This is an update to a previously posted protocol (https://ruor.uottawa.ca/handle/10393/32833).

1.) **Review Title**

Efficacy and safety of mesenchymal stromal cell therapy in pre-clinical animal models of sepsis

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6.) **Organizational Affiliation of Review**

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7.) **Review Team Members and their Organizational Affiliations**

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8.) **Funding Sources / Sponsors**

None

9.) **Conflicts of Interest**

Stewart D.J. – Northern Therapeutics, President CEO

10.) **Collaborators**

Canadian Critical Care Translational Biology Group

11.) **Review Objective**

To systematically review the effects of MSCs on death, organ dysfunction, inflammation, and pathogen clearance in in-vivo animal models of sepsis from published pre-clinical comparative MSC sepsis studies.

12.) **Searches**

The search strategies outlined below were developed in conjunction with an information specialist. Embase, BIOSIS, MEDLINE, and Web of Science will be searched. The strategy below was used to search in MEDLINE. In addition, a manual review of the bibliographies of eligible studies and relevant review articles will be performed. Relevant conference proceedings and abstracts will also be searched.

Database: Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) <1946 to October 15, 2021 Search Strategy:

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1 exp Mesenchymal Stem Cells/ (18611)
2 exp Mesenchymal Stem Cell Transplantation/ (6050)
3 exp Multipotent Stem Cells/ (20663)
4 exp Mesenchymal Stromal Cells/ (18611)
5 ((mesenchymal adj3 (stem or stroma$1 or progenitor*)) and cell$1).tw. (26133)
6 (MSC or MSCs or ADMSC or ADMSCs or BM-MSC or BM-MSCs or BMD-MSC or BMD-MSCs or BMDMSC or BMDMSCs).tw. (16474)
7 ((multipotent or multi-potent) adj3 (stroma$1 cell$1 or stem cell$1)).tw. (3216)
8 marrow stroma$1 cell$1.tw. (5729)
9 (colony-forming unit fibroblast* or CFU-F$1).tw. (677)
10 exp Mesoderm/cy (6140)
11 or1 or/1-10 (45237)
12 Stem Cell Transplantation/ (18111)
13 exp Gene Therapy/ (40256)
14 Mesenchymal.tw. (62919)
15 (12 or 13) and 14 (1644)

16 11 or 15 (45399)
17 exp Sepsis/ (96087)
18 exp Bacteremia/ (22537)
19 (sepsis* or septic* or pyaemi* or pyemi* or pyohemi*).tw. (111000)
20 shock.tw. (137049)
21 (fungemi* or fungaemi*or bacteremi* or bacteraemi* or endotoxemi* or endo-toxemi* or endotoxaemi* or endo-toxaemi*).tw. (14032)
22 (blood adj1 poison*).tw. (82)
23 ((live or viable or blood or bloodstream* or clot or clots) adj3 bacter*).tw. (9195)
24 (Cecum/ or Colon,Ascending/) and ((in or su).fs. or Punctures/ or Ligation/) (2826)
25 ((Cecum or coecum or caecum or cecal or coecal or caecal) adj3 (perforat* or ligat* or punctur* or injur*)).tw. (3603)
26 (Colon adj1 ascend* adj3 (perforat* or ligat* or punctur* or injur*)).tw. (54)
27 ((hepatic flexure or hepatic flexture) adj3 (perforat* or ligat* or punctur* or injur*)).tw. (5)
28 ((right colic flexure or right colic flexture) adj3 (perforat* or ligat* or punctur* or injur*)).tw. (0)
29 (proximal colon adj3 (perforat* or ligat* or punctur* or injur*)).tw. (7)
30 colon ascendens stent peritonitis.tw. (47)
31 (CLP or SL-CLP or CASP).tw. (4856)
32 exp systemic inflammatory response syndrome/ (99174)
33 ("systemic inflammatory response" or "inflammatory response syndrome" or SIRS).tw. (8318)
34 exp lipopolysaccharides/ (66079)
35 (lipopolysaccharide* or lipo-polysaccharide* or LPS or lipoglycan*).tw. (86697)
36 exp Peritonitis/ (24453)
37 peritonitis.tw. (25444)
38 (exp Infection/ or exp Bacterial Infections/ or exp Inflammation/) and pp.fs. (69055)
39 exp Endotoxins/ (85092)
40 (endotoxin* or ETX).tw. (33535)
41 or/17-40 (487616)
42 exp animal experimentation/ or exp models, animal/ or animals/ or mammals/ or vertebrates/ or exp fishes/ or exp amphibia/ or exp reptiles/ or exp birds/ or exp hyraxes/ or exp marsupialia/ or exp monotremata/ or exp scandentia/ or exp chiroptera/ or exp carnivora/ or exp cetacea/ or exp Xenarthra/ or exp elephants/ or exp insectivora/ or exp lagomorpha/ or exp rodentia/ or exp sirenia/ or exp Perissodactyla/ or primates/ or exp strepsirhini/ or haplorhini/ or exp tarsii/ or exp platyrrhini/ or catarrhini/ or exp cercopithecidae/ or gorilla gorilla/ or pan paniscus/ or pan troglodytes/ or exp pongo/ or exp hylobatidae/ or hominidae/ (5545600)
43 (animal$1 or chordata or vertebrate* or fish$2 or amphibian* or amphibium* or reptile$1 or bird$1 or mammal* or dog or dogs or canine$1 or cat or cats or hyrax* or marsupial* or monotrem* or scandentia or bat or bats or carnivor* or cetacea or edentata* or elephant* or insect or insects or insectivore or lagomorph* or rodent$2 or mouse or mice or murine or murinae or muridae or rat or rats or pig or pigs or piglet$1 or swine or rabbit$1 or sheep$1 or goat$1 or horse$1 or equus or cow or cows or cattle or calf or calves or bovine or sirenia or ungulate$1 or primate$1 or prosimian* or haplorhini* or tarsiiform* or simian*or platyrrhini or catarrhini or cercopithecidae or ape or apes or hylobatidae or hominid* or chimpanzee* or gorilla* or orangutan* or monkey or monkeys or ape or apes).tw. (3968954)
44 exp Drug Evaluation, Preclinical/ (177702)
45 (preclinic* or pre-clinic*).tw. (65130)
46 or/42-45 (6257584)
47 16 and 41 and 46 (514)

13.) Condition or Domain Being Studied

Septic shock is the leading cause of death in the intensive care unit with a mortality rate of approximately 20-40%[1-3]. It is caused by a severe infection and is associated with activation of inflammatory mediators initiated by the infectious pathogen. The sequelae of septic shock are mediated by a maladaptive inflammatory host response that leads to progressive endothelial dysfunction, disordered microcirculation, cellular dysfunction and ultimately organ dysfunction and death[4-7]. Mesenchymal stromal cells (MSCs, 'adult stem cells') are a subgroup of pericytes that play a role in vascular homeostasis and the inflammatory response[8, 9]. When MSCs are stimulated by injury, they act as paracrine 'factories' by producing abundant amounts of cytokines and growth factors that dampen the inflammatory response[10][11]. Evidence suggests that exogenously administered MSCs can modulate the inflammatory response, reduce organ dysfunction, increase bacterial clearance, and reduce death in pre-clinical models of sepsis[12-15]. Several pre-clinical studies examining MSCs in sepsis have been published. Recently, our team conducted and published a systematic review and meta-analysis that examined the impact of MSCs compared to a control group on death in pre-clinical sepsis models[16]. The intent of the present systematic review is to provide updated death estimates and to also examine the effect of MSCs as compared to a control group on systemic inflammation, organ dysfunction, and pathogen clearance.

14.) Participants / Population

Inclusion: Pre-clinical *in vivo* models of systemic sepsis that mimic (at least in part) the pathophysiology caused by systemic infection (e.g. cecal ligation and puncture, bacterial administration) or systemic endotoxin administration. Two hit sepsis models will also be considered (e.g. lung contusion with subsequent endotoxin administration).

Exclusion: In vitro studies, neonatal animal models of sepsis, models of direct acute lung injury (e.g. LPS instillation into trachea), co-administration of other cells/substances, and localized sepsis and sepsis-like models (e.g. intra-orbital infection).

15.) Interventions

Inclusion: Mesenchymal stromal cells (xenogenic, syngeneic, allogeneic). The International Society for Cellular Therapy position statement will be used as a guide to define MSCs[17]. To be eligible for inclusion, MSCs must be administered systemically following the induction of sepsis or endotoxemia.

Exclusion: Differentiated mesenchymal stromal cells (e.g. MSC differentiated to an endothelial cell); co-treatment with another therapy or cell type; mesenchymal stromal cells engineered to over or under express particular genes, and multiple MSC dose administration.

16.) Comparator(s)/Control

Comparator for Intervention: Treatment with vehicle (e.g. phosphate buffered saline) or other controls (e.g. normal saline, fibroblasts, no treatment).

17.) **Types of studies to be included**

Eligible studies include pre-clinical comparative studies using *in vivo* models that mimic the pathophysiology of sepsis.

18.) **Outcomes**

The primary outcome is overall mortality measured at the latest time point.

Secondary outcomes are:

a) Markers of organ dysfunction measured at ≤ 6 h, > 6 to ≤ 12 h, > 12 to ≤ 24 h, > 24 to ≤ 48 h, > 48 to ≤ 72 h, > 72 to ≤ 96 h, > 96 h post MSC/control administration.
   i. Pulmonary (Myeloperoxidase: MPO levels, and neutrophils)
   ii. Endothelial permeability (wet/dry ratio, Bronchoalveolar lavage (BAL) albumin, BAL protein, BAL IgM)
   iii. Renal (creatinine, Blood Urea Nitrogen: BUN)
   iv. Hepatic (Aspartate transaminase: AST and Alanine aminotransferase: ALT)
   v. Cardiac (echocardiographic assessment of function: Ejection Fraction and Fractional Shortening)
   vi. Hematologic (ex: INR/PTT, Platelets, Fibrinogen)
   vii. Lactate

b) Inflammatory markers in the plasma (e.g. circulating) measured at ≤ 6 h, > 6 to ≤ 12 h, > 12 to ≤ 24 h, > 24 to ≤ 48 h, > 48 to ≤ 72 h, > 72 to ≤ 96 h, > 96 h post MSC/control administration
   i. Tumour necrosis factor-alpha
   ii. Interleukin-1beta
   iii. Interferon-gamma
   iv. Interleukin-6
   v. Interleukin-8 (or equivalent homologs like: Cytokine induced neutrophil chemoattractant: CINC/KC)
   vi. Monocyte chemoattractant protein-1: MCP-1 (or equivalent homologs like chemokine C-C motif ligand 2: CCL-2)
   vii. Interleukin-10

c) Bacterial clearance measured at ≤ 6 h, > 6 to ≤ 12 h, > 12 to ≤ 24 h, > 24 to ≤ 48 h, > 48 to ≤ 72 h, > 72 to ≤ 96 h, > 96 h post MSC/control administration
   i. Colony forming units in sample organs/sites (e.g. peritoneal fluid, blood, spleen)

19.) **Data Extraction**

Two independent reviewers will review studies and extract data into standardized, pre-piloted data collection forms. Discrepancies will be resolved through discussion with the principal investigator.

20.) **Risk of Bias (Methodological Quality) Assessment**

We will use the SYRCLE too[18]l for animal studies which was adapted from the Cochrane risk of bias tool[19] to examine the methodological quality of the included studies. The ten elements of this approach that will be evaluated are:

a) Sequence generation
b) Baseline characteristics

c) Allocation concealment
d) Random housing
e) Blinding of personnel
f) Random outcome assessment
g) Blinding of outcome assessors
h) Completeness of outcome data
i) Selective outcome reporting
j) Other sources of bias (*a priori* sample size calculations and funding sources will also be assessed as suggested by Macleod and colleagues)[20].

21.) **Assessment of Construct Validity**

In pre-clinical studies, construct validity refers to the extent an animal model corresponds to the clinical entity it is intended to represent[21]. Pre-clinical to clinical generalization is threatened when the model, intervention, and outcomes do not closely match the intended clinical scenario. Since no guidelines exist to evaluate pre-clinical construct validity, we will use a previously proposed framework[22]. Items to be evaluated include use of a large animal model (e.g. pig, dog, sheep), use of adult animals, presence of intercurrent illness, use of an infectious model of sepsis, documentation of severity of illness prior to initiating therapy, follow-up duration ≥ 24 h, use of antibiotics, and use of intravenous fluid resuscitation. Each item will be assigned either a “yes” or a “no”.

22.) **Strategy for Data Synthesis**

Results from outcomes with discrete data (e.g. death) will be pooled and meta-analyses will be performed with inverse variance random effects modelling. Data will be expressed as odds ratios and 95 percent confidence intervals.

Results from outcomes with continuous data (e.g. cytokine levels) will be pooled and meta-analyses will be performed using the ratio of means method with inverse variance random effects modelling. Data will be expressed as ratio of means and 95 percent confidence intervals[23].

23.) **Analysis of Subgroups**

Subgroup analyses on the primary outcome (death) are planned to examine potential heterogeneity of the treatment effects according to the following sub groups:

a) Animal model used to induce sepsis (mice, rats, other species)
b) Sex of animal models (male, female, mixed)
c) Experimental model used (e.g. CLP, endotoxemia, live bacteria administration)
d) Source of MSCs (autologous, syngeneic, allogeneic, xenogenic)
e) Type of MSCs (bone marrow, umbilical, adipose, other)
f) Route of MSC administration (intravenous, intraperitoneal)
g) Dose of MSCs given ($<1x10^6$, $1x10^6$, $2x10^6$, $\geq 3x10^6$)
h) Route of MSC administration (intravenous, intraperitoneal)
i) Timing of MSC administration (≤1 hour, >1 to ≤6 h, >6 to 24 h post-sepsis induction)
j) Condition of MSCs (fresh (never frozen or thawed and cultured), cryopreserved cells)
k) Resuscitation used (fluids, antibiotics, fluids and antibiotics, no resuscitation),
l) Methodological quality according to SYRCLE Tool (e.g. by individual domains)
m) Construct validity (e.g. studies with less than 50% vs studies with greater than 50% of construct validity items)

These results will be considered hypothesis generating due to the **large number of analyses that will be performed.**

24.) **Current Stage of Review**

An updated search was conducted to September 2018. Modifications to the systematic review content and methods were made in the Spring of 2019 after consultation with the co-investigators and the Canadian Critical Care Translational Biology Group and an updated search for eligible studies and data collection was performed in August 2019 and October 2021.

25.) **Knowledge Dissemination**

Results will be disseminated through the Canadian Critical Care Translational Biology Group and shared with relevant stakeholders (e.g. Canadian Stem Cell Foundation, Ontario Institute for Regenerative Medicine, Sepsis Canada, Canadian Council for Animal Care), and presented at international critical care (ex: CCCF, ATS) and regenerative medicine conferences (ex: ISCT, Till and McCullough meeting).

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