

Efficacy and safety of mesenchymal stromal cell therapy in preclinical animal models of sepsis: a systematic review protocol.

Protocol also posted at CAMARADES website

(<http://www.dcn.ed.ac.uk/camarades/research.html#protocols>)

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4.) **Funding Sources / Sponsors**

None

5.) **Conflicts of Interest**

Stewart D.J. – Northern Therapeutics, President CEO

6.) **Collaborators**

Canadian Critical Care Translational Biology Group

7.) **Review Objective**

To systematically review the effects of MSCs on death, organ dysfunction, inflammation, and pathogen clearance in in-vivo animal models of sepsis

8.) **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 May 21, 2015> Search Strategy:

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-MS C or BM-MS Cs or BMD-MS C or BMD-MS Cs or BMDMS C or BMDMS Cs).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 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 bacteriaemi* 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 flexure) adj3 (perforat* or ligat* or punctur* or injur*)).tw. (5)

28 ((right colic flexure or right colic flexure) 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 tarsiform* 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)

9.) **Condition or Domain Being Studied**

Septic shock is the leading cause of death in the intensive care unit with a mortality rate of approximately 20-30%. 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. Mesenchymal stromal cells (MSCs, 'adult stem cells') are a subgroup of pericytes that play a role in vascular homeostasis and the inflammatory response. 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. Evidence suggests that exogenously administered MSCs can dampen inflammatory response and increase bacterial clearance in models of sepsis. Several preclinical studies examining MSCs in sepsis have been published. To date, however, the results of these studies have not been summarized in a methodologically rigorous manner.

10.) **Participants / Population**

Inclusion: Preclinical *in vivo* models of septic shock that mimic (at least in part) the pathophysiology caused by infection (e.g. cecal ligation and puncture, bacterial administration) or endotoxin administration.

Exclusion: In vitro studies, neonatal animal models of sepsis, models of acute lung injury, co-administration of other cells/substances.

11.) **Interventions**

Inclusion: Mesenchymal stromal cells (xenogenic; syngeneic, allogeneic) that meet the majority of criteria defined by the International Society of Cellular Therapy consensus statement (Dominici M et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006; 8:315-317). To be eligible for inclusion, MSCs must be administered 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.

12.) **Comparator(s)/control**

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

13.) **Types of studies to be included initially**

Eligible studies include preclinical controlled studies and control comparison studies using *in vivo* models that mimic the pathophysiology of sepsis.

14.) **Outcomes**

The primary outcome to be examined is death. Secondary outcomes to be examined are markers of organ dysfunction, inflammatory markers, and bacterial clearance. Specific examples are provided below.

- a) Organ dysfunction
 - i. Pulmonary (bronchoalveolar lavage (BAL) protein, MPO levels, wet/dry ratio, BAL albumin, BAL cells, infiltrating cells)
 - ii. Renal (creatinine)
 - iii. Hepatic (AST, ALT)
 - iv. Cardiac (echocardiographic assessment of function)
- b) Inflammatory markers (e.g. circulating, bronchiolar lavage, tissue)
 - i. Tumour necrosis factor-alpha
 - ii. Interleukin-1beta
 - iii. Interferon-gamma
 - iv. Interleukin-6
 - v. Interleukin-10
- c) Bacterial clearance
 - i. Colony forming units in sample organs/sites (e.g. peritoneal fluid, spleen)

A priori grouping of timing of outcomes:

- a) Death (≤ 2 days, >2 to ≤ 4 days, >4 days; overall)
- b) Organ dysfunction (≤ 6 h, >6 to ≤ 24 h, >24 h)
- c) Cytokines (≤ 6 h, >6 to ≤ 24 h, >24 h)
- d) Bacterial burden (overall)

15.) **Data Extraction**

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

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

We will incorporate The Cochrane Handbook for Systematic Reviews of Interventions approach to assessing risk of bias (Higgins JPT, Green S (editors). *Cochrane Handbook for Systematic Reviews of Interventions* Version 5.1.0. The Cochrane Collaboration, 2011.) The six elements of this approach that will be evaluated are:

- a) Random sequence generation
- b) Allocation concealment
- c) Blinding of personnel
- d) Blinding of outcome assessment
- e) Incomplete outcome data addressed
- f) Selective reporting

In addition, a priori sample size calculation and funding sources will also be assessed as suggested by Macleod and colleagues (Sena E et al. Systematic review and meta-analysis of the efficacy of tirilazad in experimental stroke. *Stroke*. 2007; 38:388-394).

17.) **Assessment of Construct Validity**

In preclinical studies construct validity refers to the extent an animal model corresponds to the clinical entity it is intended to represent (Henderson VC et al. Threats to validity in the design and conduct of preclinical efficacy studies: a systematic review of guidelines for in vivo animal experiments. PLoS Medicine. 10(7):e1001489; 2013). Preclinical 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 preclinical construct validity, we will use a previously proposed framework (Lamontagne F et al. Systematic review of reviews including animal studies addressing therapeutic interventions for sepsis. Critical Care Medicine. 38(12): 2401-8; 2010). 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, use of intravenous fluid resuscitation. Each item will be assigned either a “yes” or a “no”.

18.) **Strategy for Data Synthesis**

Results from outcomes with discrete data (e.g. death) will be pooled and meta-analysis will be performed with inverse variance random effects modeling. 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-analysis will be performed using the ratio of means method with inverse variance random effects modeling. Data will be expressed as ratio of means and 95 percent confidence intervals (Friedrich JO et al, The ratio of means method as an alternative to mean differences for analyzing continuous outcome variables in meta-analysis: A simulation study. BMC Medical Research Methodology. 2008; 8:1-15).

19.) **Analysis of Subgroups**

Subgroup analyses on the primary outcome death are planned based on the following. If different doses of MSCs are administered within one study then results from the highest dose will be used for subgroup analysis.

- 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) Route of MSC administration (intravenous, intraperitoneal, intramuscular)
- f) Dose of MSCs given ($<1 \times 10^6$, 1×10^6 , 2×10^6 , $\geq 3 \times 10^6$)
- g) Frequency of MSC dose given (1, 2, ≥ 3),
- h) Route of MSC administration (intravenous, intramuscular, intraperitoneal)
- i) Timing of MSC administration source of MSCs (≤ 1 hour, >1 to ≤ 6 h, >6 h post-sepsis induction)
- j) Condition of MSCs (fresh never cryopreserved cells, thawed and cultured, cryopreserved cells)
- k) Resuscitation used (any combination of fluid and antibiotic resuscitation versus no resuscitation),
- l) Methodological quality according to Cochrane Risk of Bias (e.g. by individual domains)
- m) Construct validity (e.g. studies with less 50% vs studies with greater than 50% of construct validity items)

22.) Current Stage of Review

A pilot search and preliminary analysis was completed in 2012 and presented at the American Thoracic Society Meeting (Lalu MM et al. Systematic review and meta-analysis of mesenchymal stromal cells in preclinical models of septic shock. American Journal of Respiratory and Critical Care Medicine. 185: A2215, 2012. We have now updated the search to May 21, 2015 and we are completing final data analysis.

23.) Knowledge Dissemination

Due to the broad scope of this review, we will present our analysis of the primary outcome (i.e. mortality) and secondary outcomes (i.e. organ dysfunction, inflammatory markers, and bacterial clearance) in separate abstracts and manuscripts. Results will be disseminated through the Canadian Critical Care Translational Biology Group and shared with relevant stakeholders (e.g. Canadian Stem Cell Foundation, Canadian Council for Animal Care).