Where next for cell-based therapy in ARDS

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Acute respiratory distress syndrome (ARDS) is a form of acute hypoxaemic respiratory failure that is characterised by non-cardiogenic pulmonary oedema which occurs as a consequence of the breakdown of the alveolar–capillary unit.[1] ARDS impacts more than 10% of all intensive care unit admissions, and pneumonia is the most common risk factor for ARDS.[2] Current standard of care aims to minimise iatrogenic lung injury from mechanical ventilation,[3 4] while clinical trials of pharmacological therapies that have targeted the underlying mechanisms driving the development of ARDS have failed to show benefit.[5]

Beyond pharmacological therapies, much interest in this field has focused on mesenchymal stromal cells (MSCs) as a potential therapeutic option for ARDS.[6] In the latest development towards a cell-based therapy for ARDS, Park *et al* report the use of an human ex vivo lung perfusion model of ARDS to investigate MSC-derived microvesicles (MVs) as a therapy for ARDS.[7] MSCs are pluripotent cells that can be extracted from various sources including bone marrow, adipose tissue and umbilical cord. In ARDS they may represent an attractive therapy because of a broad range of potentially beneficial effects, including anti-inflammatory, pro-reparative and antimicrobial effects.[8–10] Excessive host inflammation contributes to alveolar epithelial and capillary endothelial damage, and in this setting MSCs have immunomodulatory effects via the secretion of several anti-inflammatory compounds including Interleukin (IL) 10 and IL1 receptor antagonist. Loss of alveolar fluid clearance (AFC) in patients with ARDS contributes to alveolar flooding and is associated with increased hospital mortality.[11] The delivery of MSCs to injured lungs restored AFC in a human ex vivo model of lung injury. This effect was in-part mediated by the secretion of keratinocyte growth factor (KGF), one of several soluble growth factors produced by MSCs.[12] Approximately 60% of ARDS is associated with pneumonia,[2] and MSCs have direct antibacterial effects that may be beneficial in this context, as was demonstrated in a human ex vivo lung model of *Escherichia coli* pneumonia in which treatment with intravenous MSCs significantly reduced alveolar bacterial load.[13] This antimicrobial effect may be mediated by secreted peptides and increased macrophage phagocytosis.[9 10 14]

However, administration of MSCs presents logistical challenges and therefore exploring alternative compounds to harness the therapeutic benefits of MSCs have been explored. One approach is to use the key soluble mediators released by MSCs such as KGF. In a healthy volunteer, inhaled lipopolysaccharide model of ARDS, intravenous recombinant human KGF was associated with reduced alveolar injury and epithelial repair.[15] Unfortunately, when this therapy was investigated in a randomised controlled trial as a therapy for ARDS, patients treated with intravenous recombinant KGF had fewer ventilator-free days and increased mortality.[16] This suggests that the use of soluble mediators alone will not achieve the therapeutic promise of MSCs. Furthermore, MSCs are not an homogeneous entity, with significant variation between cell populations. Different cells are currently being studied in recent or planned ARDS trials (table 1). With multiple sources of heterogeneity[17] it is plausible that differences in efficacy may be seen between different cell populations, limiting the interpretation of whether effects seen in individual studies represent a class effect.

Secreted by most cells, MVs are one type of extracellular vesicle, and function to transport molecules, including proteins, messengerRNA and microRNA, between cells during cell-to-cell communication.[18 19] In a preclinical model of ARDS, MSC-derived MVs transferred mitochondria to the injured alveolar epithelium.[20] MSC extracellular vesicles have also been shown to promote an anti-inflammatory macrophage phenotype through the transfer of functional mitochondria.[21] This mechanism helps explain why alveolar macrophages are required for the antimicrobial effects of MSCs.[22] In this issue of *Thorax*, the findings from a human ex vivo model investigating intravenous MSC-derived MVs as a therapy for *E. coli*

Table 1 Characteristics of MSCs under investigation in acute respiratory distress syndrome (ARDS) clinical trials

Clinical trial	Cell source	Cell characteristics	Passage	Washed to remove cryopreservant prior to administration
Repair of Acute Respiratory Distress Syndrome by Stromal Cell Administration (REALIST) (NCT03042143)	Umbilical cord	Cells isolated based on cell surface CD362 expression	Early passage	No
A Phase 1/2 Study to Assess MultiStem® Therapy in Acute Respiratory Distress Syndrome (MUST-ARDS) (NCT02611609)	Bone marrow	Plastic adherent	Unknown	No
Human Mesenchymal Stem Cells For Acute Respiratory Distress Syndrome (START)[28] (NCT02097641)	Bone marrow	Plastic adherent	Early passage	Yes
Human umbilical cord mesenchymal stem cells therapy in ARDS (NCT03608592)	Umbilical cord	Plastic adherent	Passage 3–5	Unknown

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pneumonia are reported by Park *et al*.[7] Their findings include that MVs improved AFC, decreased lung protein permeability and were associated with a reduced alveolar neutrophil count. In the ex vivo model, MV therapy reduced alveolar bacterial count, an effect that was greatest in MVs treated with a Toll-Like Receptor 3 agonist to achieve a more immunoregulatory phenotype. Coculture of *E. coli* bacteria with isolated alveolar macrophages demonstrated that the reduction in alveolar bacterial count may have been, at least in part, mediated by upregulated alveolar macrophage phagocytosis. These findings replicate many of the beneficial effects observed from intact MSCs when studied in similar models,[12 13] suggesting that MVs may offer a therapeutic alternative to cell therapies.

Park *et al* have progressed our understanding of the potential therapeutic role that MVs may have in ARDS. This is an important translational step in the journey towards future clinical trials. The ex vivo lung perfusion model provides an opportunity to study therapies in isolated lungs, and previous studies have demonstrated that findings using this model are similar to those of healthy volunteer models of ARDS.[23] During ex vivo lung perfusion, investigators can obtain many more samples than are possible in human studies, including repeated perfusate and bronchoalveolar lavage samples and tissue samples. This offers investigators the ability to improve mechanistic understanding of disease and therapies in a way that is not possible in human clinical trials. However, it is important to highlight the potential limitations of this ex vivo lung perfusion model. Lungs are studied in isolation without an adaptive immune system or systemic organs, while the induced injury is often excessive and studied over hours. These potential limitations mean that the data derived from this model should be considered hypothesis generating.[24]

MVs may offer significant advantages over MSCs, targeting a broader range of efficacious biological mechanisms than using a single soluble mediator, while potentially presenting fewer logistical challenges which are associated with the administration of cell therapy in the critically ill. To highlight one such logistical challenge, MSCs are known to aggregate and obstruct extracorporeal circuits.[25] With the rapid increase in the use of extracorporeal therapies as an adjunctive therapy for the most unwell patients with ARDS,[26 27] MVs may offer an advantage over MSCs in the setting of extracorporeal circuits.

While the data presented by Park *et al* are promising, additional preclinical studies are required to understand in more detail the mechanism of action, dose and target population who are likely to most benefit before consideration of undertaking an early phase clinical trial of MVs in humans. As discussed by the authors, further evaluation of MVs in vivo in an animal model of pneumonia and ARDS will advance understanding of potential efficacy and adverse effects that are unable to be detected through the ex vivo lung perfusion model and will help to inform early phase clinical trials. These preclinical studies may be used to inform the target population, as well as offering investigators the opportunity to evaluate differing MV dose regimens and the timing of therapy. Prior to the delivery of MVs to humans, characterisation of the heterogeneity of MVs will be necessary to fully understand their potential effects. In keeping with the development of MSC therapies, standardisation of MV manufacturing processes and potency will be important challenges to overcome. MSCs are currently under investigation in recently completed and ongoing trials, and the results of these trials are also likely to impact on the future investigation of any potential MV therapy[28] (NCT02097641, NCT03042143, NCT02611609, NCT03608592) (table 1). The findings from these studies will also help to refine the target population for MV therapy, in addition to informing the safety profile of cell-based therapies in the critically ill.

Contributors AJB wrote the initial draft, and CMO and DFM edited and completed the final version.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests AJB declares no competing interest. DFM's institution has received a grant from the Wellcome Trust for a clinical trial of MSCs in ARDS. Outside of the submitted work, DFM reports personal fees from consultancy for GlaxoSmithKline, SOBI, Peptinnovate, Boehringer Ingelheim and Bayer, funds to his institution from grants from the UK NIHR and others and from GlaxoSmithKline for undertaking bronchoscopy as part of a clinical trial. In addition, DFM has a patent application issued to his institution. CMO's institution has received a grant from the Wellcome Trust for a clinical trial of MSCs in ARDS. Outside of the submitted work CMO reports that her spouse has received personal fees from consultancy for GlaxoSmithKline, SOBI, Peptinnovate, Boehringer Ingelheim and Bayer. CMO's institution has also received funds from grants from the Northern Ireland Health and Social Care Research and Development office.

Patient consent Not required.

Provenance and peer review Commissioned; externally peer reviewed.

To cite Boyle AJ, O'Kane CM, McAuley DF. *Thorax* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/thoraxjnl-2018-212272

Accepted 22 October 2018

► http://dx.doi.org/10.1136/thoraxjnl-2018-211576

Thorax 2018;**0**:1–3.
doi:10.1136/thoraxjnl-2018-212272

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