Therapeutic targeting of metabolic alterations in acute respiratory distress syndrome

Matthew John Robinson and Anna D. Krasnodembskaya

Affiliation: Wellcome-Wolfson Institute for Experimental Medicine, Queen's University of Belfast, Belfast, UK.

Correspondence: Anna D. Krasnodembskaya, School of Medicine Dentistry and Biomedical Sciences, Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, 97 Lisburn Road, WWIEM Building, Belfast, BT9 7BL, UK. E-mail: a.krasnodembskaya@qub.ac.uk

@ERSpublications
ARDS' biological heterogeneity is an obstacle in clinical translation of interventions. Analysis of metabolic alterations can inform development of therapeutics aimed at ARDS subpopulations. Mitochondrial dysfunction may be a potential therapeutic target. https://bit.ly/2XHfAFV

Cite this article as: Robinson MJ, Krasnodembskaya AD. Therapeutic targeting of metabolic alterations in acute respiratory distress syndrome. *Eur Respir Rev* 2020; 29: 200114 [https://doi.org/10.1183/16000617.0114-2020].

ABSTRACT Acute respiratory distress syndrome (ARDS) remains a significant source of mortality in critically ill patients. Characterised by acute, widespread alveolar inflammation and pulmonary oedema, its pathophysiological heterogeneity has meant that targeted treatments have remained elusive. Metabolomic analysis has made initial steps in characterising the underlying metabolic derangements of ARDS as an indicator of phenotypical class and has identified mitochondrial dysfunction as a potential therapeutic target. Mesenchymal stem cells and their derived extracellular vesicles have shown significant promise as potential therapies in delivering mitochondria in order to redivert metabolism onto physiological pathways.

Introduction

Acute respiratory distress syndrome (ARDS) is a severe form of respiratory failure, characterised by acute, widespread alveolar inflammation and noncardiogenic pulmonary oedema following direct or indirect injury [1]. It remains one of the most life-threatening respiratory complications in the critically ill. With no targeted treatments to its underlying pathophysiology currently available, intervention is still dominated by ventilatory assistance [1].

Since the introduction of the Berlin definition of ARDS, its pathophysiology and heterogeneity of inflammation and metabolism have been increasingly elucidated. These advances have established the need for both bedside identification of biological phenotypes and precise therapeutic targeting, with a move beyond characterisation and intervention in response only to presenting clinical appearance and radiological severity [2]. Of distinct importance has been the increased understanding of the mechanisms by which metabolic alterations contribute to ARDS; specifically, how mitochondrial dysfunction of alveolar cells drive metabolic divergence to abnormal pathways and elicit cellular dysfunction and clinical deterioration [3]. This metabolic reprogramming has been demonstrated to induce bioenergetic failure by an array of pathways including decreased oxidative phosphorylation, mitochondrial depletion, increased mitochondrial reactive oxidant species (ROS), release of mitochondrial DNA (mtDNA) and reduced secretion of pulmonary surfactant [2–5].

Provenance: Commissioned article, peer reviewed

Received: 22 April 2020 | Accepted after revision: 4 June 2020

This understanding has been due in part to the advent and application of metabolomic analytical approaches. Metabolomics involves the study and measurement of small molecular weight molecules, chemicals and metabolites within a sample [6]. Analysis provides insight into upstream physiological and pathophysiological systemic alterations [7]. Upon validation these may offer dynamic, bedside insight into specific chemical changes [8]. Metabolomics may objectively map a biological endotypes by assessing their metabolic fingerprints and use these clinical biomarkers to assess a patient's risk for developing ARDS and predict associated mortality. Ultimately, this bears optimism for the potential of improved diagnostic power and patient outcomes and has provided new therapeutic innovation in the field [9, 10]. Herein, the known metabolic abnormalities in ARDS are presented and the current developments and future directions in their targeting are discussed.

Known metabolic abnormalities in ARDS

Abnormal energy metabolism is a recognised metabolomic finding in critical illness [11, 12]. For example, in the case of sepsis-induced acute lung injury, oxidative stress, apoptosis, endothelial barrier dysfunction and disruption of energy homeostasis have a recognised set of associated metabolites [13]. Moreover, composite differences and dynamics of metabolome fingerprints characterised in septic shock have been related to disease prognosis [14]. The question remains as to what metabolites may be specifically deranged in ARDS and by which mechanism these pathways are affected.

Previously, Rogers and Matthay [8] summarised that several small sample size metabolomic analyses (total n<60 ARDS cases) of exhaled breath, bronchoalveolar lavage (BAL) and plasma in ARDS patients provided little insight into the dysfunctional metabolic pathways of ARDS or identification of the most appropriate sample type to assay. Isoprene and sphingomyelin were reported to be underrepresented in ARDS by Schubert *et al.* [15] and Stringer *et al.* [13], respectively, while an array of amino acids, glycolysis products and lipid intermediates were found to be deranged by Evans *et al.* [16]. The review concluded that metabolite heterogeneity in this population demanded an increased sample size to distinguish disease-associated variants and that analysis of BAL, plasma and urine may be the most appropriate to facilitate this, given that they may be readily obtained in large numbers of patients [8]. Izquierdo-Garcia *et al.* [11] reported significantly decreased levels of glucose, alanine, glutamine and fatty acids in serum from patients with ARDS secondary to H1N1 influenza virus pneumonia. They proposed that the decrease in tricarboxylic acid (TCA) cycle intermediates of glucose and alanine (pyruvate precursors) and glutamine (glutamate and subsequently α-ketoglutarate precursor) may be explained by energetic stress on lung epithelial cells which precludes upregulation of aerobic metabolism.

Rogers *et al.* [17] have applied a Metabolon broad-spectrum platform in 29 subjects to differentiate patients with ARDS (n=16) from patients with hydrostatic pulmonary oedema (n=13) based on the metabolic profile of undiluted oedema fluid samples. Critically, univariate analysis revealed a discriminate subset of six ARDS patients, characterised by upregulation of approximately one-third of detected metabolites (250 out of 749) across all chemical classes; meanwhile, the other ARDS patients were not distinguishable from the hydrostatic oedema group. Importantly, these metabolic changes could not be explained by differences in the sample concentrations. It was proposed that this distinct hypermetabolic subset of ARDS subjects closely mirrors the hyperinflammatory subset previously identified by Calfee *et al.* [18] in one-third of ARDS subjects. This hyperinflammatory subphenotype has been associated with raised plasma pro-inflammatory markers (interleukin (IL)-6, IL-8 and soluble tumour necrosis factor (TNF) receptor 1), coagulopathy (low protein C), endothelial injury, high prevalence of shock and metabolic acidosis, and comparatively worse clinical outcomes, including mortality, than the hypoinflammatory subphenotype, who display lower circulating inflammatory biomarkers, less acidosis and less vasopressor-dependent shock [19]. However, a definitive association between these inflammatory and metabolic groups could not be established in this study as plasma was not sampled [17].

Interestingly, pathway analysis revealed significant abnormalities in amino acid pathways, of which alanine, aspartate and glutamine metabolism met multiple comparisons correction, with false discovery rate <0.05. In addition, lysine degradation and arginine and proline metabolism pathways were differentially expressed. The group recognised a degree of consistency with the assay provided by Evans *et al.* [16] in overexpression of glutamate and proline in BAL. Notably, these pathways have established dysregulation in sepsis [20, 21]. Importantly, the study was again limited by sample size and could not benefit from wider metabolic profile comparison due to a lack of available banked undiluted pulmonary oedema fluid samples. Interestingly, metabolomic profiling was incapable of separating ARDS patients as a whole from those with hydrostatic oedema and this finding could not be explained by the low power of the study.

More recently, Bos *et al.* [22] identified two phenotypes using only plasma biomarkers in an observational cohort of patients with ARDS. These phenotypes were termed "uninflamed" and "reactive" and shared similar characteristics with the hypoinflammatory and hyperinflammatory phenotypes, respectively,

although it is still unknown whether these phenotypes are the same as described by Calfee *et al.* [18]. Subseqeuntly, Bos and co-workers [19, 23] used microarray analysis of whole-blood gene expression in an observational cohort of 210 patients with sepsis-related ARDS to investigate if the "reactive" phenotype is metabolically distinct from the "uninflamed" group. This is the first study to explore differences in gene expression and associated pathway analysis in ARDS phenotypes. Canonical pathway analysis demonstrated that the "reactive" group was characterised by enrichment of genes associated with oxidative phosphorylation and gene expression in this pathway was associated with plasma lactate levels. Collectively, these changes may be indicative of mitochondrial dysfunction. Comparatively, the "uninflamed" phenotype was associated with enrichment of mitogen-activated protein kinase 4 (MAP2K4) and Raf-1 proto-oncogene, serine/threonine kinase (RAF1) MAPK-dependent pathways, which are known to control cell proliferation, differentiation, motility and survival [23]. This study is important as it independently confirms the presence of two biologically different subpopulations within ARDS and provides insight into pathways that are relatively overexpressed in each phenotype; previously the uninflamed phenotype was defined by relatively lower levels of inflammatory mediators rather than by its own specific markers.

Recently, Viswan *et al.* [9] reported metabolic profiling of 464 subjects by nuclear magnetic resonance (NMR) in mini BAL fluid (mBALF) (ARDS n=159; non-ARDS n=40) and serum (ARDS n=197; non-ARDS n=68). In this study, ARDS samples were grouped into two categories based on clinical characteristics: 1) "subphenotype 1" based on Berlin severities (mild, moderate and severe ARDS) and 2) "subphenotype 2" based on the mechanism of lung injury, involving pulmonary aetiologies (pneumonia, lung contusion) called direct ARDS and extrapulmonary aetiologies (pancreatitis, trauma, sepsis) known as indirect ARDS. The terminology used here does not reflect the heterogeneity within ARDS population and should not be confused with ARDS hypo- and hyperinflammatory subphenotypes described by Calfee *et al.* and Bos *et al.* The control group comprised patients who were on mechanical lung ventilation for routine elective surgeries but did not develop ARDS. Interestingly, distinct subsets of metabolites were demonstrated to correlate with ARDS severity in subphenotype 1 or with pulmonary or extrapulmonary ARDS aetiology in subphenotype 2. In addition, mBALF and serum ARDS samples were demonstrated to possess an array of metabolites that were similarly upregulated in both subphenotypes, compared to non-ARDS samples. Specifically, lysine, arginine, tyrosine, threonine and branched chain amino acids were overrepresented in mBALF in ARDS group compared to non-ARDS controls, while proline, glutamate, phenylalanine and valine were upregulated in serum in ARDS group, suggesting independent pulmonary and serological metabolic processes. Importantly, high concentrations of these metabolites were positively correlated with mortality. Subsequently, the authors presented a framework for outcome predictability based on metabolic profiling. Notably, ARDS severity was determined by Berlin definition criteria, facilitating broad clinical implementation; findings were strengthened by the relatively large sample sizes. That said, there may be perceivable gain in corroborating these findings by sampling both mBALF and serum samples in each patient.

This builds on work from the same group in which NMR-based metabolomic analysis of mBALF highlighted lysine, arginine, proline, threonine, taurine and glutamate as critical biomarkers of ARDS susceptibility, severity and recovery [24]. Of particular interest was that pathway analysis suggested underlying biological processes implicated in these metabolite derangements, such as the production of carbonylated proteins secondary to hypoxia and oxidative stress in ARDS. Ultimately, Viswan *et al.* [24] concluded that this profile depicted disruptions in the TCA cycle and nitrogenous network that were associated with both ARDS pathophysiology and compensatory mechanisms switched on to regain normal biological function.

Recently, Izquierdo-García *et al.* [25] presented a pilot study of ARDS-related metabolic alterations in patients with pneumonia with and without ARDS induced by *Streptococcus pneumoniae* (ARDS n=13; non-ARDS n=17) or influenza A pneumonia (IAP) (ARDS n=12; non-ARDS n=18). They reported the ability to differentiate between patients with and without ARDS independent of the principal insulting pathogen and proposed that ARDS may drive metabolism into analogous pathway deviations, irrespective of aetiological heterogeneity. Specifically, patients with ARDS showed markedly lower serum concentrations of glucose, alanine, methylhistidine, fatty acids, creatine, citrate, valine and creatinine, while acetone concentration was significantly raised. Comparison of IAP and *S. pneumoniae* groups revealed relatively increased levels of glutamine, methylguanidine and phenylalanine in IAP patients. Comparatively, *S. pneumoniae* patients exhibited lower levels of lactate and creatinine; however, direct comparison should be cautioned due to demographic differences, notably age and baseline renal function.

Pathophysiology of metabolic alteration in ARDS

Despite progress in profiling metabolic alteration in ARDS, heterogeneity of both reported metabolites and sample media continue to preclude determining with certainty the pathways by which these abnormalities

arise. Work is required to consolidate and validate these metabolic deviations. This will help to establish whether there exists any association between metabolic fingerprints and syndrome endotypes (such as hypo/hyperinflammatory patient subsets) leading to understanding of the underlying pathophysiological mechanisms, identification of biomarkers and ultimately new therapeutic targets [18]. Unfortunately, significant barriers still persist in establishing validated biomarkers, due in part to innate heterogeneities involved in treating the critically ill. Specifically, variances in patient comorbidities, pharmaceutical and physical interventions and nutrition are all known to affect bioenergetics [26]. Moreover, an overlap between syndromes greater than currently established and a degree of wider misclassification of ARDS cannot not be excluded. That said, consensus remains that collectively these metabolomic findings demonstrate pathological mitochondrial dysfunction [9].

Indeed, metabolic reprogramming leading to deviations from the mitochondrial TCA cycle is responsible for an array of detrimental effects that are increasingly attributed to increases in morbidity and mortality. These metabolic deviations result in decreased oxidative phosphorylation and subsequent ATP yield; excessive free-radical generation of mitochondrial ROS *via* complex I and III and reactive nitrogen species *via* complex I; proinflammatory cytokine production; irreversible modification of mitochondrial proteins; reduced pneumocyte secretion of alveolar surface surfactant; disruption of mitochondrial integrity; increased mtDNA release; increased mitochondrial-related danger-associated molecular patterns (DAMPs) and ultimately apoptosis [3, 5, 9, 24, 27–31].

Metabolic reprogramming to anaerobic, mitochondrial-independent pathways of enhanced glycolysis and lactic acid fermentation have long been recognised in cancer. The Warburg effect established cells' dynamic use of metabolic pathways in response to micro-environment insults and functional organelle changes, including mitochondria [3, 32]. Since then, this phenomenon has been proposed in a range of respiratory pathologies including COPD [3] and pulmonary hypertension [30]. This may assist cells with the metabolic requirements during oxidative stress [27]. It has also been proposed that ARDS metabolic reprogramming may be a subsequence of ROS oxidative damage of the mitochondrial matrix in alveolar epithelium cells, driving metabolism to accessible anaerobic pathways following respiratory chain inhibition [30]. While the pathogenesis remains obscure, the metabolomic data reviewed above (deficient TCA cycle intermediates, precursors and ATP; increased lactate), are representative of a reliance on metabolic networks associated with glycolysis and gluconeogenesis and are collectively suggestive of mitochondrial dysfunction (table 1).

Therapeutic targeting of metabolic dysfunction

These advancements in detailing metabolic profiles and understanding the underlying pathophysiology have facilitated the advent of novel therapeutic targeting. Kiefmann *et al.* [33], for instance, proposed that alveolar hypocapnia in ARDS interfered with the mitochondrial TCA cycle by increasing isocitrate dehydrogenase subunit 3 activity and thus NADH production. This was demonstrated to induce mitochondrial calcium uptake and ROS production, leading to apoptosis of type II pneumocytes. They proposed inhalation of carbon dioxide as a novel therapeutic approach to prevent hypocapnia-induced tissue injury.

Much work has been performed in supplementing deficient organic compounds in patients with ARDS, with limited results. For example, meta-analysis of glutamine supplementation in critically ill patients was not sufficient for recovery of organ dysfunction and caused no significant improvement in mortality, with the least effect seen in the most clinically severe patients [34]. Similarly, a recent Cochrane systematic review on the effects of enteral or parenteral immunonutrition formula feeding in mechanically ventilated adults with ARDS revealed supplementation induced little or no difference in all-cause mortality [35]. These formulae are composed primarily of antioxidant vitamins, trace elements, essential fatty acids and essential amino acids, namely glutamine and arginine, included for supposed immunomodulatory properties [36].

Retrieval of normal mitochondrial activity in alveolar epithelial cells, which would support the regaining of correct pulmonary bioenergetics, is recognised as a promising therapeutic approach [37]. Indeed, the potential benefits of this restoration of mitochondrial function may extend beyond its notorious role as "the powerhouse of the cell" to a retrieval of host cell immune responses [4]. However, while work on organic supplementation as a restorative measure continues, there is growing evidence that the most critically ill patients do not demonstrate significant improvements in clinical response and that mortality persists in spite of these and other pharmacological therapeutic interventions [19]. This suggests a threshold to endothelial and epithelial cell injury, beyond which retrieval of physiological inflammatory bioenergetic functions are hindered, to the detriment of clinical outcomes [38]. This is supported by the presence of remnants of mitochondrial damage such as mtDNA: a DAMP capable of activation of the innate immune system [37].

TABLE 1 Recent studies investigating metabolic changes in acute respiratory distress syndrome (ARDS)

First author [ref.]	Cohorts compared	Sample type	Metabolic alterations in ARDS	Relationship to mitochondrial dysfunction
Izquierdo-García [11]	H1N1 influenza virus pneumonia ARDS (n=12) and non-ARDS (n=18)	Serum	↓ glucose, alanine, glutamine and fatty acids ↑ phenylalanine and methylguanidine	Decreased TCA cycle intermediates Increased utilisation of aerobic metabolism
Rogers [17]	ARDS (n=16) and non-ARDS (n=13) groups with hydrostatic pulmonary oedema	Pulmonary oedema fluid	"High metabolite" subset (n=6); distinction in 250 out of 749 identified metabolites Pathway analysis: ↑ alanine, aspartate and glutamine metabolism ↑ arginine and proline metabolism ↑ lysine degradation	Suggested correlation of high metabolite group with "hyper-inflammatory" subphenotype
Bos [23]	Sepsis ARDS: "reactive phenotype" (n=128), "uninflamed phenotype" (n=82)	Whole-blood leukocyte gene expression	"Reactive" phenotype: ↑ neutrophil activation; ↑ oxidative phosphorylation "Uninflamed" phenotype: enrichment of MAP2K4- and RAF1-dependent MAPK pathways	Heterogenic subphenotypes of ARDS, with incomparable pathophysiology Gene expression in "reactive" phenotype indicative of comparatively greater mitochondrial dysfunction
Viswan [9, 24]	mBALF: ARDS (n=159), non-ARDS (n=40) Serum: ARDS (n=197), non-ARDS (n=68)	mBALF and serum	mBALF: ↑ lysine, arginine, tyrosine, threonine, leucine, isoleucine and valine Serum: ↑ proline, glutamate, phenylalanine and valine	TCA cycle and nitrogenous network disruption
Izquierdo-García [25]	*S. pneumoniae*-induced ARDS (n=13) and non-ARDS (n=17) Influenza A pneumonia-induced ARDS (n=18) and non-ARDS (n=12)	Serum	↓ glucose, alanine, methylhistidine, fatty acids, creatine, citrate, valine and creatinine ↑ acetone	Comparable impairments to metabolic pathways irrespective of infective source Enhanced energy requirements suspected in *S. pneumoniae*-induced ARDS

TCA: tricarboxylic acid; MAPK: mitogen-activated protein kinase; RAF1: Raf-1 proto-oncogene, serine/threonine kinase; mBALF: mini bronchoalveolar lavage fluid; *S. pneumoniae*: *Streptococcus pneumoniae*.

The role of circulating mtDNA as a DAMP in ARDS pathophysiology was supported by Faust *et al.* [5], who reported a significant association of circulating mtDNA with ARDS in two critical illness populations (trauma and sepsis) 48 h after emergency department presentation. These findings support previous studies in suggesting a mechanism by which nonpulmonary insults may propagate ARDS [39, 40]. Interestingly, beyond a product of cell injury, regulated and non-necrotic mtDNA release, such as that seen in response to mitochondrial ROS signalling for inflammasome activation, also have accumulating physiological evidence. The precise mechanisms of mtDNA release into the cytoplasm and furthermore into the circulation still remain obscure and merit further work. Loss of mitochondrial membrane integrity, incomplete degradation by autophagy and mitochondria-derived vesicles have been suggested [37]. However, elevated plasma mtDNA fundamentally suggests that metabolic abnormalities in ARDS are secondary to mitochondrial damage and depletion and may explain, at least partially, why interventions to increase deficient pathway intermediates have produced limited clinical improvements.

Subsequently, the field has seen significant attention in therapeutic replenishing of mitochondria in depleted cells. Metabolically, the semblance of aerobic glycolysis in ARDS suggests irretrievable damage to pulmonary cell mitochondria warranting further investigation [41].

Mesenchymal stem cells

Mesenchymal stem/stromal cells (MSCs) are mature, multipotent, mesoderm-derived stem cells, which are widely considered to hold therapeutic promise in ARDS [42]. Although the underpinning mechanisms have not been fully elucidated, MSCs have been demonstrated to induce immunomodulation, bacterial

clearance, endothelial and epithelial cell permeability regulation, alveolar fluid clearance and antiapoptotic effects [43–45]. Consensus remains that the majority of delivered MSCs elicit their therapeutic effects through mechanisms unrelated to engraftment or indeed their progenitor function [46], and rely largely on intercellular interactions to modulate host cells.

Of considerable interest is the ability of MSCs to redress metabolic alteration of alveolar epithelium cells by the transfer of mitochondria. Mitochondrial transfer is known to occur through three means of organelle exchange, namely tunnelling nanotubes, open-ended actin-based extensions of the cell cytoplasm that facilitate cell–cell communication [47]; extracellular vesicles, a diverse group of biologically active cargo-containing membranous structures released by the cell [48]; and cellular fusion, where plasma membrane of independent cells merge, facilitating the sharing of cytosolic components [49]. Importantly, MSC delivery goes some way to mitigate the risk that delivery of free mitochondria poses in the extracellular space, which may themselves act as DAMPs and further exacerbate tissue inflammation and injury [4].

We have previously reviewed the known instances and regulation of organelle transfer between MSCs to differentiated cells [49]. The prominent work by Islam *et al.* [50] established that bone marrow derived MSCs were capable of mitochondrial transfer to type II alveolar epithelial cells (ATII) in a lipopolysaccharide-induced murine acute lung injury model. Importantly, recipient ATII cells demonstrated enhanced ATP production, restoration of surfactant secretion by ATII cells, improvement to of acute lung injury and enhanced survival. These seminal findings have been supported by an array of relevant studies which report that mitochondrial transfer induce a correction of deranged cell bioenergetics. In preclinical respiratory studies, improvements in host cell ATP generation was consistently observed in lung disease models [50–56].

Newell *et al.* [57] recently demonstrated that the increased presence of recipient cell mitochondria following transfer from human bone marrow-derived (hBM) MSCs enhanced oxidative phosphorylation in human hepatocytes. They concluded that hBM-MSC exposure resulted in enhanced ROS production at complex III and restored physiological cell signalling regulation. Significantly, subsequent metabolomic analysis revealed a significant shift to lipid unsaturation in the MSC-treated group, suggesting that the reinvigorated cell signalling and subsequent increase in ROS drove the recycling of membrane phospholipids [58]. Interestingly, no significant alteration to TCA cycle metabolites were reported, lending merit to a similar study in lung tissue.

Importantly, the increased understanding that MSC effects are largely paracrine and occur independently to engraftment has led to the isolation and delivery of extracellular vesicles as a therapeutic option. There is already significant preclinical evidence to support that in various models of ARDS therapeutic effect of MSC extracellular vesicles is at least comparable or even more prominent than the therapeutic effects seen with MSC whole-cell therapy, which we reviewed recently [48]. Significantly, extracellular vesicles may mitigate issues of tumorigenicity, immunogenicity and senescence which accompany whole-cell administration, and lend themselves to commercial use; assuming the question of large-scale biomanufacturing can be addressed [59].

In particular, we have shown that MSC extracellular vesicle mitochondria transfer resulted in macrophage shift from a proinflammatory phenotype toward an anti-inflammatory phenotype through enhancement in oxidative phosphorylation and ultimately lung injury amelioration *in vivo* [55]. In addition, we demonstrated recently that MSC extracellular vesicle mediated transfer of functional mitochondria improves mitochondrial membrane potential and ATP production in primary human distal lung epithelial cells and enhances their wound healing capacity in the ARDS inflammatory environment [60]. The therapeutic approaches and their limitations are summarised in figure 1.

Following encouraging preclinical prospects, >10 clinical trials are currently being conducted with MSCs in ARDS [61]. Results from the small number of studies that have completed early-phase trials have been promising. The first of these to publish findings (www.clinicaltrials.gov NCT01902082) demonstrated allogeneic adipose-derived MSCs to be safe in ARDS; however, doses appeared to be subtherapeutic [62]. The START trial reported no MSC-related haemodynamic or respiratory adverse events at phase 1 and 2a [63, 64]. Meanwhile, the MUST-ARDS study investigating the use of "MultiStem", patented allogenic bone marrow-derived multipotent adult progenitor cells, had a promising primary outcome in safety and, interestingly, in secondary outcomes as it appeared capable of improving 28-day mortality and lessening ventilator and intensive care unit (ICU) burden [65].

Of significance is the association between ARDS and coronavirus disease 2019 (COVID-19) and the resulting interest in MSC therapeutic applications [66]. Wang *et al.* [67] characterised 138 patients hospitalised with severe acute respiratory syndrome coronavirus-2 who tested positive for COVID-19 in

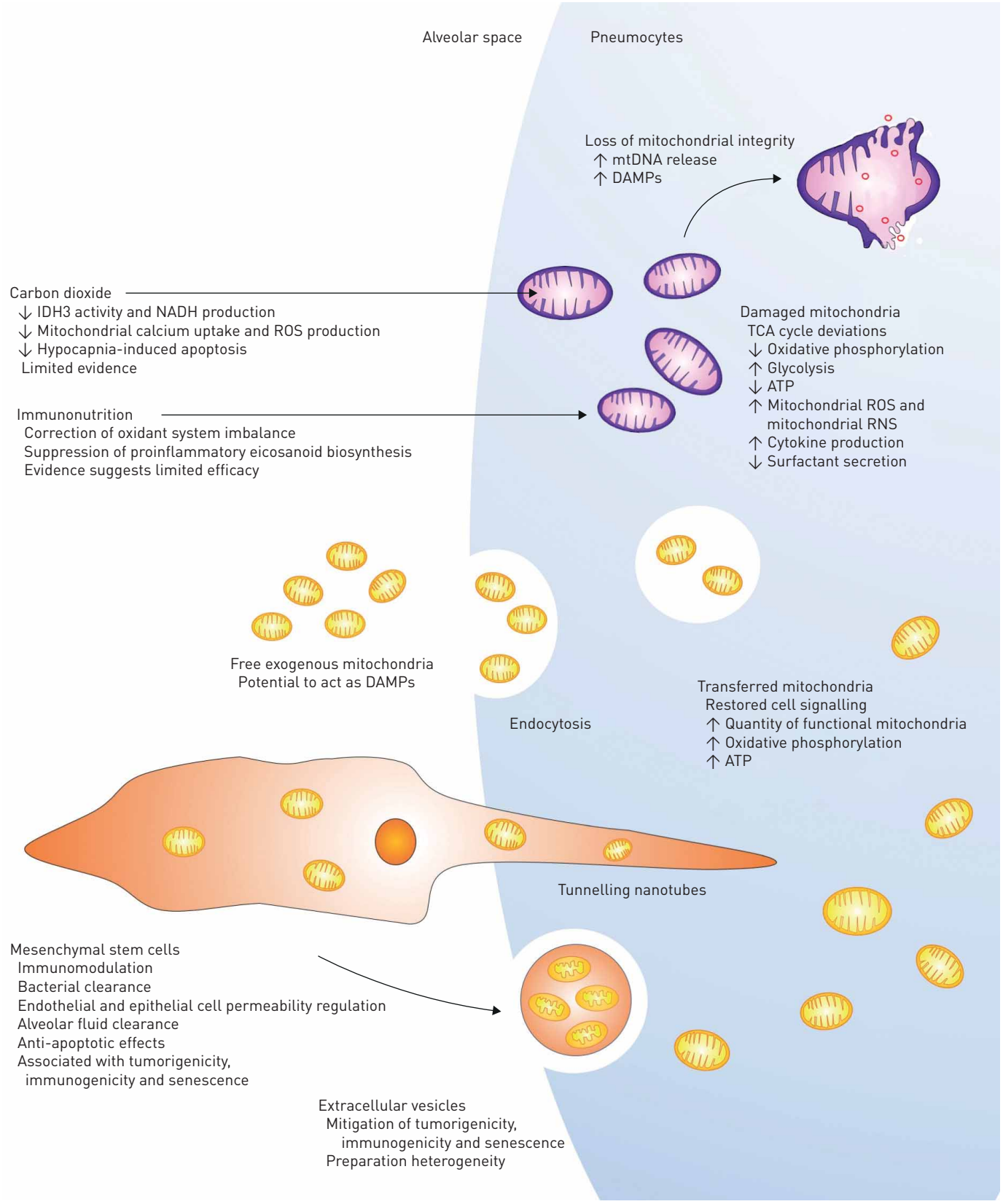

FIGURE 1 Therapeutic approaches and their limitations in the correction of metabolic alterations in acute respiratory distress syndrome. mtDNA: mitochondrial DNA; DAMPs: danger-associated molecular patterns; IDH3: isocitrate dehydrogenase subunit 3; ROS: reactive oxygen species; TCA: tricarboxylic acid; RNS: reactive nitrogen species.

Wuhan, China and found that out of 36 patients transferred to ICU, 22 (61.1%) had ARDS. More widely, there are currently >10 registered clinical trials investigating the administration of MSCs to patients with COVID-19. This includes the REALIST (NCT03042143) trial studying the administration of umbilical cord-derived CD362-positive MSCs in COVID-19-induced ARDS, which is currently recruiting for phase 2, reflecting optimism regarding the use of MSCs as a prospective treatment option [68]. Intriguingly, one registered trial (NCT04276987) is aiming to test the safety of aerosol inhalation of exosomes derived from allogenic adipose mesenchymal stem cells.

Conclusion

In conclusion, metabolic reprogramming is a significant contributor in ARDS pathophysiology and contributes to differences in phenotype, severity and prognosis. Validated metabolomic analytical approaches will help in the understanding of the dynamic metabolic derangements that occur in ARDS and may help to elucidate the syndrome's long-reported heterogeneity. The key to correcting metabolic alterations in ARDS may rely on characterising and correcting mitochondrial dysfunction. Mesenchymal stem cells, and in particular extracellular vesicles, show significant promise as future therapies in delivering mitochondria and provide hope to a field long void of optimism.

Conflict of interest: M.J. Robinson has nothing to disclose. A.D. Krasnodembskaya reports grants from the UK Medical Research Council, during the conduct of the study.

Support statement: A.D. Kransnodembskaya is supported by UK Medical Research Council Research awards (MRC MR/R025096/1 and MR/S009426/1). Funding information for this article has been deposited with the Crossref Funder Registry.

References

1 Thompson BT, Chambers RC, Liu KD. Acute respiratory distress syndrome. *N Engl J Med* 2017; 377: 562–572.
2 Sinha P, Calfee CS. Phenotypes in acute respiratory distress syndrome: moving towards precision medicine. *Curr Opin Crit Care* 2019; 25: 12–20.
3 Hiemstra PS, van der Does AM. Reprogramming of cellular metabolism: driver for airway remodelling in COPD? *Eur Respir J* 2017; 50: 1702197.
4 Schumacker PT, Gillespie MN, Nakahira K, *et al.* Mitochondria in lung biology and pathology: more than just a powerhouse. *Am J Physiol Lung Cell Mol Physiol* 2014; 306: L962–L974.
5 Faust HE, Reilly JP, Anderson BJ, *et al.* Plasma mitochondrial DNA levels are associated with ARDS in trauma and sepsis patients. *Chest* 2020; 157: 67–76.
6 Tweeddale H, Notley-McRobb L, Ferenci T. Effect of slow growth on metabolism of *Escherichia coli*, as revealed by global metabolite pool ("metabolome") analysis. *J Bacteriol* 1998; 180: 5109–5116.
7 Stringer KA, McKay RT, Karnovsky A, *et al.* Metabolomics and its application to acute lung diseases. *Front Immunol* 2016; 7: 44.
8 Rogers AJ, Matthay MA. Applying metabolomics to uncover novel biology in ARDS. *Am J Physiol Lung Cell Mol Physiol* 2014; 306: L957–L961.
9 Viswan A, Ghosh P, Gupta D, *et al.* Distinct metabolic endotype mirroring acute respiratory distress syndrome (ARDS) subphenotype and its heterogeneous biology. *Sci Rep* 2019; 9: 2108.
10 Metwaly S, Cote A, Donnelly SJ, *et al.* Evolution of ARDS biomarkers: will metabolomics be the answer? *Am J Physiol Lung Cell Mol Physiol* 2018; 315: L526–L534.
11 Izquierdo-Garcia JL, Nin N, Jimenez-Clemente J, *et al.* Metabolomic profile of ARDS by nuclear magnetic resonance spectroscopy in patients with H1N1 influenza virus pneumonia. *Shock* 2018; 50: 504–510.
12 Serkova NJ, Standiford TJ, Stringer KA. The emerging field of quantitative blood metabolomics for biomarker discovery in critical illnesses. *Am J Respir Crit Care Med* 2011; 184: 647–655.
13 Stringer KA, Serkova NJ, Karnovsky A, *et al.* Metabolic consequences of sepsis-induced acute lung injury revealed by plasma ^{1}H-nuclear magnetic resonance quantitative metabolomics and computational analysis. *Am J Physiol Lung Cell Mol Physiol* 2011; 300: L4–L11.
14 Liu Z, Triba MN, Amathieu R, *et al.* Nuclear magnetic resonance-based serum metabolomic analysis reveals different disease evolution profiles between septic shock survivors and non-survivors. *Crit Care* 2019; 23: 169.
15 Schubert JK, Müller WP, Benzing A, *et al.* Application of a new method for analysis of exhaled gas in critically ill patients. *Intensive Care Med* 1998; 24: 415–421.
16 Evans CR, Karnovsky A, Kovach MA, *et al.* Untargeted LC-MS metabolomics of bronchoalveolar lavage fluid differentiates acute respiratory distress syndrome from health. *J Proteome Res* 2014; 13: 640–649.
17 Rogers AJ, Contrepois K, Wu M, *et al.* Profiling of ARDS pulmonary edema fluid identifies a metabolically distinct subset. *Am J Physiol Lung Cell Mol Physiol* 2017; 312: L703–L709.
18 Calfee CS, Delucchi K, Parsons PE, *et al.* Subphenotypes in acute respiratory distress syndrome: latent class analysis of data from two randomised controlled trials. *Lancet Respir Med* 2014; 2: 611–620.
19 Matthay MA, Zemans RL, Zimmerman GA, *et al.* Acute respiratory distress syndrome. *Nat Rev Dis Primers* 2019; 5: 18.
20 Luiking YC, Poeze M, Ramsay G, *et al.* The role of arginine in infection and sepsis. *JPEN J Parenter Enteral Nutr* 2005; 29: S70–S74.
21 Hirose T, Shimizu K, Ogura H, *et al.* Altered balance of the aminogram in patients with sepsis – the relation to mortality. *Clin Nutr* 2014; 33: 179–182.
22 Bos LD, Schouten LR, van Vught LA, *et al.* Identification and validation of distinct biological phenotypes in patients with acute respiratory distress syndrome by cluster analysis. *Thorax* 2017; 72: 876–883.

23 Bos LDJ, Scicluna BP, Ong DSY, *et al.* Understanding heterogeneity in biologic phenotypes of acute respiratory distress syndrome by leukocyte expression profiles. *Am J Respir Crit Care Med* 2019; 200: 42–50.
24 Viswan A, Singh C, Rai RK, *et al.* Metabolomics based predictive biomarker model of ARDS: a systemic measure of clinical hypoxemia. *PLoS One* 2017; 12: e0187545.
25 Izquierdo-García JL, Nin N, Cardinal-Fernandez P, *et al.* Metabolomic profile of acute respiratory distress syndrome of different etiologies. *Intensive Care Med* 2019; 45: 1318–1320.
26 Carré JE, Orban J-C, Re L, *et al.* Survival in critical illness is associated with early activation of mitochondrial biogenesis. *Am J Respir Crit Care Med* 2010; 182: 745–751.
27 Li X, Fang P, Mai J, *et al.* Targeting mitochondrial reactive oxygen species as novel therapy for inflammatory diseases and cancers. *J Hematol Oncol* 2013; 6: 19.
28 Brealey D, Brand M, Hargreaves I, *et al.* Association between mitochondrial dysfunction and severity and outcome of septic shock. *Lancet* 2002; 360: 219–223.
29 Supinski GS, Schroder EA, Callahan LA. Mitochondria and critical illness. *Chest* 2020; 157: 310–322.
30 Ten VS, Ratner V. Mitochondrial bioenergetics and pulmonary dysfunction: current progress and future directions. *Paediatr Respir Rev* 2020; 34: 37–45.
31 Clementi E, Brown GC, Feelisch M, *et al.* Persistent inhibition of cell respiration by nitric oxide: crucial role of S-nitrosylation of mitochondrial complex I and protective action of glutathione. *Proc Natl Acad Sci USA* 1998; 95: 7631–7636.
32 Warburg O. On respiratory impairment in cancer cells. *Science* 1956; 124: 269–270.
33 Kiefmann M, Tank S, Keller P, *et al.* IDH3 mediates apoptosis of alveolar epithelial cells type 2 due to mitochondrial Ca^{2+} uptake during hypocapnia. *Cell Death Dis* 2017; 8: e3005.
34 Oldani M, Sandini M, Nespoli L, *et al.* Glutamine supplementation in intensive care patients: a meta-analysis of randomized clinical trials. *Medicine* 2015; 94: e1319.
35 Dushianthan A, Cusack R, Burgess VA, *et al.* Immunonutrition for acute respiratory distress syndrome (ARDS) in adults. *Cochrane Database Syst Rev* 2019; 1: CD012041.
36 Mizock BA. Immunonutrition and critical illness: an update. *Nutrition* 2010; 26: 701–707.
37 West AP, Shadel GS. Mitochondrial DNA in innate immune responses and inflammatory pathology. *Nat Rev Immunol* 2017; 17: 363–375.
38 Ware LB, Koyama T, Billheimer DD, *et al.* Prognostic and pathogenetic value of combining clinical and biochemical indices in patients with acute lung injury. *Chest* 2010; 137: 288–296.
39 Nakahira K, Kyung S-Y, Rogers AJ, *et al.* Circulating mitochondrial DNA in patients in the ICU as a marker of mortality: derivation and validation. *PLoS Med* 2013; 10: e1001577.
40 Zhang J-Z, Wang J, Qu W-C, *et al.* Plasma mitochondrial DNA levels were independently associated with lung injury in elderly hip fracture patients. *Injury* 2017; 48: 454–459.
41 Paliwal S, Chaudhuri R, Agrawal A, *et al.* Regenerative abilities of mesenchymal stem cells through mitochondrial transfer. *J Biomed Sci* 2018; 25: 31.
42 Ullah I, Subbarao RB, Rho GJ. Human mesenchymal stem cells – current trends and future prospective. *Biosci Rep* 2015; 35: e00191.
43 Walter J, Ware LB, Matthay MA. Mesenchymal stem cells: mechanisms of potential therapeutic benefit in ARDS and sepsis. *Lancet Respir Med* 2014; 2: 1016–1026.
44 Lee JW, Krasnodembskaya A, McKenna DH, *et al.* Therapeutic effects of human mesenchymal stem cells in *ex vivo* human lungs injured with live bacteria. *Am J Respir Crit Care Med* 2013; 187: 751–760.
45 Weiss ARR, Dahlke MH. Immunomodulation by mesenchymal stem cells (MSCs): mechanisms of action of living, apoptotic, and dead MSCs. *Front Immunol* 2019; 10: 1191.
46 Bianco P, Cao X, Frenette PS, *et al.* The meaning, the sense and the significance: translating the science of mesenchymal stem cells into medicine. *Nat Med* 2013; 19: 35–42.
47 Rustom A, Saffrich R, Markovic I, *et al.* Nanotubular highways for intercellular organelle transport. *Science* 2004; 303: 1007–1010.
48 Abraham A, Krasnodembskaya A. Mesenchymal stem cell-derived extracellular vesicles for the treatment of acute respiratory distress syndrome. *Stem Cells Transl Med* 2020; 9: 28–38.
49 Murray LMA, Krasnodembskaya AD. Concise review: intercellular communication *via* organelle transfer in the biology and therapeutic applications of stem cells. *Stem Cells* 2019; 37: 14–25.
50 Islam MN, Das SR, Emin MT, *et al.* Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury. *Nat Med* 2012; 18: 759–765.
51 Spees JL, Olson SD, Whitney MJ, *et al.* Mitochondrial transfer between cells can rescue aerobic respiration. *Proc Natl Acad Sci* 2006; 103: 1283–1288.
52 Li X, Zhang Y, Yeung SC, *et al.* Mitochondrial transfer of induced pluripotent stem cell-derived mesenchymal stem cells to airway epithelial cells attenuates cigarette smoke-induced damage. *Am J Respir Cell Mol Biol* 2014; 51: 455–465.
53 Ahmad T, Mukherjee S, Pattnaik B, *et al.* Miro1 regulates intercellular mitochondrial transport & enhances mesenchymal stem cell rescue efficacy. *EMBO J* 2014; 33: 994–1010.
54 Phinney DG, Di Giuseppe M, Njah J, *et al.* Mesenchymal stem cells use extracellular vesicles to outsource mitophagy and shuttle microRNAs. *Nat Commun* 2015; 6: 8472.
55 Morrison TJ, Jackson MV, Cunningham EK, *et al.* Mesenchymal stromal cells modulate macrophages in clinically relevant lung injury models by extracellular vesicle mitochondrial transfer. *Am J Respir Crit Care Med* 2017; 196: 1275–1286.
56 Jackson MV, Morrison TJ, Doherty DF, *et al.* Mitochondrial transfer *via* tunneling nanotubes is an important mechanism by which mesenchymal stem cells enhance macrophage phagocytosis in the *in vitro* and *in vivo* models of ARDS. *Stem Cells* 2016; 34: 2210–2223.
57 Newell C, Sabouny R, Hittel DS, *et al.* Mesenchymal stem cells shift mitochondrial dynamics and enhance oxidative phosphorylation in recipient cells. *Front Physiol* 2018; 9: 1572.
58 Girón-Calle J, Schmid PC, Schmid HH. Effects of oxidative stress on glycerolipid acyl turnover in rat hepatocytes. *Lipids* 1997; 32: 917–923.

59 Neri S. Genetic stability of mesenchymal stromal cells for regenerative medicine applications: a fundamental biosafety aspect. *Int J Mol Sci* 2019; 20: 2406.
60 Fergie N, Todd N, McClements L, *et al.* Hypercapnic acidosis induces mitochondrial dysfunction and impairs the ability of mesenchymal stem cells to promote distal lung epithelial repair. *FASEB J* 2019; 33: 5585–5598.
61 Cruz FF, Rocco PRM. Cell therapy for acute respiratory distress syndrome patients: the START study. *J Thorac Dis* 2019; 11: Suppl. 9, S1329–S1332.
62 Zheng G, Huang L, Tong H, *et al.* Treatment of acute respiratory distress syndrome with allogeneic adipose-derived mesenchymal stem cells: a randomized, placebo-controlled pilot study. *Respir Res* 2014; 15: 39.
63 Wilson JG, Liu KD, Zhuo H, *et al.* Mesenchymal stem (stromal) cells for treatment of ARDS: a phase 1 clinical trial. *Lancet Respir Med* 2015; 3: 24–32.
64 Matthay MA, Calfee CS, Zhuo H, *et al.* Treatment with allogeneic mesenchymal stromal cells for moderate to severe acute respiratory distress syndrome (START study): a randomised phase 2a safety trial. *Lancet Respir Med* 2019; 7: 154–162.
65 Bellingan G, Jacono F, Bannard-Smith J, *et al.* Primary analysis of a phase 1/2 study to assess Multistem cell therapy, a regenerative advanced therapy medicinal product (ATMP), in acute respiratory distress syndrome (MUST-ARDS). *Am J Respir Crit Care Med* 2019; 199: A7353.
66 Khoury M, Cuenca J, Cruz FF, *et al.* Current status of cell-based therapies for respiratory virus infections: applicability to COVID-19. *Eur Respir J* 2020; 55: 2000858.
67 Wang D, Hu B, Hu C, *et al.* Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA* 2020; 323: 1061–1069.
68 Chen C, Zhang XR, Ju ZY, *et al.* Advances in the research of cytokine storm mechanism induced by corona virus disease 2019 and the corresponding immunotherapies. *Zhonghua Shao Shang Za Zhi* 2020; 36: E005.