Cellular Immunotherapy for Severe Sepsis: the CISS Trial

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Cross-Appointed to Physiology; Laboratory Medicine and Pathobiology
Disclosures

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CIHR OCN 126573
CIHR MOP130331
Ministry of Research and Innovation
Early Researchers Award
PSI 0-35
Tissue Regeneration Therapeutics (TRT)

No Relevant financial relationships with a commercial interest
Restricted to the last decade: the incidence rate was 437 for sepsis and 270 for severe sepsis cases per 100,000 person-years. Mortality remains between 17-38%.

Global estimates of 31.5 million sepsis and 19.4 million severe sepsis cases, with potentially 5.3 million deaths annually.
What is The Problem?

• Septic shock is a major cause of morbidity and mortality worldwide
  – 20% of all ICU admissions
  – Leading cause of death in non-coronary ICUs
  – Mortality rates 20-40%
  – Estimated costs in USA: $24 billion annually

• > 100 Phase II and III clinical sepsis trials with little or no successful translation
  – Supportive Care remains mainstay of therapy

Pathophysiology of Sepsis

Insult
Uncontrolled infection/major trauma/circulatory shock/tissue necrosis/apoptosis/anaphylaxia

Trigger

PAMPs
LPS, LTA, lipoproteins, peptidoglycans, bacterial DNA, etc.

DAMPs
HMGB-1, heat-shock protein, DNA, uric acid, etc.

Sensors and effector cells

Complex protein systems
Complement system
Coagulation system

Vascular and tissue cells
Endothelial cells
Epithelial cells
Adipose tissue

Blood and lymphatic cells
Granulocytes
Macrophages/monocytes
Lymphocytes (T-cells, B-cells)

Mediators and biomarkers

C5a, C3a, C5αR, C5b-9, etc.
aPPT, PT, AT, Protein C etc.

Endothelial stress response: ELAM-1, ICAM-1, Selectins,

Acute phase reactants: CRP, LBP, PCT, etc.

Cytokines/chemokines
Soluble receptors: IL-6, IL-8, IL-4, IL-10
MIF, HMGB1, sTNF, suPAR, sTREM-1, etc.

Cell surface markers: mHLA DR, CD64, CD48, C5αR, etc.

Impact on organ function

Brain
Confusion

Lung
Respiratory distress

Cardiovascular system
Shock

Kidney
Oliguria/Anuria

Liver
Excretory failure

Gut
Loss of barrier function, ileus

Micro-circulation
Capillary leak edema, DIC

Mediators and biomarkers

Effective source control
Normalization of biomarker abnormalities
Resolution of organ dysfunction; recovery

Ineffective source control
Persistence of biomarker abnormalities
Multiple organ failure; death

Outcome
Supportive Care

Sepsis Resuscitation Bundle
- Blood Cultures
- Antibiotics Source Control
- Measure Lactate
- Fluids +/- vasopressors
- CVP ScvO₂

Sepsis Management Bundle
- Low dose steroids
- rhAPC
- Glucose <10 mmol/l
- 6ml/kg if ventilated
- Stress Ulcer Prophylaxis
- VTE prophylaxis
- Nutrition

Surviving Sepsis Campaign
Mesenchymal Stromal (Stem) Cells
MSC Milestones

MSCs first found to be immunosuppressive in vitro

NIH funds core lab to standardize MSC expansion

Child with GVHD is treated with allo-MSCs

Osiris launches first trials using Universal Donor MSCs

First culture-expanded allo-MSC therapy gains approval in Canada

MSC Clinical trials ARDS and Sepsis

2016

Phase I MSCs for the treatment of Sepsis

CISS

2015

Phase I MSCs for the treatment of ARDS

START

Critical Care Trials

Cumulative clinical trials (ongoing or completed)

Allogeneic

Autologous

2000

2001

2002

2003

2004

2005

2006

2007

2008

2009

2010

2011

2012

2013

2014

2000

2001

2002

2003

2004

2005

2006

2007

2008

2009

2010

2011

2012

2013

2014

MSC Milestones

Overall Mortality: Pre-Clinical MSC Sepsis Experiments

<table>
<thead>
<tr>
<th>Study Name</th>
<th>Dead / Total</th>
<th>Odds Ratio</th>
<th>Lower limit</th>
<th>Upper limit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MSC</td>
<td>Control</td>
<td></td>
<td></td>
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<tr>
<td>Gonzalez-Rey et al. 2009 A</td>
<td>5 / 18</td>
<td>8 / 10</td>
<td>0.10</td>
<td>0.01</td>
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<tr>
<td>Gonzalez-Rey et al. 2009 B</td>
<td>14 / 20</td>
<td>10 / 10</td>
<td>0.11</td>
<td>0.01</td>
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<td>Nemeth et al. 2009</td>
<td>52 / 90</td>
<td>40 / 45</td>
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<td>0.06</td>
</tr>
<tr>
<td>Bi et al. 2010</td>
<td>5 / 10</td>
<td>10 / 10</td>
<td>0.05</td>
<td>0.00</td>
</tr>
<tr>
<td>Mei et al. 2010 A</td>
<td>7 / 29</td>
<td>13 / 29</td>
<td>0.39</td>
<td>0.13</td>
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<td>Mei et al. 2010 B</td>
<td>8 / 15</td>
<td>17 / 20</td>
<td>0.20</td>
<td>0.04</td>
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<td>Liang et al. 2011</td>
<td>5 / 15</td>
<td>8 / 15</td>
<td>0.44</td>
<td>0.10</td>
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<td>Chang et al. 2012</td>
<td>10 / 16</td>
<td>6 / 16</td>
<td>2.78</td>
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<td>12 / 34</td>
<td>46 / 69</td>
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<td>18 / 40</td>
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<td>Hall et al. 2013</td>
<td>9 / 26</td>
<td>26 / 35</td>
<td>0.18</td>
<td>0.06</td>
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<tr>
<td>Zhao et al. 2013</td>
<td>9 / 24</td>
<td>18 / 27</td>
<td>0.30</td>
<td>0.09</td>
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<td>Chao et al. 2014</td>
<td>1 / 10</td>
<td>2 / 10</td>
<td>0.21</td>
<td>0.02</td>
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<td>Kim et al. 2014</td>
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<td>53 / 66</td>
<td>0.65</td>
<td>0.29</td>
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<td>Luo et al. 2014</td>
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<td>10 / 12</td>
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<td>0.02</td>
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<td>0.05</td>
<td>0.00</td>
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<tr>
<td>Yang JF et al. 2015</td>
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<td>10 / 10</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>234 / 504</strong></td>
<td><strong>337 / 446</strong></td>
<td><strong>0.27</strong></td>
<td><strong>0.18</strong></td>
</tr>
</tbody>
</table>

\( I^2 = 33\% \)

Test for overall effect: \( Z = 6.27, p < 0.00001 \)

OR < 1 favors MSCs as compared to controls
Pathogen Clearance:
Bacterial Colony Forming Units

Peritoneum

<table>
<thead>
<tr>
<th>Model</th>
<th>Time (h)</th>
<th>n (T/C)</th>
<th>RoM (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonzalez-Rey 2009</td>
<td>C 24</td>
<td>6/6</td>
<td>0.69 (0.63, 0.76)</td>
</tr>
<tr>
<td>Nemeth 2009</td>
<td>C 24</td>
<td>10/12</td>
<td>0.45 (0.12, 1.66)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16/18</td>
<td>0.69 (0.63, 0.75)</td>
</tr>
</tbody>
</table>

Blood

<table>
<thead>
<tr>
<th>Model</th>
<th>Time (h)</th>
<th>n (T/C)</th>
<th>RoM (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonzalez-Rey 2009</td>
<td>C 24</td>
<td>6/6</td>
<td>0.37 (0.25, 0.55)</td>
</tr>
<tr>
<td>Nemeth 2009</td>
<td>C 24</td>
<td>10/12</td>
<td>0.35 (0.06, 1.53)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16/18</td>
<td>0.37 (0.25, 0.54)</td>
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</table>

Spleen

<table>
<thead>
<tr>
<th>Model</th>
<th>Time (h)</th>
<th>n (T/C)</th>
<th>RoM (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonzalez-Rey 2009</td>
<td>C 24</td>
<td>6/6</td>
<td>0.39 (0.23, 0.68)</td>
</tr>
<tr>
<td>Mei 2010</td>
<td>C 28</td>
<td>10/8</td>
<td>0.21 (0.03, 1.36)</td>
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<tr>
<td></td>
<td></td>
<td>16/14</td>
<td>0.37 (0.22, 0.63)</td>
</tr>
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</table>

McIntyre et al, Unpublished Data
**Organ Failure**

**Renal: Creatinine**

<table>
<thead>
<tr>
<th>Model</th>
<th>Time (h)</th>
<th>n (T/C)</th>
<th>RoM (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nemeth 2009</td>
<td>24</td>
<td>14/13</td>
<td>0.76 (0.53, 1.07)</td>
</tr>
<tr>
<td>Yagi 2010</td>
<td>24</td>
<td>10/10</td>
<td>0.44 (0.24, 0.84)</td>
</tr>
<tr>
<td>Mei 2010</td>
<td>24</td>
<td>11/9</td>
<td>0.66 (0.50, 0.89)</td>
</tr>
</tbody>
</table>

35/32 0.66 (0.49, 0.89)

**Lung: Pulmonary Neutrophils**

<table>
<thead>
<tr>
<th>Model</th>
<th>Time (h)</th>
<th>n (T/C)</th>
<th>RoM (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xu 2007</td>
<td>24</td>
<td>4/4</td>
<td>0.61 (0.33, 1.13)</td>
</tr>
<tr>
<td>Yagi 2010 (b)</td>
<td>24</td>
<td>5/5</td>
<td>0.57 (0.27, 1.23)</td>
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</tbody>
</table>

9/9 0.59 (0.37, 0.96)

**Liver: AST**

<table>
<thead>
<tr>
<th>Model</th>
<th>Time (h)</th>
<th>n (T/C)</th>
<th>RoM (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nemeth 2009</td>
<td>24</td>
<td>14/13</td>
<td>0.85 (0.85, 0.85)</td>
</tr>
<tr>
<td>Yagi 2010</td>
<td>24</td>
<td>10/10</td>
<td>0.57 (0.39, 0.83)</td>
</tr>
<tr>
<td>Mei 2010</td>
<td>24</td>
<td>13/11</td>
<td>1.00 (0.71, 1.41)</td>
</tr>
</tbody>
</table>

37/34 0.81 (0.64, 1.02)

McIntyre et al, Unpublished Data
Mechanisms of Action

• Contact dependent
• Contact Independent
  (i) Enhancing tissue-endogenous stem/progenitor cell activity
  (ii) Secretion of paracrine factors
  (iii) Regulation of genes that modulate the response to injury and repair
  (iv) Transfer of cellular and genomic contents such as mitochondria and microRNAs
Contact-Dependent Mechanisms of Action

Subpleural depth (μm): 6 6 10
Time after mBMSC instillation (h): 1 3 3
Paracrine effectors of Beneficial effects

**Diagram:**

- **PGE\textsubscript{2}**
- **LL-37**
- **KGF**
- **MSC**
- **Bacteria**
- **Activated macrophage**
- **IL-10**
- **Epithelial repair**
- **Tight junction**
- **Endothelial repair**
- **PMN**
- **Lymphocyte**

**Annotations:**

- Increased alveolar fluid clearance
- Bacteria phagocytosis

**References:**

Exosome-mediated beneficial effect

Survival

Total Protein in BAL

Mosel, AJRCCM, 2015
Mesenchymal stem cells use extracellular vesicles to outsource mitophagy and shuttle microRNAs

hMSC-derived MV

Exosomes

Untreated
Exosomes

NF-κB

RAW 264.7 macrophages

15 μm
Immunomodulation By Therapeutic Mesenchymal Stromal Cells (MSC) Is Triggered Through Phagocytosis of MSC By Monocytic Cells

SAMANTHA F.H. DE WITTE, FRANKA LUK, JESUS M. SIERRA PARRAGA, MADHU GARGESHA, ANA MERINO, SANDER S. KOREVAAR, ANISHA S. SHANKAR, LISA O’FLYNN, STEVE J. ELLIMAN, DEBASHISH ROY, MICHEL G.H. BETJES, PHILIP N. NEWSOME, CARLA C. BAAI, MARTIN J. HOOGDUIN.
Apoptosis in mesenchymal stromal cells induces in vivo recipient-mediated immunomodulation

Antonio Galleu,¹ Yanira Riffo-Vasquez,² Cristina Trento,¹ Cara Lomas,³⁴ Luigi Dolcetti,¹ Tik Shing Cheung,¹ Malte von Bonin,⁵ Laura Barbieri,¹ Krishma Halai,¹ Sophie Ward,³⁴ Ling Weng,¹ Ronjon Chakraverty,³⁴ Giovanna Lombardi,⁶ Fiona M. Watt,⁷ Kim Orchard,⁸ David I. Marks,⁹ Jane Apperley,¹⁰ Martin Bornhauser,¹,⁵ Henning Walczak,⁴*, Clare Bennett,³⁴* Francesco Dazzi¹,10+
MSCs are phagocytosed by human monocytes
Mesenchymal stem cells enhance NOX2-dependent reactive oxygen species production and bacterial killing in macrophages during sepsis

MSCs polarize human monocytes/macrophages

MSC

PGE$_2$

EP receptor

PI3K

NADPH oxidase

ROS

Bacterial killing

M1-like macrophage

Low phagosomal ROS

Acute phagosomal acidification

Anti-inflammatory effects

CD163

M2-like macrophage
Host response to MSCs

A. GSEA Identified Mitochondrial Related Genes

B. Network Analysis Showing Mitochondrial Related Pathways Up-regulated after MSC treatment

Parkinson Disease (autosomal recessive, early onset) 7/DJ-1
CELLULAR IMMUNOTHERAPY FOR SEPTIC SHOCK: CISS Trial

Featured Interview

The world’s first in-human stem cell trial for septic shock: A bench-to-bedside journey in critical care from the perspective of Dr. Lauralyn McIntyre

Colin Suen, BMSc1,2, Loretta Cheung, BSc1

1Faculty of Medicine, University of Ottawa
2Sprott Centre for Regenerative Medicine, Ottawa Hospital Research Institute
CISS Trial Design

- **Design**: Phase I single centre, open label safety and dose escalation trial of MSCs in septic shock

- **Primary Objective**: Determine the safety profile of MSCs in septic shock
  - Determine the maximum tolerable dose of MSCs in septic shock

- **Secondary Objectives**: Examine biological effects of MSCs through measurement of serial biomarkers of inflammation and acute phase proteins
  - Examine measures of feasibility related to trial implementation and conduct
CISS Observational Arm
n = 18-24 patients

CISS eligibility met within 24 hours of ICU admission
(additional 6 hours to enrolment)

Consent obtained

Serial blood for assessment of markers of inflammation at baseline, 1, 4, 12, 24, and 72 hours

Record clinical and physiological adverse events

CISS Interventional Arm
n = 1-9 patients

CISS eligibility met within 24 hours of ICU admission
(additional 6 hours to enrolment)

Consent obtained

Low Dose (n=3):
0.3 x 10^6 cells/kg

Mid Dose (n=3):
1.0 x 10^6 cells/kg

High Dose (n=3):
3.0 x 10^6 cells/kg

* DSMC review after completion of each dose panel

Maximum Tolerated Dose Established for Phase II Trial
<table>
<thead>
<tr>
<th></th>
<th>Observational Arm (n = 21)</th>
<th>Interventional Arm (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, Median (range)</td>
<td>65.5 (22-95)</td>
<td>72.5 (39-91)</td>
</tr>
<tr>
<td>Sex (female), No (%)</td>
<td>8 (38.1)</td>
<td>5 (62.5)</td>
</tr>
<tr>
<td>APACHE II Score Median (range)</td>
<td>24 (17-34)</td>
<td>24 (13-37)</td>
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<tr>
<td>Composite MODS, Median (range)</td>
<td>6 (1-15)</td>
<td>5 (4-10)</td>
</tr>
<tr>
<td>Baseline Organ Failure/Hypoperfusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory (P/F)</td>
<td>195(80-496)</td>
<td>244(121-291)</td>
</tr>
<tr>
<td>Renal (Creatinine)</td>
<td>187 (43-459)</td>
<td>240 (72-316)</td>
</tr>
<tr>
<td>Hematologic (Platelets)</td>
<td>160 (13-342)</td>
<td>130 (45-157)</td>
</tr>
<tr>
<td>Hepatic (Bilirubin)</td>
<td>13 (3-15)</td>
<td>22 (6-83)</td>
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<tr>
<td>Lactate</td>
<td>2.3 (0.8-7.8)</td>
<td>2.5 (1.4-4.8)</td>
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<tr>
<td>Baseline Organ Support, No (%)</td>
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<tr>
<td>Vasoactive Agents</td>
<td>21 (100)</td>
<td>21 (100)</td>
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<tr>
<td>Ventilation</td>
<td>21 (100)</td>
<td>21 (100)</td>
</tr>
<tr>
<td>Dialysis</td>
<td>4 (19.1)</td>
<td>1 (11)</td>
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<tr>
<td>Infectious Source No (%)</td>
<td></td>
<td></td>
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<tr>
<td>Lung</td>
<td>7 (33)</td>
<td>3 (33)</td>
</tr>
<tr>
<td>Intra abdominal</td>
<td>6 (29)</td>
<td>3 (33)</td>
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<tr>
<td>Urinary</td>
<td>2 (10)</td>
<td>1 (11)</td>
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<tr>
<td>Other</td>
<td>6 (29)</td>
<td>2 (25)</td>
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<tr>
<td>Time to enrolment (hours), Median (Range)</td>
<td>19 (5 – 28)</td>
<td>24 (11 – 27)</td>
</tr>
</tbody>
</table>
Adverse events

- No transfusion associated adverse events
- No serious unexpected adverse events related to study drug
Cellular Immunotherapy for Septic Shock
A Phase I Clinical Trial

Lauralyn A. McIntyre\textsuperscript{1,2,3}, Duncan J. Stewart\textsuperscript{2,4}, Shirley H. J. Mei\textsuperscript{2,5}, David Courtman\textsuperscript{2,5}, Irene Watpool\textsuperscript{2}, John Granton\textsuperscript{6}, John Marshall\textsuperscript{7}, Claudia dos Santos\textsuperscript{7}, Keith R. Walley\textsuperscript{8}, Brent W. Winston\textsuperscript{9}, Kenny Schlosser\textsuperscript{2,5}, and Dean A. Fergusson\textsuperscript{2,3}; for the Canadian Critical Care Trials Group and the Canadian Critical Care Translational Biology Group.
Effects of Mesenchymal Stem Cell Treatment on Systemic Cytokine Levels in a Phase 1 Dose Escalation Safety Trial of Septic Shock Patients

Authors:
Kenny Schlosser, PhD¹, Jia-Pey Wang, MSc¹, Claudia dos Santos, MD, MSc, FRCPC²,³, Keith R. Walley, MD⁴, John Marshall, MD, FRCSC, FACS³, Dean A. Fergusson, PhD⁵, Brent W. Winston, MD, FRCPC⁶, John Granton, MD², Irene Watpool, RN, BScN⁷, Duncan J. Stewart, MD, FRCPC¹,⁸ii, Lauralyn A. McIntyre, MD, FRCPC, MHS⁷#, Shirley H. J. Mei, PhD, MSc¹*# on behalf of the Canadian Critical Care Trials Group and the Canadian Critical Care Translat
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The CISS Team

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– Duncan Stewart
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– Dean Fergusson
– Shirley Mei
– Manoj Lalu
– John Granton
– John Marshall
– Keith Walley
– Brent Winston
– Claudia dos Santos

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• Canadian Critical Care Translational Biology Group
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• Irene Watpool
Thank You