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REVIEW

MSC-Based Cell Therapy for COVID-19-Associated ARDS and Classical ARDS: Comparative Perspectives

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Abstract

Purpose of Review Despite no general conclusions regarding the therapeutic effect of MSCs on virus-induced acute lung injury in pre-clinical studies, a significant number of clinical trials using MSC-based treatment for COVID-19-associated ARDS were initiated during the global pandemic. Here, we aimed to discuss differences and similarities in clinical trials using MSC-based treatments for classical ARDS and COVID-19-associated ARDS and to raise some future perspectives.

Recent Findings Several pre-clinical studies have demonstrated that MSC treatment may not be a good treatment option for virus infections because MSCs themselves are susceptible to the virus. However, MSCs lack expression of the angiotensin-converting enzyme 2 (ACE2) receptor, suggesting that MSCs are not likely to be infected by the COVID-19 virus. Interestingly, recent meta-analyses demonstrated that an improved survival rate in patients with COVID-19-associated ARDS treated with MSCs was obtained in 24 out of 26 completed clinical trials.

Summary This review provides comparative perspectives on MSC-based therapy for COVID-19-associated ARDS and classical ARDS.

Keywords Mesenchymal stromal cells · MSCs · COVID-19 · Acute respiratory distress syndrome · Cell therapy · Lung

Introduction

The classical acute respiratory distress syndrome (ARDS) was described for the first time in 1967 by Ashbaugh et al. as a destructive lung injury with an uncontrolled inflammatory process [1]. This acute inflammatory process causes severe alveolar damage and capillary basement membrane leakage leading to a progressive respiratory failure with high morbidity and mortality burden (Reviewed in [2, 3]). Classical ARDS can result from different causes including sepsis, pneumonia, and trauma (Reviewed in [2]). During the last years, more and more studies point towards that classical ARDS is an umbrella term that includes several different ARDS phenotypes [2, 4, 5••, 6, 7]. For example, Calfee et al. described in 2014 the two subgroups hyper- and hypo-inflammatory ARDS. The hyper-inflammatory group, with hallmarks such as high plasma levels of inflammatory markers including interleukin (IL)-6, IL-8, and plasminogen activator inhibitor-1 (PAI-1), was associated with more severe disease progression and lower survival rate [4]. These two phenotypes have also been identified in other cohorts and clinical trials including for example the SAILS trial and the HARP-2 trial [8, 9] with similar findings.

During the last decades, much effort has been put into understanding the pathogenesis and pathophysiology of ARDS, and many clinical trials have been completed in the search for an effective treatment. In particular, recent clinical trials have investigated mesenchymal stromal cell (MSC)-based therapies, based on the results from very successful pre-clinical studies utilizing bacteria, endotoxin, smoke inhalation, and other models of acute lung injury. These clinical studies have all demonstrated safety but unfortunately failed to uniformly prove significantly increased clinical outcomes [10–13].

In 2019, the coronavirus disease 2019 (COVID-19)-associated ARDS was described for the first time [14, 15]. Although there are similarities between the classical ARDS and the

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COVID-19-associated ARDS pathology, an increasing number of studies demonstrate that there are also differences between the syndromes [2, 16–18], which will be summarized in the section below (Fig. 1). The first clinical investigation using MSC-based therapy to treat COVID-19-associated ARDS was initiated very early in the pandemic outbreak, and the numbers of completed studies are currently increasing (Table 1) [19••]. Similar to the results from the MSC trials on patients with classical ARDS, MSC infusions were shown to be safe for the patients. Interestingly, a pooled analysis of the clinical trials using MSCs to treat COVID-19-associated ARDS completed between January 2020 and the end of July 2022 demonstrated a relative risk reduction for all-cause COVID-19 mortality (RR = 0.63) [19••]. However, the number of studies is still fairly small, and results derived from the different studies are difficult to compare to each other since the standard clinical treatment strategies changed during the pandemic, and sometimes also during an ongoing study [19••, 20, 21].

In the first part of this review, we will discuss the differences and similarities between ARDS and COVID-19-associated ARDS pathology. In the second part, we will summarize, discuss, and compare the results from the clinical trials using MSC-based treatment for ARDS and COVID-19-associated ARDS.

Differences in Classical ARDS and COVID-19-Associated ARDS Pathophysiology

Classical ARDS and COVID-19-associated ARDS share several similarities in their pathology including significant lung inflammation with fluid accumulation in the alveoli, respiratory failure, and excessive immune response, but there are also important differences between the two syndromes (Fig. 1) [2, 16–18, 22]. The most obvious difference is that COVID-19-associated ARDS is exclusively caused by the SARS-CoV-2 virus, while the classical ARDS can have different etiologies including for example trauma, sepsis, and aspiration [2, 3, 14, 15, 22]. Moreover, other significant differences include differences in respiratory mechanics where higher respiratory system compliance and increased dead space fractions have been reported in patients with COVID-19-associated ARDS compared to patients with the classical ARDS [16, 23••, 24]. Moreover, increased levels of thrombotic mediators and lower expression of interferons have been reported in COVID-19-associated ARDS compared to the classical ARDS [25, 26]. A reduction of neutrophil-to-lymphocyte ratio with an impaired or delayed lymphocyte activation has also

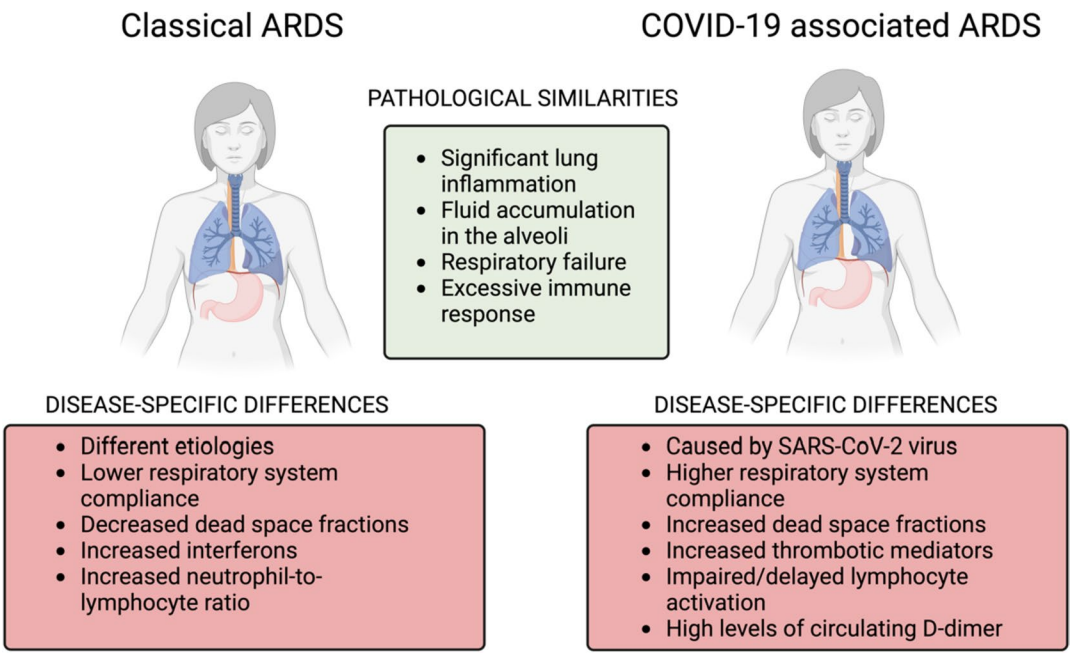

Fig. 1 Important pathological similarities and differences between classical ARDS and COVID-19-associated ARDS. Classical ARDS and COVID-19-associated ARDS share several similarities in their pathology including significant lung inflammation with fluid accumulation in the alveoli, respiratory failure, and excessive immune response, but there are also important differences between the two syndromes which have been summarized in this figure. Understanding these differences is important for the clinical management and the development of therapeutic strategies for both classical ARDS and COVID-19-associated ARDS. Abbreviations: ARDS, acute respiratory distress syndrome; COVID-19, coronavirus disease 2019; SARS-CoV-2 virus, severe acute respiratory distress syndrome coronavirus 2. This figure was illustrated using Biorender.com

Table 1 COVID-19 clinical trials

Journal	First author	Published (year)	Disease	Trial registration number	Type	Dosing	Condition of cells	Follow-up time	Objectives	No. of patients	Outcomes	MSC source		Additional therapy
Sci Rep	Bintang Soetjahjo	2023	Severe COVID-19-related ARDS	NCT04333368	Double-blind, multicentric, randomized, placebo-controlled trial	3 injections (1×10^6 /kg body weight per time point)	na	na	Safety and effectiveness	42 (21 MSC, 21 ctrl)	Duration of hospitalization, PCT, ESR, and CRP level assessment	Normoxic-allogenic umbilical cord	Allogenic	Standard COVID-19 therapy according to national guidelines
Stem Cell Res Ther	Morteza Zarrabi	2023	COVID-19-related ARDS	IRCT20200217046526N2	Phase II randomized, multicentric clinical trial	For MSC group, 2 infusions (100×10^6cells); for MSC plus EVs, 1 infusion of 100×10^6cells and 1 infusion of EVs (inhalation)	na	28 days	Safety and efficacy	43 (11 MSC, 8 MSC plus EVs, 24 ctrl)	Clinical symptoms, laboratory parameters, and inflammatory markers at baseline and 48 h	Perinatal tissue	Allogenic	na
EBioMedicine	Tian-Tian Li	2023	Severe COVID-19-related ARDS	NCT04288102	Follow-up study, randomized, double-blind, placebo-controlled	3 infusions, (4×10^7 cells per infusion)	na	2 years	Efficacy, safety	100 (65 MSC, 35 ctrl)	6-MWD, lung imaging, quality of life, COVID-19-related symptoms, titers of SARS-CoV-2 neutralizing antibodies, tumor markers, AEs	Umbilical cord	Allogeneic	na
AM J Respir Crit Care Med	Michael E. Bowdish	2022	Moderate to severe COVID-19-related ARDS	NCT04371393	Randomized	2 infusions (2×10^6 MSCs per kg)	Cryopreserved (viability 78–93%)	12-month follow-up	Efficacy, safety	222 (112 MSC, 110 ctrl)	30-day mortality, ventilator-free survival within 60 days	na	Allogeneic	Combined with low dose of remestemcel-L and standard care
Stem Cell Research & Therapy	Hamid Reza Aghayan	2022	COVID-19-related ARDS pneumonia	IRCT20200621047859N4	Phase I, non-blinded, randomized	1 infusion (IV) (1×10^6 cells/kg)	Cryopreserved	28 days	Safety, tolerability	20 (10 MSC, 10 ctrl)	Mortality, adverse events	Placenta-derived	Allogeneic	na
Front Immunol	Céline Grégoire	2022	Severe COVID-19-related ARDS pneumonia	NCT04445454	Phase I/II, retrospective	3 infusions (IV) ($1.5–3\times10^6$ cells/kg)	Cryopreserved (viability 56–93%)	60 days	Safety, efficacy	32 (8 MSC, 24 ctrl)	Adverse events, 28- and 60-day mortality	Bone marrow-derived	Allogeneic, no HLA matching	Dexamethasone, anticoagulation, and standard supportive therapy

Table 1 (continued)

Journal	First author	Published (year)	Disease	Trial registration number	Type	Dosing	Condition of cells	Follow-up time	Objectives	No. of patients	Outcomes	MSC source		Additional therapy
Stem Cell Res Ther	Najmeh Kaffash Farkhad	2022	Mild to moderate COVID-19-related ARDS	IRCT20160809029275N1	Phase I, control-placebo, single-center, open-label	3 infusions (IV) (1×10^6 cells/kg)	Fresh cells viability 95–98%	17 days	Safety, inflammatory biomarkers	20 (10 MSC, 10 ctrl)	Allergic reactions, biological assays (e.g., CRP, inflammatory and pro-inflammatory cytokines), CT scan	Umbilical cord-derived	Allogeneic	Standard care
Stem Cell Rev Rep	Meiping Chu	2022	Mild ($n=5$) and severe ($n=2$) COVID-19 pneumonia	ChiCTR2000030261	Pilot trial	Nebulization therapy (7.66e + 0.8 to 7.00e + 0.7 particles/ml), 2 times per day	MSC exosomes	na	Safety, infection, allergic reactions, adverse events	7 MSC	Allergic reactions, adverse events, CRP, CT scan	Umbilical cord-derived	Allogeneic	Ritonavir orally, abidol orally, interferon nebulization, or chloroquine phosphate orally
Stem Cell Res Ther	Ying-Gang Zhu	2022	Severe COVID-19-related pneumonia	MEX-COVID, NCT04276987	Phase IIa, single-arm, open-labeled, interventional	Nebulization therapy (total 2.0×10^8 particles per day), 5 days inhalation	MSC exosomes, stored − 20 °C	7 days	Safety, tolerability	7 MSC	Inhalation-associated events, serious adverse events	Adipose-derived	Allogeneic	Anti-viral therapy and other supportive care
Stem Cell Res Ther	Muhammad Karyana	2022	Non-severe COVID-19	NCT04535856	Phase I, double-blind, placebo-controlled, randomized	1 infusion (IV) (5.0×10^7 (low dose) or 1.0×10^8 (high dose) cells)	na	28 days	Safety, feasibility, tolerability	9 (3 MSC low, 3 MSC high, 3 ctrl)	Adverse events, survival rate, duration of hospitalization, and clinical improvement	Embryonic stem cells (DW-MSCs)	Allogeneic	na
Stem Cell Res Ther	Carmen Lúcia Kuniyoshi Rebelatto	2022	Severe COVID-19	UTN code-U1111-1254–9819	Phase I/II, prospective, single-center, randomized, double-blind, placebo-controlled	3 infusions (IV) (5×10^5 cells/kg)	Cryopreserved	4 months	Safety, long-term improvement	17 (11 MSC, 6 ctrl)	Clinical assessment, viral load, cytokines, CT scans	Umbilical cord-derived	Allogeneic	Standard treatment with anti-coagulants, steroids, and antibiotics (if evidence of bacterial infection)
Stem Cell Res Ther	Fathi-Kazerooni	2022	Severe COVID-19-related pneumonia	NCT05019287	Phase I/II, prospective, randomized, double-blinded, placebo-controlled	5 infusions (5 ml secretome per infusion), 5 days	MSC secretome (MSC cryopreserved before secretome collection)	28 days	Safety, efficacy	30 (15 MSC secretome, 15 ctrl)	Adverse events, laboratory parameters, duration at hospitalization, improved clinical symptoms, serial chest images	Menstrual blood-derived	Allogeneic	na

Table 1 (continued)

Journal	First author	Published (year)	Disease	Trial registration number	Type	Dosing	Condition of cells	Follow-up time	Objectives	No. of patients	Outcomes	MSC source		Additional therapy
Crit Care	Antonie Monsel	2022	Mild, moderate, or severe COVID-19-induced ARDS	STROMA-CoV-2, NCT04333368	Phase II, multi-center, double-blind, randomized, placebo-controlled	3 infusions (1 × 106 cells/kg)	Cryopreserved	28 days	Safety, respiratory improvement	45 (21 MSC, 24 ctrl)	Adverse events, PaO_2/FiO_2, inflammatory and immunity biomarkers	Umbilical cord-derived	Allogeneic	Anticoagulant, dexamethasone
J Cell Mol Med	Bahnam Sadeghi	2021	COVID-19-induced ARDS	na	Safety and efficacy study	2 infusions (range 1–3) (1.02×10^6 cells/kg)	Cryopreserved (viability 90–96%)	na	Safety, efficacy	10	Survival, oxygenation, effects on cytokine levels	Placenta-derived decidua	Allogeneic	Anti-viral treatment, corticosteroid
Stem Cell Res Ther	Mahshid Saleh	2021	Severe COVID-19	IRCT20190717044241N2	Phase I, open-label, single-center	3 infusions (IV) (150×10^6 cells per injection)	na	30 days	Safety, efficacy	5	Adverse events, hematology parameters, biochemistry, and inflammation tests	Wharton's jelly-derived	Allogeneic	na
Cell Transplant	G Adas	2021	Moderate and critically ill COVID-19	NCT04392778)	Clinical trial, interventional, prospective, three-parallel armed	3 infusions (IV) (3×10^6 cells/kg)	Cryopreserved	na	Safety, effectiveness	30 (10 MSC, 10 ctrl, 10 ctrl)	Adverse events, mortality, mechanism of action	Wharton's jelly-derived	Allogeneic	Conventional therapy
Stem Cell Transl Med	Ismail Hadisoebroto Dilogo	2021	Critically ill COVID-19-related pneumonia	NCT04457609	Multicentered, double-blinded, randomized	1 infusion (IV) (1×10^6 cells/kg)	na	na	Survival rate, and/or length of ventilator usage, clinical and laboratory improvements	40 (20 MSC, 20 ctrl)	Adverse events, baseline parameters, CRP, cytokines, mortality, length of stay in the ICU	Umbilical cord	Allogeneic	Standard therapy
Clin Transl Med	Xiaowei Xu	2021	Severe and critical-ill COVID-19	ChiCTR2000029606	Exploratory clinical trial, multicenter, open-label, non-randomized, parallel-controlled, phase I	3 infusions (3×10^7 cells per infusion)	Cryopreserved	1 month	Safety, efficacy, tolerability	44 (26 MSC, 18 ctrl)	Laboratory measurements, survival rate, viral test, CT scans, inflammatory markers, etc	Menstrual blood-derived	Allogeneic	Comprehensive treatment

Table 1 (continued)

Journal	First author	Published (year)	Disease	Trial registration number	Type	Dosing	Condition of cells	Follow-up time	Objectives	No. of patients	Outcomes	MSC source		Additional therapy
Stem Cell Res Ther	Seyed-Mohammad Reza Hashemian	2021	Critically ill COVID-19	na	Phase 1	3 infusions (IV) (600×10^6 in total)	Placenta (fresh) umbilical cord (cryopreserved). Viability 88.7–94.2%	60 days	Safety	11 (6 umbilical cord MSC, 5 placenta-derived MSC)	Adverse events, biomarkers, CT scans	Placenta—and umbilical cord-derived	Allogeneic	Standard medication
Stem Cell Transl Med	Giacomo Lanzoni	2021	COVID-19-induced ARDS	NCT04355728	Double-blinded, phase I/II, randomized, controlled trial	2 infusions (IV) ($100\pm20\times10^6$)	Cryopreserved	1 month	Safety, efficacy	24 (12 MSC, 12 ctrl)	Adverse events, inflammatory cytokines, survival, time to recover, laboratory testing	Umbilical cord-derived	Allogeneic	Best standard care
Signal Transduct Target Ther	Fanping Meng	2020	Moderate to severe COVID-19	na	Phase I, parallel, controlled, non-randomized	3 infusions (3×10^7 cells per infusion)	Cryopreserved	na	Safety	18 (9 MSC, 9 ctrl)	Adverse events, duration of clinical symptoms, laboratory parameters, length of hospitalization, serial chest computed tomography (CT) images, the PaO_2/FiO_2 ratio, dynamics of cytokines, and IgG and IgM anti-SARS-CoV-2 antibodies	Umbilical cord-derived	Allogeneic	Standard care
Stem Cell Res Ther	Lei Shu	2020	Severe COVID-19	ChiCTR2000031494	Single-center, open-label, individually randomized, standard-controlled	2×10^6 cells/kg (IV)	na	14 days	Safety, efficacy	41 (12 MSC and 29 ctrl)	28-day mortality, clinical symptom improvement, time to clinical symptom improvement, hematologic indication, image changes	Umbilical cord-derived	Allogeneic	na
Stem Cells Dev	Vikram Sengupta	2020	Severe COVID-19 and moderate to severe ARDS	na	Prospective, non-randomized, open-label, cohort study	15 ml exosomes (IV)	na	14 days	Safety, efficacy	27	Adverse events, survival rate, clinical status, oxygenation improvements	Bone marrow-derived MSCs, exosomes	Allogeneic	Hydroxychloroquine, azithromycin

been observed in COVID-19-associated ARDS compared to classical ARDS, where the reduced neutrophil-to-lymphocyte ratio has been correlated to an increased disease severity [25, 26]. There is also evidence that patients with COVID-19-associated ARDS have elevated levels of circulating D-dimer [23••]. Understanding these differences is important for the clinical management and the development of therapeutic strategies for both classical ARDS and COVID-19-associated ARDS.

MSC-Based Therapies for Classical ARDS and COVID-19-Associated ARDS

What Is Known from Pre-clinical Experiments on MSC Treatment for Virus-Induced Acute Lung Injury?

There is a large body of literature demonstrating the efficacy of MSC administration in pre-clinical models of acute lung injury; however, most of them have focused on endotoxin- or bacterial-induced lung injury [27, 28] and not so much focus, so far, has been on virus-induced lung disease. Nevertheless, a few papers on MSC-based treatment for virus-induced acute lung injury have been published with contradictory results. For example, in a recent paper, Tan et al. investigated the effect of MSC treatment in H1N1 influenza virus-induced acute lung injury. Here, the authors reported that MSC treatment decreased the total cell count in bronchoalveolar lavage fluid and increased the number of infiltrating $CD4^+$, $CD8^+$, B-cells, T-cells, and monocyte in the alveolar space, but did not result in an improved survival rate or reduced viral load compared to untreated control cells [29••]. Similar results, i.e., no improved survival rate and no reduction in viral load, have also been reported by other groups [30, 31]. In contrast, Qin et al. demonstrated that MSC treatment reduced herpesvirus-68-induced pneumonia with decreased lung damage, decreased levels of inflammatory markers, and inhibition of viral replication compared to untreated control mice [32]. Similar results were reported by Chan et al., where they reported that mice infected with influenza A/H5N1 treated with MSCs had an increased survival rate compared to controls treated with control fibroblasts [33]. As such, there is no general conclusion regarding the therapeutic effect of MSCs on virus-induced acute lung injury. One potential explanation for this could be that MSCs are effective against specific viruses. For example, Tan et al. demonstrated that the majority of MSCs in their study expressed α-2,6-linked SA (influenza A/H1N1 virus binding receptors) and were highly susceptible to infection of the virus. Interestingly, it has been reported that human MSCs do not express the angiotensin-converting enzyme 2 (ACE2) receptor [34••], suggesting that MSCs might not be susceptible to infection of the COVID-19 virus.

What Is Known from Clinical Trials?

MSC-Based Clinical Trials in Patients with Classical ARDS

Since the two first phase I trials on MSC-based treatments for classical ARDS in 2014–2015 [10, 11], several other trials have been completed which all demonstrated that MSCs were well-tolerated in this patient group, but no significant improved lung function or other clinical relevant outcomes were consistently observed [12, 35, 36••, 37]. The data obtained in the clinical trials mentioned above, except the two latest publications, have been extensively summarized by us and others [38–40] and will therefore not be covered here. In the more recent study by Wick et al., the authors measured potential biomarkers in the airspace and in circulation in ARDS patients included in the START trial study 48 h after treatment with MSC or placebo. Here, they found that there was a decrease in the airspace proteins Ang-2, IL-6, and sTNFR1 in patients treated with MSC compared to patients in the placebo group. Interestingly, the levels of measured biomarkers in the circulation differed very much from those measured in the mini-bronchoalveolar lavage fluid samples. This is important information to consider when collecting samples for treatment evaluation and biological understanding, as biomarkers isolated from the plasma versus the airspace most likely reflect different biological processes [37]. The most recent completed clinical trial in classical ARDS was published in 2022; here, the authors used multipotent adult progenitor cells to treat classical ARDS (moderate-to-severe) in a multicenter, randomized, double-blind, dose-escalation, placebo-controlled phase 1/2 trial. The patients were given either 300×10^6 or 900×10^6 cells diluted in 300 ml PlasmaLyte-A or placebo through a 200-µm blood filter tubing set as a single peripheral or central venous infusion. Similar to the other completed trials, the cells were demonstrated to be well-tolerated, and no acute safety concerns were observed. There was one death that occurred in the cell-treated group; however, it was determined by the data and safety monitoring board (DSMB) to be unrelated to the cell therapy. At day 28 after treatment, there was an increased number of treatment-emergent adverse events in the group that received the cells compared to the placebo group (91.3% in patients receiving 900×10^6 cells vs. 60% for placebo); however, there was a lower mortality rate in the cell-treated group both at day 28 (25% in patients receiving 900×10^6 cells vs. 40% for placebo) and at day 365 (40% in patients receiving 900×10^6 cells vs. 50% for placebo) compared to placebo [36••].

MSC-Based Clinical Trials in Patients with COVID-19-Associated ARDS

After the COVID-19 outbreak, the enthusiasm for using MSCs as cell-based therapy was once again raised leading to a dramatic increase in clinical trials using MSCs as therapy for COVID-19-associated ARDS. Searching on the PubMed database for published clinical trials through October 2, 2023, using the keywords "COVID-19" and "mesenchymal stromal cells," we identified 24 published studies (summarized in Table 1). In this section, we will briefly summarize and discuss the more recent papers and highlight some interesting lessons that can be learned from these trials as well as discuss differences and similarities with completed MSC-based trials on patients with classical ARDS.

In 2022, Kirkham et al. published a meta-analysis of controlled trials of MSC-based treatment for patients with COVID-19-associated ARDS. This was a systematic search of the literature conducted on studies published until November 15, 2021. Based on the results from the reviewed studies, the authors concluded that MSCs likely can reduce mortality in patients with critical or severe COVID-19 because they found evidence that MSC-based treatment reduced the relative and absolute risk of death at the study endpoint [41•]. All studies included in this meta-analysis were however very small and different investigational protocols were used [41•]. Earlier this year, 2023, Soetjahjo et al. published a double-blind, randomized, placebo-controlled, multicenter trial (NCT04333368) involving severe COVID-19 patients in which they gave three injections of umbilical cord-derived MSCs with 1×10^6 cells/kg body weight per time point. The study enrolled 42 patients who were randomly assigned into two equal groups and aimed to investigate the safety and effectiveness of MSC-based treatment. No decrease in the length of hospitalization was seen in the MSC-treated group compared to the control group. However, the MSC-treated group had a significant increase in oxygenation index and a smaller increase in procalcitonin values compared to the control group [42•]. In the trial by Zarrabi and colleagues (IRCT20200217046526N2), MSC treatment was combined with a dose of extracellular vesicles (EVs) derived from MSCs. In this randomized, multicentric, phase II clinical trial, 43 patients with severe COVID-19 were enrolled (MSC alone, $n=11$; MSC combined with EVs, $n=8$; control group, $n=24$), and the study aimed to assess safety and efficacy of two doses of perinatal tissue-derived MSC or one dose of MSCs followed by a dose of MSC-derived EVs. The authors reported the treatments to be safe with minimal adverse events, and a decreased serum level of inflammatory markers was seen in all study groups; however, there was a more prominent change in the MSC alone and MSC combined with EVs compared to controls [43•]. Li et al. published their 2-year follow-up results from a randomized, double-blind, placebo-controlled trial (NCT04288102) [44], in which 100 patients with severe COVID-19 were included. The patients received either 3 MSC infusions ($n=65$, 4×10^7 cells per infusion) or placebo ($n=35$) on days 0, 3, and 6 in combination with standard of care. The authors observed that MSC administration was safe 2 years after treatment; however, the efficacy of MSC treatment reported at the 1-year follow-up [45] was not significantly sustained at the 2-year follow-up according to 6-min walking distance data, quality of life, and extent of lung damage. There were no significant differences in pulmonary fibrosis based on the CT images between the MSC group and the placebo group at 24-month follow-up [44]. Taken together, it is very difficult to draw any conclusions from these different studies on COVID-19-associated ARDS because since they were performed during the pandemic, they are small studies, the standard of care changed between different trials, and sometimes within one trial, different MSC sources, doses, and criteria were used. However, there are several clinical trials that report at least some beneficial effects, and similar to the MSC trials on classical ARDS, they demonstrate that MSC-based therapy is safe also for COVID-19-associated ARDS. As suggested by Kirkham et al., one option would be to develop a "master protocol" to ensure consistency of cell product production and manufacturing and dosing strategies to simplify the ability to compare results between different clinical trials [41•]. However, creating a "master protocol" for ensuring consistent product manufacturing among the clinical trials would entail several difficulties including for example intellectual patent rights associated with each pharmaceutical industry and differences in regional laws and regulations.

So far, only first-generation MSC products have been used in all MSC therapy for classical and COVID-19-associated ARDS; however, a large body of literature indicates that pre-activating MSCs with appropriate cues prior to infusion could enhance their therapeutic potency [46–48]. For example, IFN-γ pre-treated MSCs have been demonstrated to inhibit T-cell proliferation as well as inhibit T-cell production of IFN-γ, TNF-α, and IL-2 in vitro [46]. However, contradictory results have been published on the actual in vivo effect of IFN-γ pre-treated MSCs in experimental graft versus host disease models [48, 49]. In another study, Bustos et al. pre-treated MSCs with serum obtained from ARDS patients and found that pre-treated MSCs produced increased levels of anti-inflammatory cytokines such as IL-10 and IL-11RN and decreased levels of pro-inflammatory cytokines such as IL-6, IL-8, IFN-γ, and IL-1β [47]. An altered secretome profile has also been demonstrated by MSCs treated with bronchoalveolar lavage fluid samples obtained from ARDS patients [50]. An increasing number of publications suggest that the MSC therapeutic function depends on the microenvironment they encounter [47, 50–53]. Therefore, it is essential to understand how MSC function is altered after entering a COVID-19 infectious environment containing large

concentrations of pro-inflammatory cytokines (cytokine storm) and neutralizing antibodies and B-cell responses. In a recently published study, it was demonstrated that MSCs inhibit B-cell differentiation and block pan-antibody secretion, findings that may have implications for B-cell-mediated anti-viral responses [54]. Another important factor is the increased levels of D-Dimer observed in COVID-19 patients [23••]. In a few case reports, elevation levels of D-dimer have been observed after MSC treatment and linked with serious side effects such as pulmonary embolisms and venous clots [55, 56]. However, the pooled analysis on the clinical trials using MSCs to treat COVID-19-associated ARDS found that MSC-based treatment was safe for patients with COVID-19-associated ARDS [19••]. However, further studies are warranted before we can understand the exact impact of the COVID-19-associated ARDS environment on infused MSCs.

Summary and Final Remarks

The completed clinical trials have all demonstrated that MSC-based treatment is safe to be used as treatment for patients with classical ARDS and COVID-19-associated ARDS, despite the different etiologies and differences in pathophysiology. Some of the clinical trials published during the last years and recent meta-analyses suggest that MSCs could potentially reduce mortality in patients with severe COVID-19-associated ARDS.

After several decades of progression in the field of MSC-based therapies for respiratory diseases with good pre-clinical outcomes and very stimulating results, we have now reached a plateau phase without a well-defined track forward. After several years with many completed clinical trials reporting no significant improved outcomes, it is easy to be critical and question if MSC-based therapies would be a likely future treatment option for patients with respiratory failure or severe acute lung disorders. However, we strongly believe that MSC-based therapy will be a future therapeutic option for at least subgroups of patients within specific inflammatory lung disorders such as ARDS and COVID-19-associated ARDS. But to advance to the next step, it is important to take a step back. We need to return to do some bench work and to repeat many of the in vitro and pre-clinical experiments with all the advanced techniques and instruments that are now available to us, because we believe that it is crucial that we understand (i) the MSC biology, (ii) the MSC–host environment interaction, (iii) the plasticity of in vivo MSCs, and (iv) which subgroups of patients that truly have a chance of benefit from this type of treatment before we can obtain significantly improved outcomes in future MSC-based clinical trials for acute inflammatory lung disorders.

Author Contribution SRE and DJW conceived the design and concept, performed the literature search, and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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