Updated Living Systematic Review and Meta-analysis of Controlled Trials of Mesenchymal Stromal Cells to Treat COVID-19: A Framework for Accelerated Synthesis of Trial Evidence for Rapid Approval—FASTER Approval

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Abstract

Background: Mesenchymal stromal cells (MSCs) may reduce mortality in patients with COVID-19; however, early evidence is based on few studies with marked interstudy heterogeneity. The second iteration of our living systematic review and meta-analysis evaluates a framework needed for synthesizing evidence from high-quality studies to accelerate consideration for approval.

Methods: A systematic search of the literature was conducted on November 15, 2021, to identify all English-language, full-text, and controlled clinical studies examining MSCs to treat COVID-19 (PROSPERO: CRD42021225431).

Findings: Eleven studies were identified (403 patients with severe and/or critical COVID-19, including 207 given MSCs and 196 controls). All 11 studies reported mortality and were pooled through random-effects meta-analysis. MSCs decreased relative risk of death at study endpoint (RR: 0.50 [95% CI, 0.34-0.75]) and RR of death at 28 days after treatment (0.19 [95% CI], 0.05-0.78) compared to controls. MSCs also decreased length of hospital stay (mean difference (MD: −3.97 days [95% CI, −6.09 to −1.85]), n = 5 studies) and increased oxygenation levels at study endpoint compared to controls (MD: 105.62 mmHg O₂ [95% CI, 73.9-137.3], n = 3 studies). Only 2 of 11 studies reported on all International Society for Cellular Therapy (ISCT) criteria for MSC characterization. Included randomized controlled trials were found to have some concerns (n = 2) to low (n = 4) risk of bias (RoB), while all non-randomized studies were found to have moderate (n = 5) RoB.

Interpretation: Our updated living systematic review concludes that MSCs can likely reduce mortality in patients with severe or critical COVID-19. A master protocol based on our Faster Approval framework appears necessary to facilitate the more accelerated accumulation of high-quality evidence that would reduce RoB, improve consistency in product characterization, and standardize outcome reporting.

Key words: mesenchymal stromal cells; mesenchymal stem cells; extracellular vesicles; MSC-EVs exosomes; microvesicles; coronavirus disease 2019; COVID-19; severe acute respiratory syndrome coronavirus 2; SARS-CoV-2; acute respiratory distress syndrome; ARDS; sepsis; pneumonia.
Graphical Abstract

a.

| Study or Subgroup | Experimental Events | Control Events | Weight | Risk Ratio M-H, Random, 95% CI |
|-------------------|---------------------|----------------|--------|-------------------------------|
| Adas 2021         | 3                   | 10             | 13.3%  | 0.50 [0.17, 1.46]             |
| Dileo 2021        | 10                  | 20             | 64.0%  | 0.63 [0.38, 1.02]             |
| Haberle 2021      | 1                   | 5              | 4.7%   | 0.36 [0.06, 2.18]             |
| Lanzoni 2020      | 1                   | 12             | 4.1%   | 0.14 [0.02, 0.99]             |
| Leng 2020         | 0                   | 7              | 1.7%   | 0.17 [0.01, 3.24]             |
| Meng 2020         | 0                   | 9              | Not estimable |               |
| Shi 2020          | 0                   | 65             | Not estimable |               |
| Shu 2020          | 0                   | 12             | 1.8%   | 0.33 [0.02, 5.94]             |
| Wei 2021          | 1                   | 12             | 1.6%   | 3.23 [0.14, 72.46]            |
| Xu 2021           | 2                   | 26             | 7.0%   | 0.23 [0.05, 1.02]             |
| Zhu 2021          | 0                   | 29             | 1.7%   | 0.20 [0.01, 3.99]             |
| Total (95% CI)    | 207                 | 196            | 100.0% | 0.50 [0.34, 0.75]             |
| Total events      | 18                  | 51             |        |                               |
| Heterogeneity: Tau^2 = 0.00; Chi^2 = 6.62, df = 8 (P = 0.58); I^2 = 0% |
| Test for overall effect: Z = 3.42 (P = 0.0006) |

Forest plot demonstrating significantly decreased relative (a) and absolute (b) risk of death at the study endpoint in patients administered MSCs (experimental) compared to patients not administered MSCs (control). Control groups received standard of care for COVID-19 at the time of hospital admission, which varied depending on the institution.

b.

| Study or Subgroup | Experimental Events | Control Events | Weight | Risk Difference M-H, Random, 95% CI |
|-------------------|---------------------|----------------|--------|------------------------------------|
| Adas 2021         | 3                   | 10             | 5.9%   | -0.30 [-0.72, 0.12]               |
| Dileo 2021        | 10                  | 20             | 8.5%   | -0.30 [-0.58, -0.02]              |
| Haberle 2021      | 1                   | 5              | 5.8%   | -0.36 [-0.77, 0.06]               |
| Lanzoni 2020      | 1                   | 12             | 7.6%   | -0.50 [-0.82, -0.18]              |
| Leng 2020         | 0                   | 7              | 4.7%   | -0.33 [-0.84, 0.17]               |
| Meng 2020         | 0                   | 9              | 10.6%  | 0.00 [-0.19, 0.19]                |
| Shi 2021          | 0                   | 65             | 13.3%  | 0.00 [0.04, 0.04]                 |
| Shu 2020          | 0                   | 12             | 11.4%  | -0.10 [-0.26, 0.05]               |
| Wei 2021          | 1                   | 12             | 10.4%  | 0.08 [-0.12, 0.28]                |
| Xu 2021           | 2                   | 26             | 9.4%   | -0.26 [-0.50, -0.02]              |
| Zhu 2021          | 0                   | 29             | 12.4%  | -0.07 [-0.18, 0.04]               |
| Total (95% CI)    | 207                 | 196            | 100.0% | -0.15 [-0.29, -0.02]              |
| Total events      | 18                  | 51             |        |                                   |
| Heterogeneity: Tau^2 = 0.03; Chi^2 = 60.91, df = 10 (P < 0.00001); I^2 = 84% |
| Test for overall effect: Z = 2.24 (P = 0.03) |

Lessons Learned
- Mesenchymal stromal cells can likely reduce mortality in patients with severe or critical COVID-19.
- To overcome differences between study designs and to enhance the consistency of the cell product used in these studies, a master protocol should be considered.
- This could accelerate the ability to pool results from more similar studies to support regulatory approval of mesenchymal stromal cell to treat COVID-19.

Significance Statement
Mesenchymal stromal cells (MSCs) may reduce mortality in patients with COVID-19 based on early evidence from a few studies. Our systematic review identified 11 controlled studies. Random effects meta-analysis revealed that MSCs decreased relative and absolute risk of death at study endpoint and relative risk of death at 28 days after treatment. Only 2 studies reported on standard criteria for MSC characterization. We conclude that MSCs can likely reduce mortality in patients with severe or critical COVID-19. A master protocol would enhance pooling of evidence from studies that report outcomes in a similar manner and use MSCs that meet standard criteria.
**Introduction**

Since its initial emergence in December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes coronavirus disease 2019 (COVID-19), has killed millions of people and infected many hundreds of millions more.1 Despite intensive measures implemented to contain its spread, SARS-CoV-2 continues to infect people across the globe at an unprecedented rate.2,3 Although safe and effective vaccines have been developed and administered to billions of people,4,5 many areas are struggling to vaccinate enough of their population.7,8 Furthermore, COVID-19 vaccines display reduced efficacy in certain patient populations, such as those with autoimmune conditions, blood cancers, or transplant recipients.9,10 Moreover, SARS-CoV-2 variants of concern and potential future variants pose a continued risk to disease control.11,12 Taken together, there remains a continued urgent need for safe and effective therapies to reduce the morbidity and mortality associated with COVID-19.

From the onset of the pandemic, mesenchymal stromal cells (MSCs) were quickly viewed as a promising therapy to treat COVID-19 due to their proven immunomodulatory and regenerative capabilities across a wide variety of related diseases and conditions.13,14 Many clinical trials of MSC-based therapy have been registered but remain underpowered to determine safety and efficacy on their own due to the small sample size and there is considerable heterogeneity in terms of study design, intervention characteristics, and planned primary and secondary outcomes.

To determine the preliminary safety and efficacy of MSCs in the context of COVID-19, we performed an initial systematic review and meta-analysis examining all clinical studies (controlled and uncontrolled) examining the use of MSCs for COVID-19.16 Our initial search identified 9 studies (4 controlled), and meta-analysis demonstrated that MSCs significantly decreased the risk of death at study endpoint compared to controls. Moreover, MSCs were found to be safe, with no severe adverse events reported in any of the patients. However, this initial analysis had several limitations including a small number of controlled studies, heterogeneity in study design and intervention characteristics, potential ROB, and limited reporting of MSC product characterization in accordance with criteria from the International Society of Cellular Therapy (ISCT).17 We proposed a framework for the selection of high-quality studies that would yield a more robust evidence base that could be used in support of requests for regulatory approval of MSC products and that could accelerate more widespread use and availability of MSCs to treat COVID-19. This update to our living systematic review leverages our proposed framework for the accelerated synthesis of trial evidence for rapid approval—or FASTER Approval—that will provide more clear guidance on the extent to which MSC-based therapy could be considered for approval to treat COVID-19. Moreover, we identify potential elements of a master protocol that would ensure the rapid generation of high-quality evidence to support the timely launch of future informative trials of MSC-based applications.

**Methods**

This systematic review is reported in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) guidelines (Supplementary Table S3).
Outcomes that were reported in less than 2 controlled studies or where adequate data for inclusion in the meta-analysis was not provided were analyzed in a descriptive manner using summary tables. Statistical heterogeneity was assessed using the I² statistic. The thresholds for interpretation of I² were: 0-40% (low heterogeneity), 50-60% (moderate heterogeneity), 50-90% (substantial heterogeneity), and 75-100% (considerable heterogeneity). Potential subgroup analyses (MSCs isolated from different tissue sources, MSCs compared to their secretome, MSCs that have been treated and/or genetically modified before administration or isolation of paracrine factors, according to COVID-19 severity, presence of comorbidities, patient age, patient sex, based on geographic location and by type of funding support for the study) were determined a priori in our study protocol and were described in the first edition of our living systematic review. Publication bias was assessed using funnel plots for outcomes reported in 10 or more studies by plotting the effect measure (RD, SMD, etc.) against the standard error.

Role of the Funding Source
The funding sources had no role in the study design, analysis, interpretation of data, writing of the manuscript, or decision to submit the manuscript for publication.

Results

Literature Search
Our systematic search of the literature yielded 602 unique citations. After full text screening, 11 studies met all criteria for inclusion in our review. Reasons for study exclusion at the full-text stage were related to publication type (n = 25), outcomes reported (n = 18), non-MSC-based therapy (n = 6), did not involve patients with COVID-19 (n = 2), uncontrolled study design (n = 3) and language other than English (Chinese n = 2, Russian n = 2) (Fig. 1).

Study Characteristics
The characteristics of the 11 included studies are summarized in Table 1. Five of the studies were RCTs and 6 were non-randomized controlled trials. Study publication date ranged from March 9, 2020, to October 26, 2021. Seven of the studies were conducted in China, one in the US, one in Indonesia, one in Germany and one in Turkey.

Participant Characteristics
In total, there were 403 patients (mean age 58 ± 7; 229 male) enrolled across all study groups, with 207 patients (mean age 57 ± 9; 115 male) being administered MSCs and 196 patients

Figure 1. Results of systematic search of the literature. MEDLINE and Embase and Cochrane Central Register of Controlled Trials, searched from 1947 up to November 15, 2021.
1.**Table 1.** Characteristics of patients enrolled in clinical studies of MSCs as a therapeutic intervention for COVID-19.

| Patient characteristics | All groups | Control groups | MSC groups |
|-------------------------|------------|----------------|------------|
| Total patients, n       | 403        | 196            | 207        |
| Sex, male, %            | 56.9       | 55.4           | 58.4       |
| Mean age, years (SD)    | 58.4 (7.1) | 60.1 (5.3)     | 56.7 (8.5) |
| COVID-19 severity, n (%)|            |                |            |
| Mild                    | 39 (9.7)   | 19 (9.7)       | 20 (9.7)   |
| Moderate                | 20 (5.0)   | 10 (5.1)       | 10 (4.8)   |
| Severe                  | 251 (62.3) | 124 (36.3)     | 127 (61.4) |
| Critical                | 93 (23.1)  | 43 (41.9)      | 50 (24.2)  |

**Co-morbidities, n (%)**

| Co-morbidities                        | All groups | Control groups | MSC groups |
|---------------------------------------|------------|----------------|------------|
| Hypertension                          | 109 (27.0) | 60 (30.6)      | 49 (23.7)  |
| Diabetes                              | 70 (17.4)  | 34 (17.3)      | 36 (17.4)  |
| Obesity                               | 16 (4.0)   | 5 (2.6)        | 11 (5.3)   |
| Chronic obstructive pulmonary disease | 4 (1.0)    | 1 (0.5)        | 3 (1.4)    |
| Coronary artery disease               | 9 (2.2)    | 6 (3.1)        | 3 (1.4)    |
| Congestive heart failure              | 10 (2.5)   | 6 (3.1)        | 4 (1.9)    |
| Chronic kidney failure                | 7 (1.7)    | 5 (2.6)        | 2 (1.0)    |
| Other*                                | 45 (11.2)  | 26 (13.3)      | 19 (9.2)   |
| Mean follow up, days (range)          | 27.7 (14-60)| 26.9 (14-60)  | 28.8 (14-60)|

*Includes current smoker, ex-smoker, pre-diabetes, asthma, tuberculosis, chronic bronchitis, hemorrhagic cerebral infarction, coronary heart disease, and chronic atrial fibrillation.

(mean age 60 ± 5; 114 male) serving as controls. The overall distribution of COVID-19 disease severity was similar for patients in the intervention and control groups, with 62% and 23% of all patients having severe and critical disease severity, respectively (Table 1).

In terms of patient co-morbidities, there was a greater proportion of patients with hypertension in the control group compared to the intervention group (31% vs. 24%), a greater proportion of obese patients in the MSC group compared to the control group (5% vs. 3%), and a greater proportion of patients with other co-morbidities in the control group compared to the MSC group (13% vs. 9%). However, all other co-morbidities including diabetes, COPD, coronary artery disease, and chronic kidney failure were well balanced between the control and intervention groups (Table 1).

**Intervention Characteristics**

Intervention characteristics are summarized in Table 2. All 11 studies used MSCs, with no studies using MSC secreted factors (MSC-Extracellular Vesicles [MSC-EVs], MSC-Conditioned Medium [MSC-CM]). All MSCs were derived from allogeneic human tissues, including umbilical cord blood and/or tissue (n = 8),24-26,28-31,33 menstrual blood (n = 1)27 and bone marrow mononuclear cells (n = 1).32 One study did not report the specific tissue source from which its MSCs were derived.23 The passage number in ex vivo culture of the MSCs varied widely between studies (passage 3 to passage 6) (see Table 2), with 4 of the studies28-30,32 not reporting how many passages were performed before harvesting MSCs from ex vivo culture. In terms of the extent to which studies reported on specific ISCT criteria17 for MSC characterization, only 2 of the 11 studies reported sufficient information regarding all 4 minimal criteria established by the ISCT.17 Specific details regarding the number of studies reporting on each of the 4 individual ISCT criteria17 can be found in Table 2.

MSC dosing and administration were reported differently between studies. The 2 formats in which MSC dosage were reported included cells/kg of body weight (n = 7; 1–3 × 10^6 cells/kg) and total cells/injection (n = 4; 30–100 × 10^6 cells). In 4 studies, MSCs were cryopreserved prior to administration and in 1 other study, the MSCs were either fresh or cryopreserved. The remaining studies (n = 7, 67%) did not report whether the MSCs they administered were fresh or cryopreserved. All 11 studies administered their MSCs intravenously. Most patients (n = 112, 54.1%) received 3 infusions of MSCs, although other studies reported administering 1 or 2 MSC infusions (see Table 2). The reported time from COVID-19 diagnosis to intervention was similar between control groups (median

2.**Table 2.** Intervention characteristics for clinical studies of patients administered MSCs as a therapeutic intervention for COVID-19.

| Intervention                          | Total studies, n |
|---------------------------------------|-----------------|
| MSC tissue source                     |                 |
| Umbilical cord blood or tissue        | 8               |
| Menstrual blood                       | 1               |
| Bone marrow mononuclear cells         | 1               |
| Not described                         | 1               |
| MSCs fresh or frozen/cryopreserved    |                 |
| Fresh only                            | 0               |
| Fresh or cryopreserved                | 1               |
| Cryopreserved                         | 4               |
| Not stated                            | 6               |
| Product dose                          |                 |
| MSCs, cells/kg (n=7)                  | 1·3 × 10^6      |
| MSCs, total cells (n=4)               | 30-100 × 10^6   |
| Route of administration               |                 |
| Intravenous                           | 11              |
| No. MSC infusions, patients (%)       |                 |
| 1                                     | 80 (38.6)       |
| 2                                     | 15 (7.2)        |
| 3                                     | 112 (54.1)      |
| ISCT criteria                         |                 |
| Fully met criteria (A–D below), n     | 2               |
| (A) Plastic adherence                 | 3               |
| (B) Trilineage differentiation        | 5               |
| (C) Positive/negative surface markers | 8               |
| (D) MSC viability                     | 6               |

Abbreviations: NA, not applicable; ISCT, International Society of Cellular Therapy.
9.8, range 1–47 days) and intervention groups (median 10.4, range 1–45) in the controlled studies.

Patients were administered other therapeutic agents in addition to MSCs in 10 of the 11 studies (91%). The specific therapeutic agents administered to patients varied considerably between studies and are summarized in Table 5. Two of the studies stated that they used other therapeutics in addition to MSCs but did not specify exactly which therapeutic agents were used.25,28 The mean follow-up period was 27.7 days (range 14–60), with mean follow-ups of 26.9 (range 14–60) and 28.8 (range 14–60) for the control and experimental groups respectively (Table 1).

**Outcome Reporting**

Outcome reporting across studies was variable, with some outcomes like mortality and pro-inflammatory cytokines being reported in all 11 studies, while other outcomes including progression of clinical symptoms and viral load were reported in less than half of the studies. An overview of outcomes reported across studies is summarized in Table 3.

**Primary Outcome Analysis: Mortality**

All 11 studies reported mortality at study endpoint, which ranged from 14 to 60 days. The mortality rate at study endpoint for patients in the control groups was 51 of 196 patients (26%), compared to 18 of 207 patients (8.7%) in the groups administered MSCs. In meta-analysis (n = 11 studies), patients administered MSCs had a significant decrease in relative risk of mortality (RR: 0.50 [95% CI, 0.34-0.75, P = .0006, I²=0%]) (Fig. 2a) and absolute risk of death (RD: −0.15 [95% CI, −0.29 to −0.02, P = .03, I² = 84%]) at study endpoint compared to controls (Fig. 2b). Meta-analysis of studies reporting risk of death at 28 days (n = 5 studies) also demonstrated a significant decrease in relative risk of death in patients administered MSCs compared to controls (RR: 0.19 [95% CI, 0.05-0.78, P = .02, I² = 0%]) (Supplementary Fig. S1a) whereas no significant difference in absolute risk of death at 28 days was observed (RD: −0.09 [95% CI, −0.23 to 0.04, P = .19, I² = 82%]) (Supplementary Fig. S1b).

We addressed several potential factors in subgroup analysis to assess their impact on mortality. In all cases, the relative risk of death was reduced to a similar extent compared to controls (see Supplementary Figs. S2-S6). Specifically, we examined differences in the relative risk of death in subgroups of studies where the study endpoint was <28 or ≥28d, whether patients had ARDS or did not have ARDS, whether studies included patients with any severity of disease or just severe or critical disease, and whether cord blood tissue versus other cell sources were used to generate and expand MSCs, and whether a single infusion of MSCs compared to multiple infusions were administered.

**Secondary Outcomes**

**Hospitalization**

Four of the 11 studies reported on the number of patients still requiring hospital admission at study endpoint. No difference in relative risk (RR: 0.90 [95% CI, 0.65-1.26, P = .55, I² = 46%]) (Supplementary Fig. S7) of requiring hospital admission at study endpoint for patients administered MSCs compared to controls was demonstrated in meta-analysis.
However, length of stay in hospital (reported in 5 studies) was reduced in MSC groups compared to controls (MD: −3.97 days [95% CI, −6.09 to −1.85, \(P = .0002\), \(I^2 = 0\%\)]; MSC: MoM = 25.7 days (IQR = 10.6); control: MoM = 28.4 days (IQR = 11.9)) (Supplementary Fig. S8).

Oxygenation Levels
Eight studies reported changes in oxygenation levels from baseline to endpoint in their patients. Many measures were used to quantify the change in oxygenation, including \(\text{PaO}_2/\text{FiO}_2\), \(\text{FiO}_2\) (%), \(\text{SpO}_2\) (%), maximum forced vital capacity (\(V_{\text{cmax}}\)) and diffusion lung capacity for carbon monoxide. Three of the studies reported \(\text{PaO}_2/\text{FiO}_2\) and could be combined in meta-analysis which demonstrated that patients administered MSCs had a significantly higher oxygenation index at endpoint compared to controls (MD: 105.62 mmHg [95% CI, 73.9-137.3, \(P < .0001\), \(F = 0\%\)]; MSC: MoM = 73.8 (IQR = 51.9); control: MoM = 50.8 (IQR = 99.7)) (Supplementary Fig. S8).

Pro-inflammatory and Anti-inflammatory Cytokine Levels
All 11 studies reported changes in a range of pro-inflammatory cytokines from baseline to the end of the study period. Seven of the studies reported interleukin-6 (IL-6) levels, and meta-analysis revealed no significant decrease in IL-6 levels at study endpoint compared to controls (SMD: −0.22 [95% CI, −0.60-0.16, \(P = .25\), \(I^2 = 43\%\)]; MSC: MoM = 73.8 (IQR = 51.9); control: MoM = 50.8 (IQR = 99.7)) (Supplementary Fig. S12). However, in a subset of studies, MSCs significantly reduced IL-6 levels compared to controls when measured 3–7 days following MSC administration (SMD: −0.62 [95% CI, −1.03 to −0.21, \(P = .003\), \(F = 0\%\)]; MSC: MoM = 29.5 (IQR = 34.5); control: MoM = 59.6 (IQR = 97.1)) (Supplementary Fig. S13). Five of the studies reported C-reactive protein (CRP) levels at study endpoint, and meta-analysis revealed that patients administered MSCs had significantly lower C-reactive protein (C-RP) levels at study endpoint compared to controls. (SMD: −0.41 [95% CI, −0.77 to −0.05, \(P = .02\), \(I^2 = 84\%\); MSC: MoM = 37.20 mmHg (IQR = 19.4); control: MoM = 278.3 mmHg (IQR = 32.1)) (Fig. 3).

\[\text{Figure 2.} \text{ Forest plot demonstrating significantly decreased relative (a) and absolute (b) risk of death at study endpoint in patients administered MSCs (experimental) compared to patients not administered MSCs (control). Control groups received standard of care for COVID-19 at the time of hospital admission, which varied depending on the institution.}\]
Other Outcomes

Other outcomes measured across studies including admission to the intensive care unit, need for mechanical ventilation and/or ventilation parameters, immune cell levels, radiological parameters, virological and/or antibody responses, and clinical scale scores are summarized in the supplementary materials (pp. 1–4).

Adverse Events

Adverse event reporting was described for all studies and is summarized in Table 4. Adverse events occurred in 4 studies in association with MSC infusion. Meta-analysis demonstrated no significant difference in the relative risk of adverse events between treated and control groups (RR: 0.78 [95% CI, 0.58–1.06, P = .11, I² = 43%]) (Fig. 4.). Specific adverse events occurring in patients administered MSCs included facial flushing, transient fever, hypoxemia, palpitations, and dizziness. However, these symptoms resolved in all patients spontaneously or with minimal supportive treatment following MSC administration. Only one of the studies reported the occurrence of treatment-related severe adverse events. However, this study did not specify which or how many severe adverse events occurred, only that “more serious adverse events were recorded for the placebo group than for the MSC group; however, the difference was not statistically significant.”29

Risk of Bias (RoB)

RoB was assessed for each outcome reported in RCTs using the Cochrane Risk of Bias 2 tool (ROB 2). The RoB analysis for our primary outcome of risk of death at the study endpoint can be seen in Supplementary Table S1. Four studies26,29,30,32 were found to have low RoB and 2 studies had an RoB of “some concerns,” as the method of randomization was unclear for both studies, and it was unclear whether there were deviations from intended interventions or selection of reported results in one of the studies. With regards to other outcomes that were subject to meta-analysis (eg, risk of death at 28 days, levels of pro-inflammatory cytokines, length of stay in the hospital, risk of requiring mechanical ventilation at study endpoint, etc.), each study had the same RoB classification for both individual RoB domains and overall RoB (n = 4 low RoB, n = 2 “some concerns” RoB; Supplementary Table S1). RoB was assessed for the non-randomized controlled studies using the ROBINS I tool.21 All studies23,25,27,28,31 were found to have a moderate RoB overall (Supplementary Table S2). All studies had potential bias due to confounding, measurement of outcomes (as studies did not mention blinding), and selection of reported results (as none of the studies reported a pre-registered protocol). Funnel plots for the primary analysis of mortality yielded asymmetrical distributions for both RR (Supplementary Fig. S19a) and RD Supplementary (Fig. S19b) effect measures, suggesting possible publication bias.

FASTER Approval Criteria Evaluation

In terms of the extent to which the overall evidence meets the criteria for high-quality evidence outlined in our proposed framework described in our first iteration of the living systematic review,16 referred to as the FASTER Approval criteria (Table 6), 2 of the domains were considered satisfactory (sample size, study populations), four of the domains were considered unclear (number of studies, study characteristics, outcome measurement, and RoB) and one of the domains was considered unsatisfactory (product characterization).

Discussion

This second update to our living systematic review and meta-analysis suggests MSCs are safe and may be effective for the
The number of studies in our analysis remains limited (n = 11), however, and most studies did not report sufficient characterization of MSC products in accordance with published ISCT minimal criteria. In particular, plastic adherence, cell viability, and tri-lineage differentiation potential were not reported in most studies. Moreover, outcome reporting varied between studies which limit the ability to perform meaningful meta-analyses regarding measures of clinical benefit. Some aspects of the study design can be further addressed to reduce the potential risk of bias and improve confidence in the evidence. Enhancing the likelihood of MSC-based therapy achieving regulatory approval and more widespread clinical use will require greater consistency in product characterization, outcome reporting, and overall study design, which could be facilitated with the design and implementation of a master protocol that could be shared by investigators working toward a common goal.

MSCs decreased both the relative and absolute risk of death at the study endpoint compared to controls in our meta-analysis and MSCs decreased the relative risk of death at 28 days. Although a limited number of other therapeutics including remdesivir and bamlanivimab have also demonstrated promise in terms of decreasing mortality in patients suffering from COVID-19, their mechanisms of action appear more limited in scope. Although the specific mechanisms and pathways interrogated by MSCs in patients with COVID-19 have yet to be confirmed, work in other related diseases and conditions indicates that MSCs ameliorate COVID-19 through a combination of immunomodulatory, antibacterial, tissue regenerative, and anti-apoptotic mechanisms. The pleiotropic effects of MSCs could explain the significant reduction in mortality observed for COVID-19 patients. More insight regarding specific mechanisms could allow further optimization of MSC therapy by leveraging key therapeutic mechanisms.

Oxygenation at the study endpoint was improved in patients administered MSCs compared to controls and subgroup analysis indicated that MSCs were effective in reducing the risk of death at the study endpoint for patients experiencing ARDS. Mechanisms of how MSCs could improve oxygenation and ameliorate COVID-19-induced ARDS remain unknown but may involve attenuation of the cytokine storm and/or healing of damaged and dysfunctional lung tissue. Optimal management of COVID-19-induced ARDS is an area of active research and further studies that involve MSCs appear warranted.

MSCs did not decrease the levels of pro-inflammatory cytokines and immune biomarkers including IL-6, D-dimer,
ferritin, and IFN-γ when measured at various time points after treatment. Given the highly dynamic nature of these biomarkers at various points before, during, and after the height of the cytokine storm that can be influenced by IL-6 amplifier (IL-AMP) and by activated pro-inflammatory immune cells,41-44 we observed that patients administered MSCs had significantly reduced IL-6 levels compared to controls at early time points (<7 days), but not at later periods after MSC administration.

Interestingly, patients administered MSCs had significantly reduced CRP levels at the study endpoint compared to controls. CRP is an acute-phase protein that is produced by the liver and increases rapidly in response to inflammation, cell injury, and tissue necrosis.45-47 Binding of CRP to

| Study (ref) | Antiviral agents | Antibiotic agents | Glucocorticoids | Transfusion based interventions | Other interventions |
|------------|------------------|-------------------|-----------------|---------------------------------|-------------------|
| (Leng)23   | None             | None              | None            | None                            | None              |
| (Shu)24    | Abidor/          | Moxifloxacin      | Yes             | None                            | None              |
| (Lopinavir/Ritonavir) | None | Yes | None | None | None |
| (Shu)26    | Antiviral drugs  | Antibiotics       | Yes             | None                            | None              |
| (Shi)30    | Antiviral therapy| Antimicrobial treatment | None | Extracorporeal blood purification system | Multiple' |
| (Wei)28    | Arbidol, Lopinavir-Ritonavir | None | Yes | None | None |
| (Zhu)29    | α-Interferon, Ribavirin, Ganciclovir | Moxifloxacin, Piperacillin tazobactam, Levofloxacin | Yes | None | None |
| (Lanzoni)30 | BAT              | BAT               | BAT             | BAT                             | BAT               |
| (Dilogo)31 | Osemtamivir      | Azithromycin      | None            | None                            | None              |
| (Haberle)32| None             | None              | None            | None                            | Multiple'         |
| (Adas)33   | Favipiravir, HCQ | Piperacillin-tazobactam, | Yes | None | Enoxaparin |

Table 5. Concomitant therapies reported in studies. Italics indicates the wording used in the reports.

| Number of studies | Study characteristics | Sample size | Study populations | Outcome measurement | Product characterization | Risk of bias |
|-------------------|-----------------------|-------------|-------------------|---------------------|--------------------------|--------------|
| 11                | • Sufficient number and similar enough to perform meta-analysis that achieves the required power for determining efficacy. See sample size. | 403 total, with 207 patients in treatment (MSC) arm. | Severe or critical COVID-19 in hospitalized patients (most common.). | • Mortality at day 28. | • MSCs produced and characterized according to ISCT criteria. | • Studies with high risk of potential bias should not be included in meta-analysis. |
molecules on microorganisms activates the complement system, which plays an important role in innate host immunity. CRP levels are prognostic in patients with COVID-19 with higher CRP levels associated with significantly worse disease severity, worse clinical outcomes, and higher mortality rates.\(^{48-53}\) Moreover, patients with elevated CRP levels have been associated with higher rates of extrapulmonary COVID-19 complications including venous thromboembolism (VTE) and acute kidney injury (AKI).\(^{54}\)

Subgroup analysis from our review suggested that MSCs from umbilical cord tissue were inferior to MSCs from other tissue sources in reducing the risk of death at the study endpoint. Interestingly, previous reports have suggested that MSCs derived from umbilical cord tissue could be optimal for the treatment of COVID-19 given the ease of expansion and rapid doubling times along with low levels of surface ACE-R which should mitigate against infection by SARS-CoV-2.\(^{55,56}\) Moreover, many publications also suggest that cord tissue-derived MSCs are rich in anti-inflammatory cytokines.\(^{57}\) Given this contrast in perspectives, more work appears necessary to determine the optimal tissue source for MSCs to treat COVID-19.\(^{58,59}\)

All patients received allogeneic MSCs. Allogeneic MSCs have advantages over autologous MSCs, including greater ease of acquiring the source material for product manufacturing and improved therapeutic efficacy compared to autologous MSCs, especially if isolated from individuals with advanced age or pre-existing medical conditions like diabetes or autoimmune diseases.\(^{60-63}\) Most importantly, third-party allogeneic MSCs may be stored and used when needed.\(^{64}\) This avoids the long process of manufacturing small personalized autologous MSC products—paramount for rapidly progressing diseases like COVID-19, where delayed treatment leads to increased morbidity and mortality.\(^{65,66}\) Although allogeneic MSCs appear to be effective in the context of COVID-19, the therapeutic implications of HLA-matching of MSCs between donors and recipients are currently less well understood and may be an important direction for future study to optimize MSC therapy.\(^{67}\) HLA matching of MSCs between donors and recipients may help avoid rejection of MSCs by the immune system and/or the occurrence of adverse events.\(^{68}\)

Adherence to ISCT criteria\(^{17}\) remains low amongst studies included in our analysis, with only 2 studies reporting on all 4 criteria. Incomplete adherence and reporting of ISCT criteria are not restricted to the studies identified in our review, with 1 recent analysis demonstrating that 33% of MSC-based clinical trials included no ISCT characterization data.\(^{69}\) Poor adherence to ISCT criteria has been hypothesized to be one of the reasons why the success of MSC therapies in preclinical studies fails to translate to clinical settings.\(^{70,71}\) Of note, 6 of the 11 studies reported MSC viability, a criterion added to the most recent update to the ISCT guidelines.\(^{17}\) Administering viable MSCs increases the likelihood that cells will persist in vivo upon administration, which is likely critical to ensuring therapeutic success.\(^{71}\) Greater adherence and reporting of MSC product characterization according to all 4 ISCT criteria\(^{17}\) would augment confidence in the consistency of MSC products across studies.

The optimal timing of MSC administration to patients with COVID-19 remains uncertain.\(^{41,72}\) With their potential to attenuate the cytokine storm early in COVID-19 disease progression, some suggest administering MSCs at the onset of hypoxia to avoid intubation or mechanical ventilation.\(^{41,72}\) Concern regarding potential exacerbation of a hypercoagulable state, however, has been expressed\(^{73}\) given that MSCs from certain tissue sources are rich in tissue factor (TF/CD142).\(^{73}\) Future subgroup analysis examining the clinical outcomes and occurrence of adverse events in patients administered MSCs at different stages of COVID-19 disease would be informative and may help in the design of an optimized dosing schedule.

Our study has limitations worthy of mention. First, the number of studies and patients included in our analysis remains relatively small. This modest number of studies and patients limits our confidence in the observed effects. Slow accrual and delays in the completion and publication of studies remain an issue that impedes more rapid clinical translation of results. Indeed, a large number of registered controlled clinical trials were identified in a scoping review of the literature but remain unpublished.\(^{74}\) As more of these registered trials reach completion and are published, more refined estimates should be possible with regards to the safety and efficacy of MSC-based products as a therapeutic intervention for COVID-19. Given the limited study size of future RCTs, it appears meta-analysis will remain central to understanding the benefit of MSC therapy. Variable outcome reporting across the studies included in our review was also a major limitation and limited meta-analysis. The only outcome reported across all studies was mortality but standard time points for assessing mortality were not uniformly reported. Future studies should strive toward consensus on outcome reporting. Additional outcomes of interest could include the number of days free from mechanical ventilation, although we acknowledge that practices in ventilator support of critically ill patients with COVID continue to evolve. Moreover, only 2 studies reported on whether MSC products met all ISCT criteria,\(^{17}\) further limiting the confidence in our pooled results. Lastly, asymmetry was detected in the funnel plots for mortality which suggests possible publication bias.

In conclusion, this second iteration of our living systematic review and meta-analysis demonstrates continued promise with regard to the use of MSCs as a therapeutic intervention for COVID-19. Challenges persist, however, which limit the certainty of our conclusions, including a limited number of published studies, modest patient enrollment, and substantial interstudy heterogeneity with regard to study design, patient characteristics, outcome reporting, and adherence to ISCT criteria. Future iterations of our systematic review will analyze data only from studies that meet the criteria for high-quality studies outlined in our FASTER Approval framework. The development of a master protocol for MSC therapy should leverage aspects of our framework to ensure future studies report high-quality evidence. A strategy that ensures higher quality evidence for knowledge synthesis appears necessary to leverage the results from modest-sized trials and where evidence from meta-analysis could be used to support applications for regulatory approval and to the accelerate more widespread clinical application of MSC therapy for COVID-19.

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Conflict of Interest

D.S.A. is a paid medical consultant with Canadian Blood Services. The other authors declared no potential conflicts of interest.

Author Contributions

A.M.K. and D.S.A.: conceived the study design; A.M.K., A.J.M.B., and M.M.: performed study selection, data extraction, and data analysis. A.M.K. and D.S.A. were responsible for the initial drafting of the manuscript; D.S.A., A.J.M.B., M.M.L., and D.A.F.: provided important revisions to the protocol development as well as data extraction questions and reviewed data analysis and synthesis. All authors were involved in manuscript revisions before final approval.

Data Availability

Datasets available upon request from the corresponding author.

Supplementary Material

Supplementary material is available at Stem Cells Translational Medicine online.

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