residual donor nonclassical monocytes (NCM) within the pulmonary vasculature, and their ability to recruit recipient neutrophils into the lung allograft.

Donor derived NCMs are retained within the pulmonary vasculature following procurement of a lung allograft, despite adequate measures to rid the allograft of donor immune cells [48]. Upon IRI, these NCMs within the

pulmonary vasculature through MyD88 signaling are responsible for CXCL2 production which attracts recipient neutrophils to the newly transplanted allograft upon implantation. These NCMs additionally release IL-1β through pyroptosis (NLRP3 dependent) which activates alveolar macrophages, and which through CCL2 signaling recruits classical monocytes (CM) to the allograft as well. Once recruited to the area of injury, CM produces IL-1β through PKC- and NF-κB–dependent but PI3K-independent pathway [14]. The presence of IL-1β within the pulmonary vasculature activates endothelial cells to downregulate vascular endothelial cadherin (VE-cadherin) transcription, which results in the destruction of tight junctions that are critical to endo-epithelial integrity [49]. Ultimately, the loss of barrier function allows for neutrophil extravasation into the alveolar space, pulmonary edema, loss of pulmonary function and ultimately PGD [14, 48]. While IRI is a trigger for pyroptosis, and ultimately leads to PGD development and adverse reactions in the immediate post-operative period, it can also have detrimental long-term effects which are also pyroptotic involved as well.

PGD can progress to chronic lung allograft dysfunction (CLAD), which exists along a clinical spectrum that ranges from transient dyspnea to hypoxemic respiratory failure needing re-transplantation. The definition of CLAD has evolved over the past 5–8 years and now encompasses the older bronchiolitis obliterans syndrome (BOS) and the newer restrictive allograft syndrome (RAS). CLAD is a spectrum of chronic dysfunction inclusive of RAS and BOS with fibrosis as a significant contributor [50–52]. Though allograft rejection is mediated by many factors, acquired immunity plays an important role. Type 1 T helper (Th1) and Type 17 Thelper (Th17) cellular response can be modified by both IL-1β and IL-18, thus inhibiting regulatory T-cells, contributing to the development of CLAD [53]. Though current studies surrounding pyroptosis and lung transplant have shown its longitudinal involvement, they are still limitations.

There are many cell types in the pulmonary parenchyma, e.g., alveolar macrophages, interstitial macrophages, AT1 and AT2 pneumocytes, neutrophils and fibroblasts [54]. Though pyroptosis during lung IRI or lung transplant has been implicated in several cell types, further research is needed to see if other cell types are involved as well. Though not transplant specific, there are several other disease pathophysiology that offer key insights. In a disease with a pathophysiology most closely related to PGD, acute respiratory distress syndrome (ARDS), GSDMD was evident in pneumocytes [55]. Within other pulmonary vascular diseases, such as pulmonary artery hypertension, pyroptosis has been found to contribute to endothelial cell dysfunction and breakdown of this important barrier [56]. Specifically, hypoxia caspase-1 mediated damage has been implicated in several studies [57, 58]. Additionally, caspase-4/11 mediated pyroptosis has been implicated as well [59]. While none of these studies were specific to transplant associated IRI, hypoxic injury has been shown to induce pyroptosis within the endothelium, adding to the body of evidence that this injury pattern would be prevalent in peri-operative period during lung transplantation.

Thus, as PGD leads to significant adverse outcomes, agents providing effective mitigation of pyroptosis are novel and of therapeutic benefit. There are no Food and Drug Administration (FDA) approved treatments to heal lung endothelial and epithelial barrier functions, and therefore investigation of therapeutics that inhibit pyroptosis is of scientific value.

Mitigation of pyroptosis and novel therapeutics within lung transplantation

The injury, activation and subsequent dysfunction of the endothelium as well as associated immune cells are central to the pathophysiology of IRI, pyroptosis and PGD. While all lung allografts suffer a degree of IRI, this injury is becoming more prevalent. In solid organ transplantation, there is a critical shortage of adequate donor organs [1, 2]. For all organs, the waitlist is much larger than the number of available organs. In 2020, there were 107,925 total waitlist candidates, but only 39,035 total transplants were performed, leading to an average of 15.5 patients dying on the waitlist daily [2]. Clearly, only a small fraction of potential patients can access transplantation, and increased access could provide a significant public health benefit. To meet the gap in demand and prevent death, transplant teams increasingly turn to marginal or extended criteria donors [2]. In particular, DCD is associated with increased warm ischemic injury [8]. Furthermore, marginal donor organs will make up a larger proportion of transplants in the future as patients in need of life-saving transplants are steadily increasing [1, 6–10]. A primary factor preventing expanded access is that marginal quality donor organs suffer disproportionately worse IRI and activation of pyroptosis [60].

Currently, organ viability is assessed largely by chest X-ray and arterial blood gases which are crude metrics for PGD. As we are not able to predict the deleterious PGD, as a transplant community, we discard ~80 % of potential donor

lung out of fear of PGD [61]. PGD leads to pulmonary vascular endothelial cell activation and increased vascular permeability, which results in increased extravascular water/edema and, ultimately respiratory failure. Thus, as we are not able to predict PGD and it leads to significant adverse outcomes [62], agents providing effective mitigation of inflammation and PGD are a novel and therapeutic benefit. Utilizing extended criteria donors (i.e., DCD donors), may ultimately help us bridge the gap in available donors. However, there are no current Food and Drug Administration-approved therapies to ameliorate the damage inherent in the DCD donor use and to mitigate the formation of PGD. Thus, improving the function of these organs prior to transplantation is critical to improving transplant outcomes. There are however several potential therapeutic treatments undergoing investigation (Figure 3).

Caspase-1 inhibitors

There are several caspase-1 inhibitors that have been used in the past to combat pyroptosis [63–65]. VX-740 and VX-765 both reversibly catalyze the active site of caspase-1, which showed decreased release of IL-1β and IL-18 [65], as well as disease mitigation (arthritis) [64]. VX-165 has been studied in IRI, and has been shown to decrease Kupffer cell damage and pyroptosis [66], neuronal ischemic insult [67] as well as pulmonary endothelial damage [68]. In both *in vitro* and *in vivo* models, VX-165 was able to decrease markers of pyroptosis, inflammatory and preserve lung tissue architecture. While this study did not involve transplantation as a specific *in vivo* model, *in vivo* IRI still offers valuable preclinical information that can inform clinicians to create further studies.

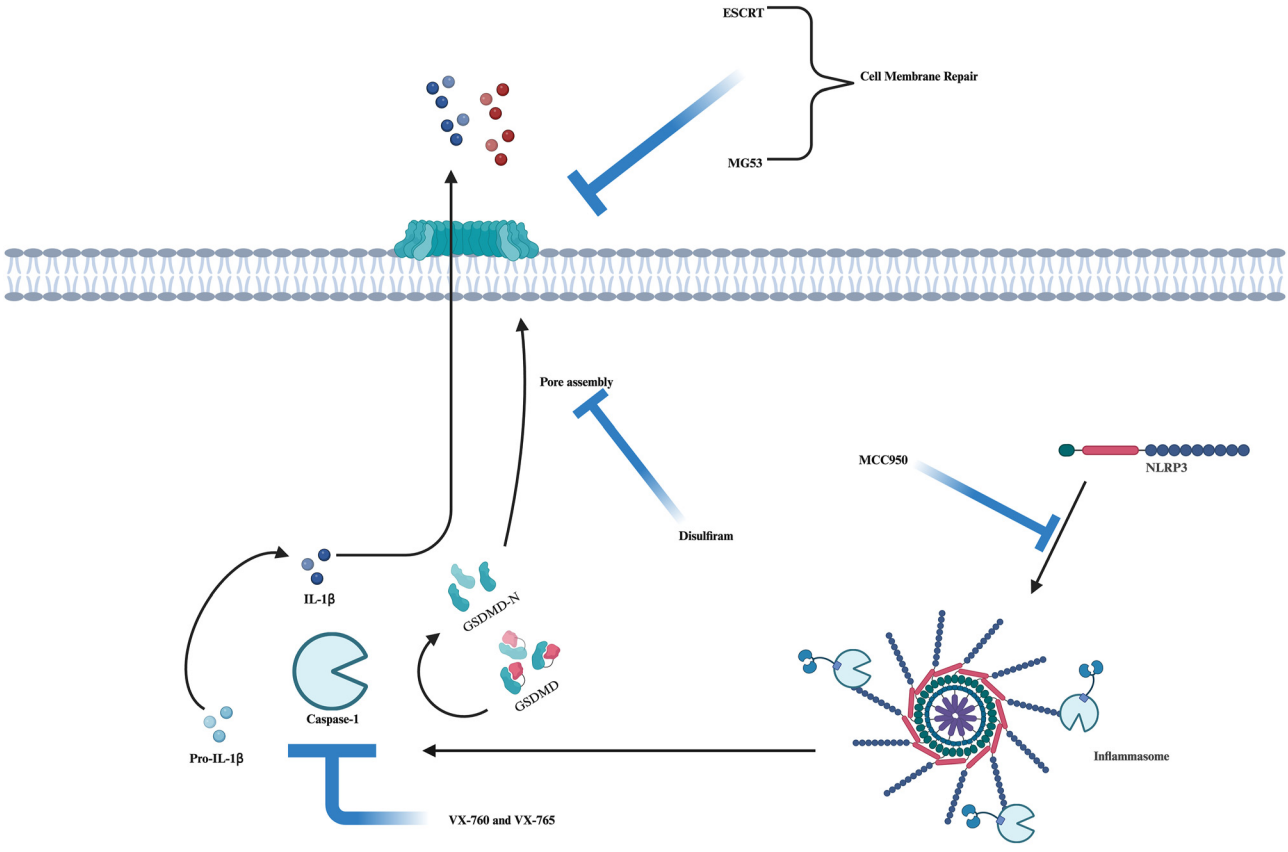

Figure 3: Mitigation of pyroptosis and novel therapeutics. *Caspase-1 Inhibitors*: VX-740 and VX-765 both reversibly catalyze the active site of caspase-1. *NOD-like receptors family pyrin domain containing 3 (NLRP3) Inhibitors*: MCC950 is a small molecular inhibitor that inhibits NLRP3 activation in both the canonical and non-canonical pathway by direct inhibition of the NATCH domain and further motifs that prevent oligomerization. *Gasdermin (GSDMD) Inhibitors*: Disulfiram blocks GSDMD pore formation during pyroptosis. Hu et al. discovered that disulfiram covalently modifies Cys191/192 in GSDMD, thus inhibiting pore formation while still allowing for the formation of IL-1β. *Cell Membrane Repair*: Endosomal sorting complexes required for transport (ESCRT) machinery can repair the plasma membrane upon GSDMD activation. Mitsugumin 53 (MG53) with tripartite motif-containing (TRIM) family protein also repairs cell membranes.

NLRP3 inhibitors

MCC950 is perhaps the best studied NLRP3 inhibitor in regard to transplantation in general [69–71]. MCC950 is a small molecular inhibitor that inhibits NLRP3 activation in both canonical and non-canonical pathway by direct inhibition of the NATCH domain and further motifs that prevent oligomerization [72, 73]. In a large animal model of DCD liver transplantation, the treatment of MCC950 results in improved allograft function as well decreased markers of inflammation [70]. More relevant to pulmonary anatomy and physiology, Xu et al. investigated MCC950 and its ability to preserve function during heart transplantation. The addition of MCC950 either after transplantation or during *ex vivo* perfusion prior to transplantation resulted in decreased oxidative stress, preservation of function and ultimately ameliorated cardiac IRI [71]. While there are no studies pertaining directly to lung transplantation, MCC950 has been shown to mitigate injury from pneumonia [74].

Gasdermin inhibitors

Disulfiram is an inhibitor of acetaldehyde dehydrogenase and is approved in the treatment of alcohol abuse. Additionally, Disulfiram was found to block GSDMD pore formation during pyroptosis. Hu et al. discovered that Disulfiram covalently modifies Cys191/192 in GSDMD, thus inhibiting pore formation while still allowing for the formation of IL-1β [75]. This is particularly exciting as Disulfiram is already FDA-approved, which could lead to more rapid studies within humans to determine its therapeutic efficacy in decreasing pyroptotic inflammation. Again, no specific lung transplant studies exist focusing on disulfiram mitigation of pyroptosis, and fibrotic injury within lung parenchyma following toxic administration has been shown to be mitigated by its use [76].

There are several pathways that have been shown to directly decrease GSDMD pore formation as well. Endosomal sorting complexes required for transport (ESCRT) machinery have been proposed to repair the plasma membrane upon GSDMD activation [77]. Rühle et al. found that ESCRT machinery was involved in the cellular membrane repair process following GSDMD pore formation, and that lack of ESCRT greatly enhanced pyroptotic effects within a cell. Though there are no current therapeutics that enhance the ESCRT pathway in order to mitigate pyroptosis, cell membrane repair is a promising avenue as well to mitigate GSDMD pore formation. Mitsugumin 53 (MG53) with tripartite motif-containing (TRIM) family protein is also involved in cell membrane repair. MG53 functions by sensing the oxidized extracellular environment upon injury, subsequently undergoing oligomerization, formatting a repair complex via tethering of intracellular vesicles and fusing these vesicles with the plasma membrane to form the repair patch [78, 79]. MG53 has been found in alveolar epithelial cells [80], and administration of an exogenously produced human recombinant MG53 is a potential novel therapeutic for the preservation of the pulmonary parenchyma by decreasing pyroptosis after IRI during transplantation [15, 81].

Limitations with current therapies

Unfortunately, none of these therapies have undergone human testing within the realm of lung transplantation. Therefore, it is difficult to say which class of inhibitor (GSDMD, caspase-1, NLRP3, etc.) would have the most efficacy during lung transplantation. Perhaps the most promising avenue would in fact be GSDMD inhibitors, i.e., disulfiram, as this is already an FDA approved therapy (and has known tolerability/side effects), albeit its use in lung transplantation would be 'off-label' [75, 76]. Though a dose for this drug has been established (for its FDA approved use), it would still take additional studies to determine what dose would effectively mitigate pyroptosis, and what additional side effects could potentially arise. Perhaps another promising GSDMD inhibitor could be human recombinant MG53 (rhMG53) as well. As previously mentioned, MG53 is an endogenous protein, and therefore exogenous administration of rhMG53 could be a viable therapy with minimal side effects and tolerable treatment profile [15, 79–81]. However, again this must be further substantiated in additional preclinical and clinical studies.

Caspase-1 inhibitors, though having very favorable preclinical results, have not had good long-term results in humans. VX-765 has in fact undergone human testing in several clinical trials for a variety of diseases, and has poor tolerability with an increased rate of adverse side effects [82]. VX-740 has also been tested in humans, showing mitigation of inflammation, though long term has been shown to have increased rates of liver toxicity [82]. Like caspase-1 inhibitors, MCC950 (NLRP3 inhibitor) has also shown liver toxicity as a side effect in human trials [83]. Ultimately, further studies are needed to develop pyroptotic inhibitors which are tolerable, safe, and can effectively mitigate disease.

Ex vivo lung perfusion and the future of allograft repair and modification

While the invention and efficacy of creating novel therapeutics to combat pyroptosis is still a developing field, clinicians should also be focusing on ways to deliver such drugs. The obvious answer is intravenous delivery to either the donor or recipient upon implantation, or a possible oral form drug is given to the recipient over several days following transplantation. However, these delivery strategies pose several issues. The delivery of a drug to a donor poses multiple logistical issues. As these donors are often at community hospitals or potentially several hundreds of miles away at a facility that might not carry novel therapeutics or have the necessary storage equipment, like a quaternary referral center might, it is difficult to imagine this strategy to be the most fruitful in mitigating injury. Additionally, treating the donor immediately upon reperfusion may be able to prevent the reperfusion injury while it cannot mitigate the ischemic insult. Finally, as pyroptotic injury occurs quickly (within 24 h), treating the recipient over several days following transplantation might not be the most feasible way to ameliorate this injury pattern.

One new and aggressive approach to meeting this need for adequate donor organs for transplantation is utilizing normothermic *ex vivo* lung perfusion (EVLP) as a way to assess the potential allograft function, resuscitate the allograft, and maintain metabolic viability to support a successful transplant [40, 84]. EVLP allows for enhanced evaluation of potential extended criteria donor allografts in a closed circuit outside the body to determine if the organ is in fact suitable for use, thus increasing the number of transplanted lungs [85]. The closed circuit is achieved by creating a ventilatory circuit and vascular circuit. Ventilation is achieved by attaching the trachea to a ventilator, and a vascular circuit is creating by connecting the left atrium to the pulmonary artery with plastic tubing allowing acellular solution to flow with the use of a centrifugal pump [86, 87].

EVLP also allows for the delivery of therapeutics. Currently, EVLP is part of standard clinical practice throughout the world [86, 88, 89], and is used by both transplant programs and external perfusion centers [90] to actively repair and resuscitate potential donor allografts. This practice has not only increased the donor pool to make transplantation more achievable for all but has done so safely with minimal adverse effects in recipients [85, 86, 91]. Currently, EVLP perfusates use either cellular (with red blood cell addition) or acellular perfusates. These perfusates are comprised of low-potassium dextran, glucose, bicarbonate, human serum albumin and additional steroids and antibiotics [92]. Currently, this perfusate is sufficient to rehabilitate prospective lung allografts, mitigate damage and resuscitate them to be suitable for transplantation [93, 94]. However, the next evolution of this technology is to apply innovative therapeutics and strategies within the platform that will be able to better assess, repair and modify organs that are severally damage (i.e., unsuitable to transplantation) and make them safe for transplantation [84, 87].

Currently there are several exciting applications of EVLP as they pertain to modifying and resuscitating potential allografts. Mesaki et al. have demonstrated that through CRISPR modulation with IL-10 gene therapy, rodent lungs were able to overexpress IL-10 leading to protection, decreased inflammation and ultimately proof of concept that gene therapy is possible through this exciting technology [95]. Taking this a step further, Cypel et al. were able to demonstrate similar results in human lungs [96]. Beyond gene therapy, mesenchymal stromal cell therapy is also possible with EVLP as well [97]. In addition to gene and cell therapy, Wang et al. showed that enzymatic additives within the perfusate administered during EVLP could change red blood cell groups during 4-h of clinical EVLP in human lungs [98].

Thus, EVLP is an ideal treatment modality to apply novel therapeutics to repair and resuscitate damaged lung allografts. We envision that the previously described treatment modalities will be able to be added to current perfusates and allow for mitigation of pyroptosis. An added benefit of *ex vivo* repair is that there are minimal off-target effects, thus potentially mitigating poor tolerability of NLRP3 and caspase-1 inhibitors. Ultimately, developing novel therapeutic regiments to combat pyroptosis to be used during EVLP will increase the donor pool, thus making this life saving therapy achievable for all.

Conclusions

Pyroptosis is a significant pathway that contributes to injury early in the post-operative phase following lung transplantation and can affect the development of CLAD as well. Pyroptosis can be triggered by many stimuli, but during transplantation this is often the result of IRI. Once cells sense DAMPs (as a result from IRI), inflammasome assembly occurs which leads to GSDMD cleavage into GSDMD-N, as well as IL-1β and IL-18 release. IL-1β release is also crucial in the innate cellular response that leads to PGD as well. Long-term, PGD and further activation of the acquire immune response via IL-1β and IL-18 contributes to the development of CLAD. Thus, pyroptosis is implicated

in all phases of lung transplantation, though its most harmful effects happen early in the post-operative period. Pyroptosis can be mitigated through several different avenues to include caspase-1, NLRP3, GSDMD inhibition and direct cellular membrane repair. Though many of these inhibitors have been tested in humans, few are promising as of yet, emphasizing the investigation of therapeutics that inhibit pyroptosis is of scientific value. To deliver novel therapeutics there are several methods and timepoints, but considering logistic constraints as well as optimal current modern day therapeutic approaches, EVLP offers an ideal approach. By mitigating the harmful effects of pyroptosis, transplantation will become safer for patients, thus furthering the potential of this life altering treatment.

Research ethics: Not applicable.
Informed consent: Not applicable.
Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Competing interests: Authors state no conflict of interest.
Research funding: BAW is supported through the National Institute of Health (NIH) grant R01 HL143000 and serves on the Clinical Events Committee of TransMedics OCS. Hua Zhu is supported by the NIH grants: HL153876, EY030621, EY032583 and the American Heart Association grant: 23TPA1142638. This research was also generously supported through the Jewel and Frank Benson Family Endowment and The Jewel and Frank Benson Research Professorship at The Ohio State University.
Data availability: Not applicable.

References

1. OPTN/SRTR 2019 annual data report: introduction. Am J Transplant. 2021;21:11–20.
2. UNOS. United network for organ sharing; 2022. Available from: https://unos.org/.
3. Colvin-Adams M, Valapour M, Hertz M, Heubner B, Paulson K, Dhungel V, et al. Lung and heart allocation in the United States. Am J Transplant 2012;12:3213–34.
4. Valapour M, Skeans MA, Heubner BM, Smith JM, Hertz MI, Edwards LB, et al. OPTN/SRTR 2013 annual data report: lung. Am J Transplant 2015; 15:1–28.
5. Valapour M, Lehr CJ, Schladt DP, Smith JM, Goff R, Mupfudze TG, et al. OPTN/SRTR 2021 annual data report: lung. Am J Transplant 2023;23: S379–442.
6. Colvin M, Smith JM, Ahn Y, Skeans MA, Messick E, Goff R, et al. OPTN/SRTR 2019 annual data report: heart. Am J Transplant 2021;21:356–440.
7. Hart A, Lentine KL, Smith JM, Miller JM, Skeans MA, Prentice M, et al. OPTN/SRTR 2019 annual data report: kidney. Am J Transplant 2021;21: 21–137.
8. Israni AK, Zaun D, Rosendale JD, Schaffhausen C, McKinney W, Snyder JJ. OPTN/SRTR 2019 annual data report: deceased organ donors. Am J Transplant 2021;21:521–58.
9. Kwong AJ, Kim WR, Lake JR, Smith JM, Schladt DP, Skeans MA, et al. OPTN/SRTR 2019 annual data report: liver. Am J Transplant 2021;21: 208–315.
10. Valapour M, Lehr CJ, Skeans MA, Smith JM, Miller E, Goff R, et al. OPTN/SRTR 2019 annual data report: lung. Am J Transplant 2021;21:441–520.
11. Whitson BA, Prekker ME, Herrington CS, Whelan TP, Radosevich DM, Hertz MI, et al. Primary graft dysfunction and long-term pulmonary function after lung transplantation. J Heart Lung Transplant 2007;26: 1004–11.
12. Whitson BA, Nath DS, Johnson AC, Walker AR, Prekker ME, Radosevich DM, et al. Risk factors for primary graft dysfunction after lung transplantation. J Thorac Cardiovasc Surg 2006;131:73–80.
13. Chacon-Alberty L, Fernandez R, Jindra P, King M, Rosas I, Hochman-Mendez C, et al. Primary graft dysfunction in lung transplantation: a review of mechanisms and future applications. Transplantation 2023; 107:1687–97.
14. Kurihara C, Lecuona E, Wu Q, Yang W, Núñez-Santana FL, Akbarpour M, et al. Crosstalk between nonclassical monocytes and alveolar macrophages mediates transplant ischemia-reperfusion injury through classical monocyte recruitment. JCI Insight 2021;6:e147282.
15. Gouchoe DA, Lee YG, Kim JL, Zhang Z, Marshall JM, Ganapathi A, et al. Mitsugumin 53 mitigation of ischemia reperfusion injury in a mouse model. J Thorac Cardiovasc Surg 2023;167:e48–58.
16. Burdette BE, Esparza AN, Zhu H, Wang S. Gasdermin D in pyroptosis. Acta Pharm Sin B 2021;11:2768–82.
17. Zychlinsky A, Prevost MC, Sansonetti PJ. *Shigella flexneri* induces apoptosis in infected macrophages. Nature 1992;358:167–9.
18. Chen Y, Smith MR, Thirumalai K, Zychlinsky A. A bacterial invasin induces macrophage apoptosis by binding directly to ICE. EMBO J 1996; 15:3853–60.
19. D'Souza CA, Heitman J. Dismantling the Cryptococcus coat. Trends Microbiol 2001;9:112–3.
20. Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol Cell 2002;10:417–26.
21. Kovacs SB, Miao EA. Gasdermins: effectors of pyroptosis. Trends Cell Biol 2017;27:673–84.
22. Wang Y, Gao W, Shi X, Ding J, Liu W, He H, et al. Chemotherapy drugs induce pyroptosis through caspase-3 cleavage of a gasdermin. Nature 2017;547:99–103.
23. Newton K, Wickliffe KE, Maltzman A, Dugger DL, Reja R, Zhang Y, et al. Activity of caspase-8 determines plasticity between cell death pathways. Nature 2019;575:679–82.
24. Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 1972;26: 239–57.
25. Chen X, He WT, Hu L, Li J, Fang Y, Wang X, et al. Pyroptosis is driven by non-selective gasdermin-D pore and its morphology is different from MLKL channel-mediated necroptosis. Cell Res 2016;26:1007–20.
26. Rudel T, Bokoch GM. Membrane and morphological changes in apoptotic cells regulated by caspase-mediated activation of PAK2. Science 1997;276:1571–4.
27. Tang R, Xu J, Zhang B, Liu J, Liang C, Hua J, et al. Ferroptosis, necroptosis, and pyroptosis in anticancer immunity. J Hematol Oncol 2020;13:110.
28. Fink SL, Cookson BT. Pillars Article: caspase-1-dependent pore formation during pyroptosis leads to osmotic lysis of infected host macrophages. Cell Microbiol 2006;8:1812–25.

29. Shi J, Zhao Y, Wang Y, Gao W, Ding J, Li P, et al. Inflammatory caspases are innate immune receptors for intracellular LPS. Nature 2014;514: 187–92.
30. Frank D, Vince JE. Pyroptosis versus necroptosis: similarities, differences, and crosstalk. Cell Death Differ 2019;26:99–114.
31. Thi HTH, Hong S. Inflammasome as a therapeutic target for cancer prevention and treatment. J Cancer Prev 2017;22:62–73.
32. Strowig T, Henao-Mejia J, Elinav E, Flavell R. Inflammasomes in health and disease. Nature 2012;481:278–86.
33. Bergsbaken T, Fink SL, Cookson BT. Pyroptosis: host cell death and inflammation. Nat Rev Microbiol 2009;7:99–109.
34. Swanson KV, Deng M, Ting JPY. The NLRP3 inflammasome: molecular activation and regulation to therapeutics. Nat Rev Immunol 2019;19: 477–89.
35. Sollberger G, Strittmatter GE, Garstkiewicz M, Sand J, Beer HD. Caspase-1: the inflammasome and beyond. Innate Immun 2014;20: 115–25.
36. Sborgi L, Rühl S, Mulvihill E, Pipercevic J, Heilig R, Stahlberg H, et al. GSDMD membrane pore formation constitutes the mechanism of pyroptotic cell death. EMBO J 2016;35:1766–78.
37. Thornberry NA, Bull HG, Calaycay JR, Chapman KT, Howard AD, Kostura MJ, et al. A novel heterodimeric cysteine protease is required for interleukin-1 beta processing in monocytes. Nature 1992;356: 768–74.
38. Liu X, Zhang Z, Ruan J, Pan Y, Magupalli VG, Wu H, et al. Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. Nature 2016;535:153–8.
39. Hou J, Zhao R, Xia W, Chang CW, You Y, Hsu JM, et al. PD-L1-mediated gasdermin C expression switches apoptosis to pyroptosis in cancer cells and facilitates tumour necrosis. Nat Cell Biol 2020;22:1264–75.
40. Whitson BA, Hayes D Jr. Indications and outcomes in adult lung transplantation. J Thorac Dis 2014;6:1018–23.
41. Cypel M, Levvey B, Van Raemdonck D, Erasmus M, Dark J, Love R, et al. International society for heart and lung transplantation donation after circulatory death registry report. J Heart Lung Transplant 2015;34: 1278–82.
42. Van Slambrouck J, Van Raemdonck D, Vos R, Vanluyten C, Vanstapel A, Prisciandaro E, et al. A focused review on primary graft dysfunction after clinical lung transplantation: a multilevel syndrome. Cells 2022;11: 745.
43. Wong A, Zamel R, Yeung J, Bader GD, Dos Santos CC, Bai X, et al. Potential therapeutic targets for lung repair during human *ex vivo* lung perfusion. Eur Respir J 2020;55:1902222.
44. Zhou P, Song N-C, Zheng Z-K, Li Y-Q, Li J-S. MMP2 and MMP9 contribute to lung ischemia–reperfusion injury via promoting pyroptosis in mice. BMC Pulm Med 2022;22:230.
45. Zhou P, Guo H, Li Y, Liu Q, Qiao X, Lu Y, et al. Monocytes promote pyroptosis of endothelial cells during lung ischemia-reperfusion via IL-1R/NF-κB/NLRP3 signaling. Life Sci 2021;276:119402.
46. Cypel M, Kaneda H, Yeung JC, Anraku M, Yasufuku K, de Perrot M, et al. Increased levels of interleukin-1β and tumor necrosis factor-α in donor lungs rejected for transplantation. J Heart Lung Transplant 2011;30: 452–9.
47. Liu X, Xia S, Zhang Z, Wu H, Lieberman J. Channelling inflammation: gasdermins in physiology and disease. Nat Rev Drug Discov 2021;20: 384–405.
48. Zheng Z, Chiu S, Akbarpour M, Sun H, Reyfman PA, Anekalla KR, et al. Donor pulmonary intravascular nonclassical monocytes recruit recipient neutrophils and mediate primary lung allograft dysfunction. Sci Transl Med 2017;9:eaal4508.
49. Xiong S, Hong Z, Huang LS, Tsukasaki Y, Nepal S, Di A, et al. IL-1β suppression of VE-cadherin transcription underlies sepsis-induced inflammatory lung injury. J Clin Invest 2020;130:3684–98.
50. Verleden GM, Glanville AR, Lease ED, Fisher AJ, Calabrese F, Corris PA, et al. Chronic lung allograft dysfunction: definition, diagnostic criteria, and approaches to treatment–A consensus report from the Pulmonary Council of the ISHLT. J Heart Lung Transplant 2019;38:493–503.
51. Glanville AR, Verleden GM, Todd JL, Benden C, Calabrese F, Gottlieb J, et al. Chronic lung allograft dysfunction: definition and update of restrictive allograft syndrome–A consensus report from the Pulmonary Council of the ISHLT. J Heart Lung Transplant 2019;38:483–92.
52. Snell GI, Yusen RD, Weill D, Strueber M, Garrity E, Reed A, et al. Report of the ISHLT Working Group on primary lung graft dysfunction, part I: definition and grading-A 2016 consensus group statement of the International Society for heart and lung transplantation. J Heart Lung Transpl 2017;36:1097–103.
53. Sun J, Li Y. Pyroptosis and respiratory diseases: a review of current knowledge. Front Immunol 2022;13:920464.
54. Sikkema L, Ramírez-Suástegui C, Strobl DC, Gillett TE, Zappia L, Madissoon E, et al. An integrated cell atlas of the lung in health and disease. Nat Med 2023;29:1563–77.
55. Li S, Jiang L, Li X, Lin F, Wang Y, Li B, et al. Clinical and pathological investigation of patients with severe COVID-19. JCI Insight 2020;5: e138070.
56. Zhang M, Xin W, Yu Y, Yang X, Ma C, Zhang H, et al. Programmed death-ligand 1 triggers PASMCs pyroptosis and pulmonary vascular fibrosis in pulmonary hypertension. J Mol Cell Cardiol 2020;138:23–33.
57. Udjus C, Cero FT, Halvorsen B, Behmen D, Carlson CR, Bendiksen BA, et al. Caspase-1 induces smooth muscle cell growth in hypoxia-induced pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol 2019;316: L999–1012.
58. Cero FT, Hillestad V, Sjaastad I, Yndestad A, Aukrust P, Ranheim T, et al. Absence of the inflammasome adaptor ASC reduces hypoxia-induced pulmonary hypertension in mice. Am J Physiol Lung Cell Mol Physiol 2015;309:L378–87.
59. Wu Y, Pan B, Zhang Z, Li X, Leng Y, Ji Y, et al. Caspase-4/11-Mediated pulmonary artery endothelial cell pyroptosis contributes to pulmonary arterial hypertension. Hypertension 2022;79:536–48.
60. Akande O, Chen Q, Toldo S, Lesnefsky EJ, Quader M. Ischemia and reperfusion injury to mitochondria and cardiac function in donation after circulatory death hearts-an experimental study. PLoS One 2020; 15:e0243504.
61. Punch JD, Hayes DH, LaPorte FB, McBride V, Seely MS. Organ donation and utilization in the United States, 1996-2005. Am J Transplant 2007;7: 1327–38.
62. Gouchoe DA, Whitson BA, Rosenheck J, Henn MC, Mokadam NA, Ramsammy V, et al. Long-term survival following primary graft dysfunction development in lung transplantation. J Surg Res 2024;296: 47–55.
63. Boxer MB, Shen M, Auld DS, Wells JA, Thomas CJ. A small molecule inhibitor of caspase 1. Probe reports from the NIH molecular libraries program. Bethesda (MD): National Center for Biotechnology Information (US); 2010.
64. Rudolphi K, Gerwin N, Verzijl N, van der Kraan P, van den Berg W. Pralnacasan, an inhibitor of interleukin-1beta converting enzyme, reduces joint damage in two murine models of osteoarthritis. Osteoarthritis Cartilage 2003;11:738–46.
65. Wannamaker W, Davies R, Namchuk M, Pollard J, Ford P, Ku G, et al. (S)-1-((S)-2-{[1-(4-amino-3-chloro-phenyl)-methanoyl]-amino}-3,3-dimethyl-butanoyl)-pyrrolidine-2-carboxylic acid ((2R,3S)-2-ethoxy-

5-oxo-tetrahydro-furan-3-yl)-amide (VX-765), an orally available selective interleukin (IL)-converting enzyme/caspase-1 inhibitor, exhibits potent anti-inflammatory activities by inhibiting the release of IL-1beta and IL-18. J Pharmacol Exp Ther 2007;321:509–16.
66. Dai Q, Jiang W, Liu H, Qing X, Wang G, Huang F, et al. Kupffer cell-targeting strategy for the protection of hepatic ischemia/reperfusion injury. Nanotechnology 2021;32:265101.
67. Tang B, Li Y, Xu X, Du G, Wang H. Electroacupuncture ameliorates neuronal injury by NLRP3/ASC/Caspase-1 mediated pyroptosis in cerebral ischemia-reperfusion. Mol Neurobiol 2024;61:2357–66.
68. Wu S, Li Z, Ye M, Liu C, Liu H, He X, et al. VX765, a specific caspase-1 inhibitor, alleviates lung ischemia reperfusion injury by suppressing endothelial pyroptosis and barrier dysfunction. BioMed Res Int 2021; 2021:4525988.
69. Matsuoka T, Yoshimatsu G, Sakata N, Kawakami R, Tanaka T, Yamada T, et al. Inhibition of NLRP3 inflammasome by MCC950 improves the metabolic outcome of islet transplantation by suppressing IL-1β and islet cellular death. Sci Rep 2020;10:17920.
70. Yu Y, Cheng Y, Pan Q, Zhang YJ, Jia DG, Liu YF. Effect of the selective NLRP3 inflammasome inhibitor mcc950 on transplantation outcome in a pig liver transplantation model with organs from donors after circulatory death preserved by hypothermic machine perfusion. Transplantation 2019;103:353–62.
71. Xu L, Zeng Z, Niu C, Liu D, Lin S, Liu X, et al. Normothermic *ex vivo* heart perfusion with NLRP3 inflammasome inhibitor Mcc950 treatment improves cardiac function of circulatory death hearts after transplantation. Front Cardiovasc Med 2023;10:1126391.
72. Tapia-Abellán A, Angosto-Bazarra D, Martínez-Banaclocha H, de Torre-Minguela C, Cerón-Carrasco JP, Pérez-Sánchez H, et al. MCC950 closes the active conformation of NLRP3 to an inactive state. Nat Chem Biol 2019;15:560–4.
73. Coll RC, Hill JR, Day CJ, Zamoshnikova A, Boucher D, Massey NL, et al. MCC950 directly targets the NLRP3 ATP-hydrolysis motif for inflammasome inhibition. Nat Chem Biol 2019;15:556–9.
74. Ye WD, Wang HM, Xu ZJ, Liang DS, Huang AR, Xu ZW, et al. MCC950 ameliorates acute exogenous lipoid pneumonia induced by sewing machine oil in rats via the NF-κB/NLRP3 inflammasome pathway. *In Vivo* 2023;37:2533–42.
75. Hu JJ, Liu X, Xia S, Zhang Z, Zhang Y, Zhao J, et al. FDA-approved disulfiram inhibits pyroptosis by blocking gasdermin D pore formation. Nat Immunol 2020;21:736–45.
76. Hamidi N, Feizi F, Azadmehr A, Zabihi E, Khafri S, Zarei-Behjani Z, et al. Disulfiram ameliorates bleomycin induced pulmonary inflammation and fibrosis in rats. Biotech Histochem 2023;98:584–92.
77. Rühl S, Shkarina K, Demarco B, Heilig R, Santos JC, Broz P. ESCRT-dependent membrane repair negatively regulates pyroptosis downstream of GSDMD activation. Science 2018;362:956–60.
78. Cai C, Masumiya H, Weisleder N, Matsuda N, Nishi M, Hwang M, et al. MG53 nucleates assembly of cell membrane repair machinery. Nat Cell Biol 2009;11:56–64.
79. Gouchoe DA, Whitson BA, Zhu H. The next Frontier in lung transplantation: protecting the endothelium and repairing organs for transplant utilizing MG53. Clin Transl Discov 2023;3:e255.
80. Jia Y, Chen K, Lin P, Lieber G, Nishi M, Yan R, et al. Treatment of acute lung injury by targeting MG53-mediated cell membrane repair. Nat Commun 2014;5:4387.
81. Gouchoe DA, Yi T, Kim JL, Lee YG, Black SM, Breuer C, et al. MG53 mitigates warm ischemic lung injury in A murine model of transplantation. J Thorac Cardiovasc Surg 2023;S0022–5223:01013–9.
82. Dhani S, Zhao Y, Zhivotovsky B. A long way to go: caspase inhibitors in clinical use. Cell Death Dis 2021;12:949.
83. Li H, Guan Y, Liang B, Ding P, Hou X, Wei W, et al. Therapeutic potential of MCC950, a specific inhibitor of NLRP3 inflammasome. Eur J Pharmacol 2022;928:175091.
84. Whitson BA, Black SM. Commentary: organ ARCs: assessment and repair centers in transplantation as gateways to meaningful therapeutic interventions. JTCVS Open 2020;3:173–4.
85. Peel JK, Pullenayegum EM, Naimark D, Aversa M, Liu M, Del Sorbo L, et al. Evaluating the impact of *ex vivo* lung perfusion on organ transplantation: a retrospective cohort study. Ann Surg 2023;278: 288–96.
86. Cypel M, Yeung JC, Liu M, Anraku M, Chen F, Karolak W, et al. Normothermic *ex vivo* lung perfusion in clinical lung transplantation. N Engl J Med 2011;364:1431–40.
87. Gouchoe DA, Shukrallah B, Eggeman C, Igoe D, Ralston J, Mast D, et al. XPS™ Jensen lung as a low-cost, high-fidelity training adjunct to *ex-vivo* lung perfusion. Artif Organs 2023;47:1794–7.
88. Cypel M, Neyrinck A, Machuca TN. *Ex vivo* perfusion techniques: state of the art and potential applications. Intens Care Med 2019;45:354–6.
89. Wang A, Ali A, Keshavjee S, Liu M, Cypel M. *Ex vivo* lung perfusion for donor lung assessment and repair: a review of translational interspecies models. Am J Physiol Lung Cell Mol Physiol 2020;319: L932–l40.
90. Mallea JM, Hartwig MG, Keller CA, Kon Z, Iii RNP, Erasmus DB, et al. Remote *ex vivo* lung perfusion at a centralized evaluation facility. J Heart Lung Transplant 2022;41:1700–11.
91. Peel JK, Keshavjee S, Naimark D, Liu M, Del Sorbo L, Cypel M, et al. Determining the impact of *ex-vivo* lung perfusion on hospital costs for lung transplantation: a retrospective cohort study. J Heart Lung Transplant 2023;42:356–67.
92. Loor G. EVLP: ready for prime time? Semin Thorac Cardiovasc Surg 2019;31:1–6.
93. Jeon JE, Huang L, Zhu Z, Wong A, Keshavjee S, Liu M. Acellular *ex vivo* lung perfusate silences pro-inflammatory signaling in human lung endothelial and epithelial cells. J Transl Med 2023;21:729.
94. Huang L, Vellanki RN, Zhu Z, Wouters BG, Keshavjee S, Liu M. *De novo* design and development of a nutrient-rich perfusate for *ex vivo* lung perfusion with cell culture models. Int J Mol Sci 2023;24: 13117.
95. Mesaki K, Juvet S, Yeung J, Guan Z, Wilson GW, Hu J, et al. Immunomodulation of the donor lung with CRISPR-mediated activation of IL-10 expression. J Heart Lung Transplant 2023;42: 1363–77.
96. Cypel M, Liu M, Rubacha M, Yeung JC, Hirayama S, Anraku M, et al. Functional repair of human donor lungs by IL-10 gene therapy. Sci Transl Med 2009;1:4ra9.
97. Nykänen AI, Liu M, Keshavjee S. Mesenchymal stromal cell therapy in lung transplantation. Bioengineering 2023;10:728.
98. Wang A, Ribeiro RVP, Ali A, Brambate E, Abdelnour-Berchtold E, Michaelsen V, et al. *Ex vivo* enzymatic treatment converts blood type A donor lungs into universal blood type lungs. Sci Transl Med 2022;14: eabm7190.