CELLULAR IMMUNO-THERAPY FOR COVID-19 RELATED ARDS: THE CIRCA-19 TRIALS

CellCAN Webinar June 29, 2020

DR. DUNCAN J. STEWART

EXECUTIVE VICE PRESIDENT, RESEARCH, THE OTTAWA HOSPITAL; CEO AND SCIENTIFIC DIRECTOR, OTTAWA HOSPITAL RESEARCH INSTITUTE

PRESIDENT AND SCIENTIFIC DIRECTOR, ONTARIO INSTITUTE FOR REGENERATIVE MEDICINE

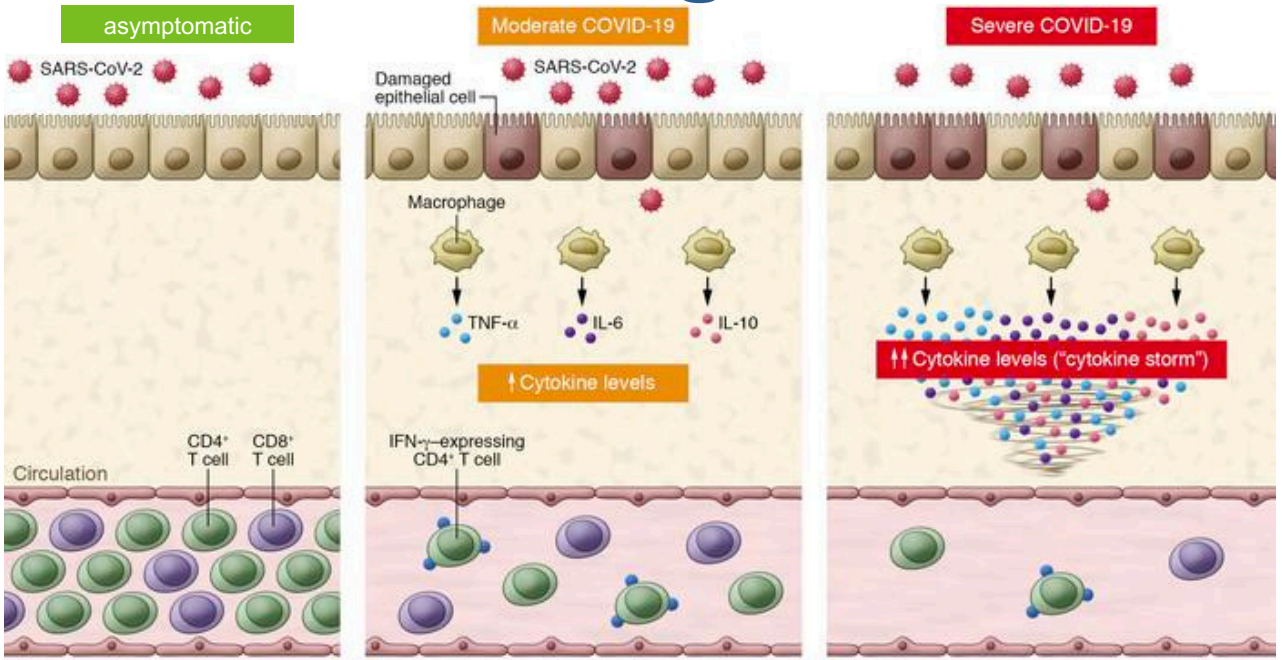
CIHR IRSC
Canadian Institutes of Health Research
Instituts de recherche en santé du Canada

oirm Ontario Institute for Regenerative Medicine

Ontario

STEM CELL NETWORK
RÉSEAU DE CELLULES SOUCHES

COVID-19 Disease Stages

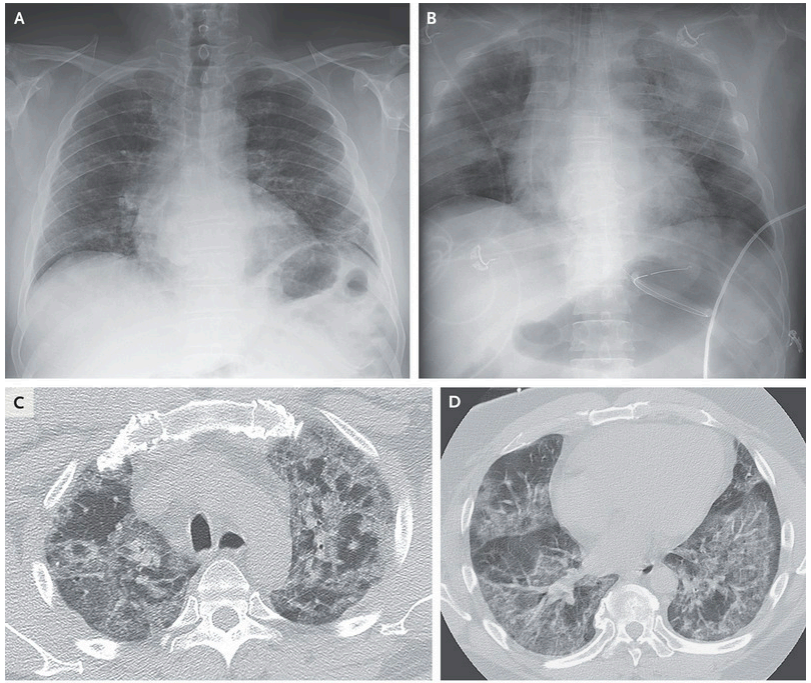


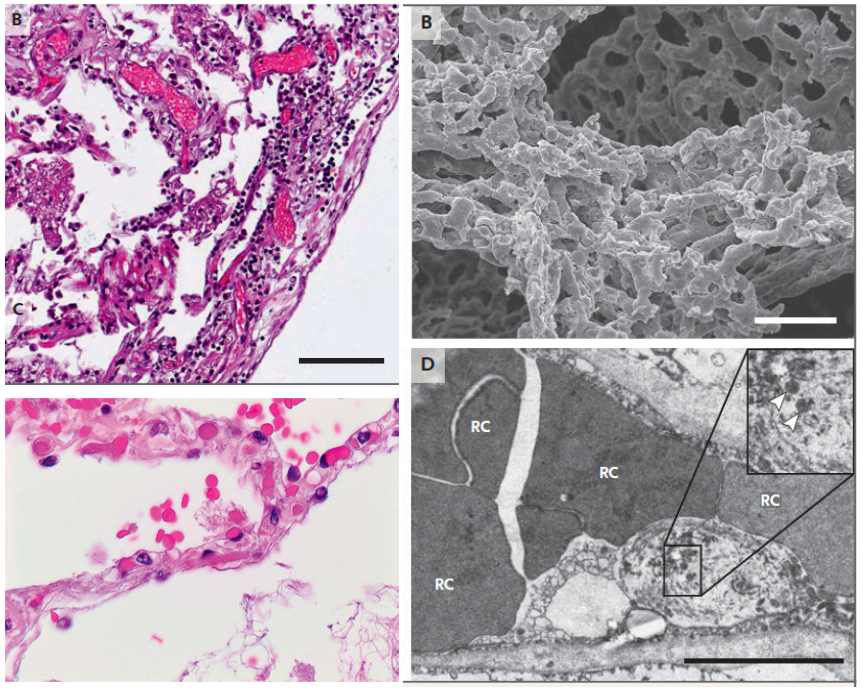
Symptoms:	None	Fever, cough, fatigue, myalgia	Fever, cough, fatigue, myalgia, dyspnea, ARDS, respiratory failure
Cytokines/biomarkers:	Normal	↑IL-6	↑↑IL-6, LDH, D-dimer, procalcitonin, CRP, ferritin, sIL-2R, IL-10, TNF-∝
Inflammatory cells:	Normal	↓Lymphocytes (CD4+ and CD8+ T cells,	↓↓Lymphocytes (CD4+ and CD8+ T cells,) ↓IFNγ-expressing CD4+ T cell, NK cells

Modified from J Clin Invest. 2020;130(5):2202–2205

COVID-19 induced Adult Respiratory Distress Syndrome (ARDS)

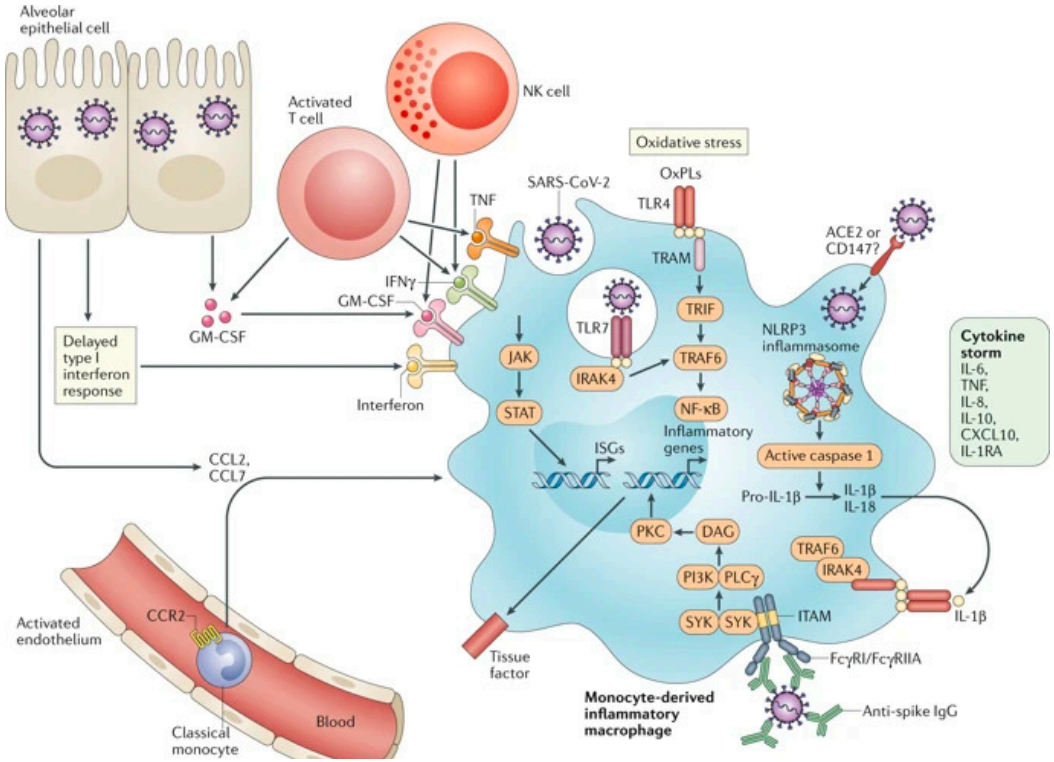
Severe ‘endothelialitis’ and intravascular coagulation





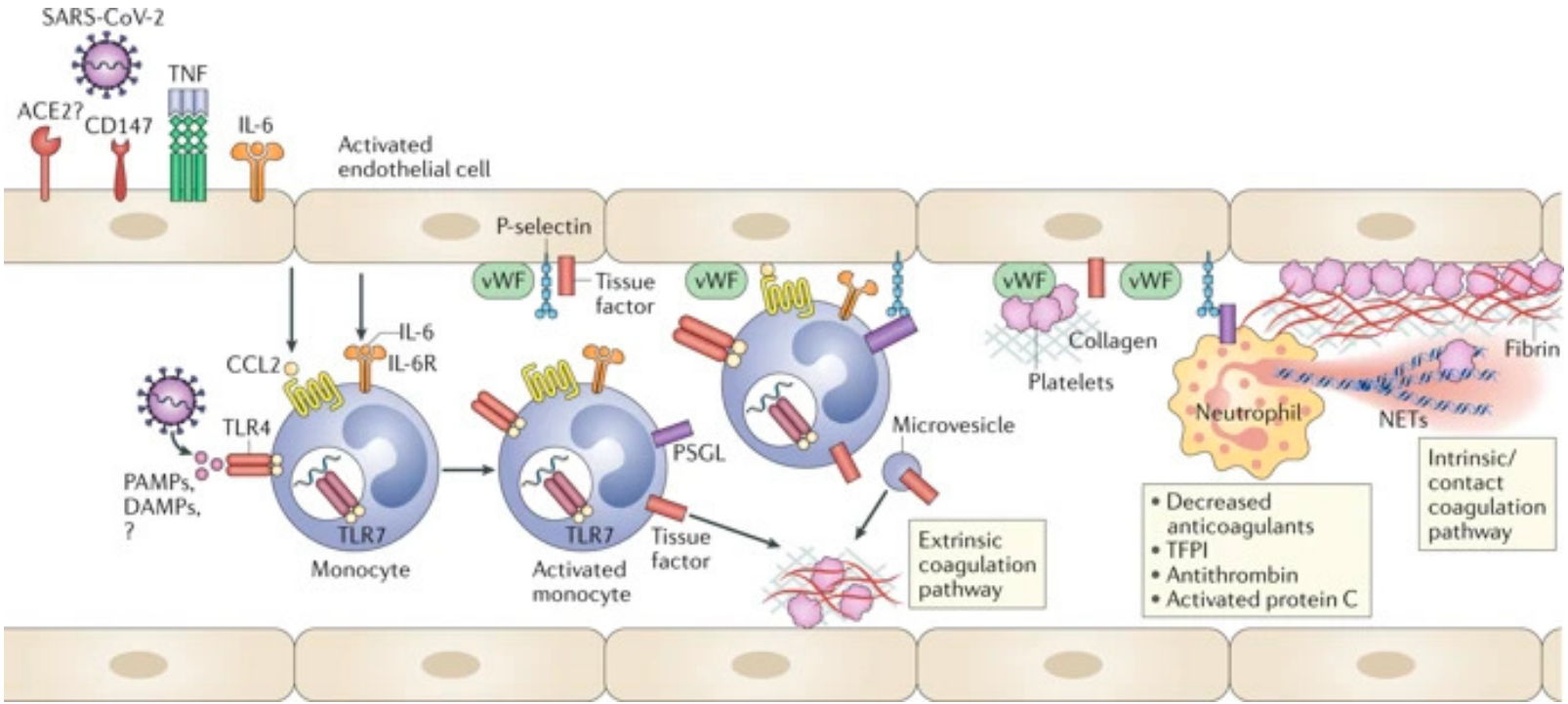
N Engl J Med 2020; 382:2012-2022

Monocyte/ macrophage hyper-activation drives hyper-inflammation in COVID-19 ARDS



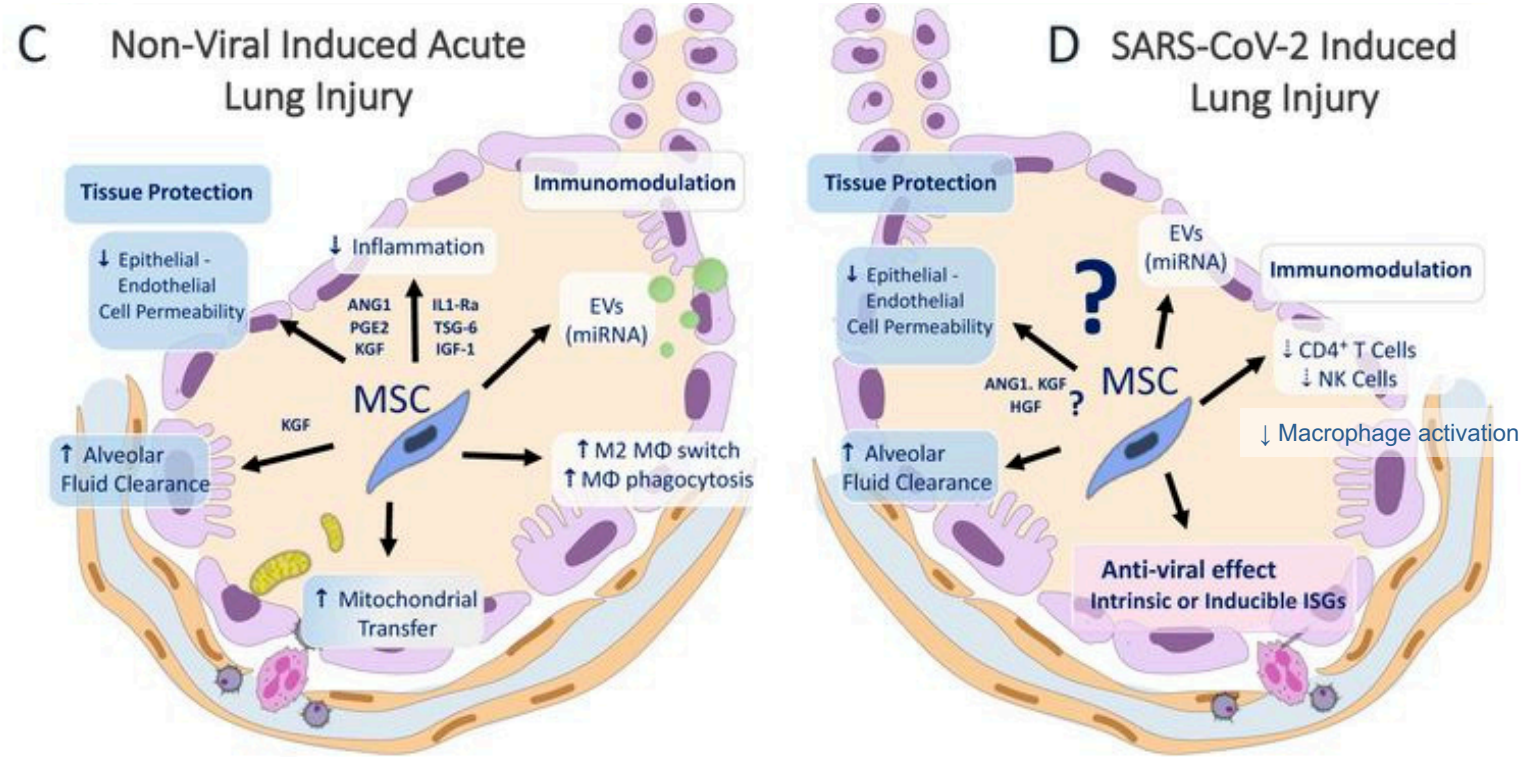
Nature Reviews | Immunology; Volume 20 | June 2020

POSSIBLE CONTRIBUTION OF HYPERACTIVATED MONOCYTES TO COAGULATION IN COVID-19



Nature Reviews | Immunology; Volume 20 | June 2020

IMMUNOMODULATORY CELL THERAPY WITH MESENCHYMAL STROMAL CELLS (MSCs)



Khoury et al. N Engl J Med. 2020

MSC Research at OHRI

- MSCs in sepsis and ARDS models
  - ↓ pro-inflammatory cytokine pathways, ↑ pathogen clearance[1,2]
  - ↓ lung injury and improved survival[1,2]

[1]Mei SH et al. 2010;
[2]Mei SH et al. 2007

- Cellular Immunotherapy for Septic Shock (CISS-1) Trial
  - Dose escalation phase 1 trial
    - Three panels (0.3 – 3M cells/kg); 3 patients/panel, 9 patients total
  - Results
    - Up to 250 million cells/patient well tolerated[3]
    - Efficacy signals in metabolomic and microRNA studies[4,5]

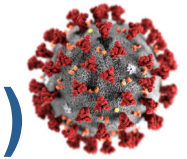
[3]McIntyre LA et al. 2018
[4]McIntyre LA et al. 2018,
[5]Schlosser et al. 2019

Safety profile of MSCs

- 55 randomized clinical trials (2696 patients)
- No SAE directly attributable to MSCs
- Increase in acute fevers → all self-limited

Adverse Event	Risk Ratio, 95% CI
Fever	2.48 (1.27 – 4.86)
Acute infusional toxicity	1.16 (0.70 – 1.91)
Infection	0.99 (0.81 – 1.21)
Thrombotic events	1.14 (0.67-1.95)
Tumour/Malignancy	0.93 (0.60-1.45)
Death	0.78 (0.65 – 0.94)

Immune modulatory cell therapy in Clinical Trials for non-COVID related ARDS (ISCT2020)



- MUST-ARDS - Phase 1/2 Trial, Multistem® (MAPCs)

MUST-ARDS– 28 Day hospitalization Data

All Subjects	MultiStem	Placebo
Number	20	10
Ventilator-free days	12.9 (10.7)	9.2 (9.6)
ICU-free days	10.3 (8.9)	8.1 (8.9)
Mortality (d28)	25%	40%

Patients with more severe ARDS (Prospective Analysis)

Patients w/ PaO2/FiO2 < 150 mmHg at baseline	MultiStem	Placebo
Number	8	8
Ventilator-free days (mean)	14.6 (9.8)	8.0 (9.5)
ICU-free days (mean)	11.4 (8.1)	5.9 (8.5)
Mortality (d28)	25%	50%

Data are n(%) or mean(SD)

Athersys data presented at ISCT2020

COVID-19 Immunomodulatory Cell Therapy Trials (ISCT2020)

- Case series of seven COVID patients treated with MSCs with encouraging results[1]
- Four trials currently registered on ClinicalTrials.gov

Company	Product	Phase	subjects	Cell dose	Route	Primary outcome
Pluristem	Placental cells	2	140	200-600M	IM	Ventilator-free days
Orbsen	MSCs	2	75	400M	IV	Oxygenation index
Mesoblast	MSCs	3	300	2M/kg repeated within 4Ds	IV	Mortality
Athersys	MAPCs	2/3	~400	900M – 2B	IV	Ventilator-free days

[1]Zikuan Leng RZ et al. 2020; [2]Bellingan G et al. 2019

Cellular Immuno-Therapy for COVID-19 related ARDS (CIRCA-19) Trials


Shane English


Dean Fergusson

First Phase 1 trial *(CIRCA-19 Vanguard, n=9)*

- BM-MSCs, rapid deployment, dose excalation, feasibility, tolerability

BM-MSCs

Second Phase 1 trial *(CIRCA-1901, n=6)*

- UC-MSCs, tolerability

UC-MSCs

Phase 2a Trial open-label trial *(CIRCA-1902, n=12)*

- UC-MSCs, tolerability, early signals of potential efficacy

UC-MSCs

Phase 2b Trial Randomized-controlled trial *(CIRCA-19 RCT, n=54)*

UC-MSCs

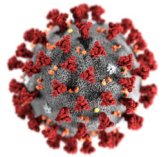
Total projected enrolment = 81 patients

Josee Champagne

Irene Watpool

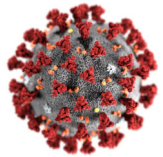
CIRCA-19

CIRCA-19 Vanguard trial:
Safety of multiple dosing of BM-MSCs



- **Open label, dose-escalating, safety trial: 3+3+3 design**
  - **9 patients:** 3 repeated 'unit doses' of BM-MSCs over 3 consecutive days
    - *Panel 1: 25 million cells/unit dose (cumulative dose: 75 million MSCs)*
    - *Panel 2: 50 million cells/unit dose (cumulative dose: 150 million MSCs)*
    - *Panel 3: up to 90 million cells/unit dose (cumulative dose: 270 million MSCs)*
- **Primary objective:**
  - ***Determine maximum feasible tolerated dose*** (MFTD) of BM-MSCs in COVID-19 ARDS patients
- **Secondary objectives:**
  - Assess the safety of increasing repeated doses of BM-MSC
  - Verify surrogate and clinical endpoints (i.e. preliminary evidence of early efficacy) vs. historic and contemporary cohorts
  - Assess immune-monitoring endpoints (i.e. better define the target population)

CIRCA-1901: Phase 1 Safety Trial (UC-MSCs) – 6 patients



Primary objective:

- Determine safety of UC-MSCs in COVID-19 ARDS patients (using MFTD)

Secondary objectives:

- Verify surrogate and clinical endpoints (i.e. preliminary evidence of early efficacy) vs. historic and contemporary cohorts
- Assess immune-monitoring endpoints (i.e. better define the target population)

CIRCA-1902: Phase 2a Safety/Efficacy Trial (UC-MSCs) – Open-label extension of CIRCA-1901 (12 patients)

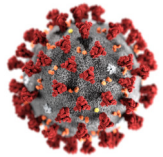
Primary objective:

- Determine safety of UC-MSCs in COVID-19 ARDS patients (using MFTD)

Secondary objectives:

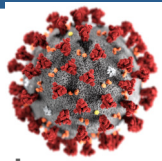
- Assess early signals of mortality and major morbidity vs. historic and contemporary cohorts
- Assess immune-monitoring endpoints (i.e. better define the target population)

CIRCA-19 RCT: Phase 2b efficacy trial



- **Multicentre randomized, placebo-controlled trial** (2:1 randomization; 54 patients)
  - The Ottawa Hospital (PI Shane English)
  - St Michael's Hospital (PI Claudia Do Santos)
  - Centre Hopitalier Universite de Montréal (PI Michäel Chasse)
- **Primary endpoint**
  - Ventilator-free days in patients with COVID-19 ARDS
- **Secondary endpoints**
  - Mortality and major measures of morbidity (organ support and organ failure, infection, ICU and hospital length of stays)
  - biologic measures related to systemic inflammatory and thrombotic response, cardiac and other organ injury, and endothelial function
  - Safety of MSC therapy for COVID-19 ARDS

Selected Eligibility Criteria



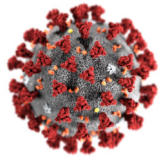
Inclusion criteria: Patients will be eligible for inclusion if they have documented COVID-19 infection with a clinical diagnosis of ARDS criteria as per the Berlin ARDS definition and meet all the criteria listed below

- ✓ Age >18 years
- ✓ Laboratory-confirmed SARS-CoV-2 (PCR)
- ✓ On Invasive Mechanical ventilation ≤72h
- ✓ ARDS (P/F ratio < 300 on FiO2≥0.5, with PEEP ≥5cm H20)

Exclusion Cx:

- × Pregnant or lactating
- × Presence of any active malignancy (other than non-melanoma skin cancer)
- × Any other irreversible disease (6-month mortality >50%)
- × Patient, surrogate, or physician not committed to full support
- × Severe chronic respiratory disease with a PaCO2 > 50 mm Hg or the use of home oxygen

Pooled outcome analyses of all 81 patients



- **Safety/tolerability outcomes for all CIRCA-19 trials:**
  - Adverse events and serious adverse events (AEs and SAEs) will be documented.
    - **Number**, **grade**, **timing, expectedness** and **relatedness** will be captured
    - **Physiologic AEs:** occurring within 30 minutes of infusion (death, cardiac arrest, anaphylaxis)

- **Efficacy outcomes for all CIRCA-19 trials:**
  - Number of Ventilator-free Days at Day 28
  - ICU Mortality

Cell Manufacturing Facility at OHRI

- Five suites for cGMP processing (~2,000 ft^2) – FACT accredited
- 'Isolator' technology to enhance work-flow, limit PPE use, and increase product quality
- Continuous monitoring of critical environmental parameters including: temperature, pressure, CO_2 and O_2 levels etc. Hypoxic conditions can be continuously applied
- Founding member of CellCAN, the Canadian-wide network of cell manufacturing facilities

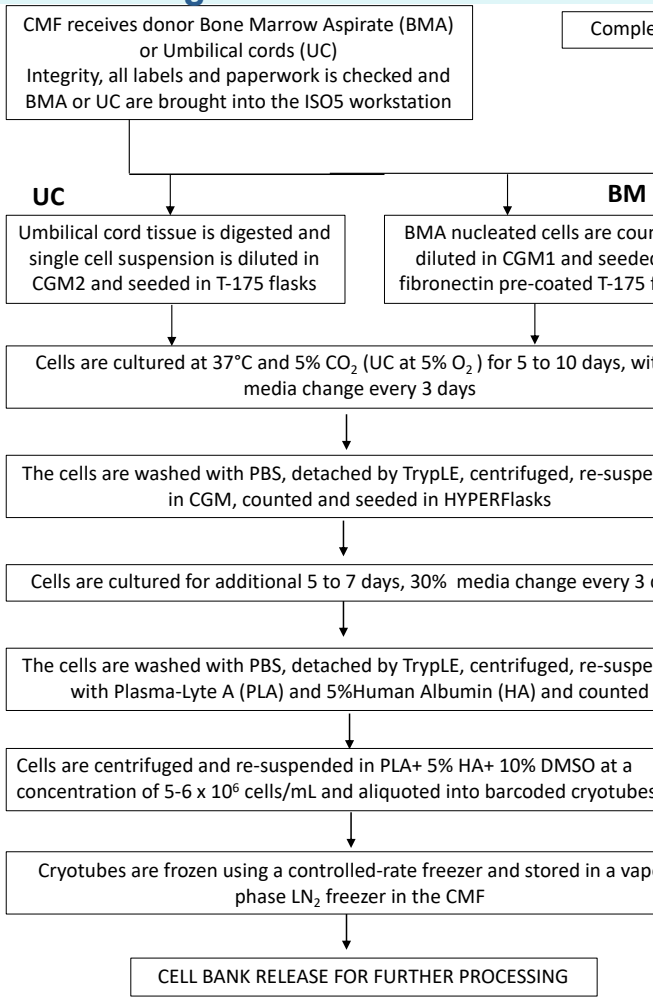
Center for Regenerative Therapies Dresden (CRTD),
Technische Universität, Dresden, Germany

- Licensed according to GMP (Art. 111(5) of Directive 2001/83/EC and Art. 15 of Directive 2001/20/EC).
- Licenced for hematopoietic stem cells, bone marrow-derived MSCs and human islet cells.
- Implemented a regulatory-approved quality management system and is regularly audited by the national regulatory bodies

Two stage manufacturing process

1) Creation of the ‘Working Cell’ Banks (WCB)
   - WCB cryopreserved and fully qualified
2) Manufacture of the Final Cell Products (FCP) from the WCB
   - Cell culture for 2-5 days
   - FCP will be delivered ‘fresh’ (no cryopreservation)

Creation of Working Cell Banks from Donor M



In process controls:

- Examination of morphology of adherent cells by light microscopy
- Microscopic inspection of attached cells for signs of contamination
- 1mL spent media aliquots (for sterility testing if necessary)

In process Q/C:

- Cell viability by trypan blue exclusion test

20 x 10^6 cells are removed for Q/C testing

In-process Q/C (in-house):

- Cell viability by trypan blue exclusion test and Annexin V and PI staining
- Surface Marker Staining
- Endotoxin level detection
- Absence of contamination through 5-day bacterial culture
- Mycoplasma detection
- In vitro potency assay
- Tri-lineage differentiation potential

Third party (out-sourced) Q/C

- SNP-based genotyping to detect major chromosomal aberrations
- Adventitious agent testing using multiple regulated assays
- Mycoplasma detection using a regulated assay

Final Study Products from Working Cell Banks (BM-hMSCs or UC-hMSCs)

MSCs in cell banks are removed from the LN_2 freezer, integrity, labels and paperwork are checked and MSCs are thawed in the ISO5 workstation

Complete Growth Media (CGM1 BM or CGM2 UC) is prepared in the ISO5 workstation

MSCs are diluted in CGM1 or CGM2 and seeded in T-175 or HYPERFlasks

Cells cultured at 37°C and 5% CO_2 for (UC at 5% O_2) 2 to 5 days, with a 30% media change on day 3

Cells are washed with PBS, detached by TrypLE diluted in Plasma-Lyte A (PLA), centrifuged and resuspended with 5% Human Albumin (HA) and PLA and centrifuged again

Cells are re-suspended in 5% Human Albumin (HA) and PLA, and counted

Remaining cell suspension is centrifuged and re-suspended in 5 % HA-PLA to a concentration of 2.5 x 10^6 cells/mL

Final Study Product (2.5 x 10^6 cells/mL in 5 % HA-PLA) is loaded into bag

FINAL PRODUCT RELEASE

In process controls:

- Examination of morphology of adherent cells by light microscopy in sentinel flask
- Microscopic inspection of attached cells for signs of contamination in sentinel flask
- 1mL spent media aliquots (for sterility testing if necessary)

5-10 x 10^6 cells are removed for in-house Q/C testing

Process Q/C (in-house):

- Cell viability by trypan blue exclusion test and Annexin V and PI staining
- Endotoxin level detection
- Absence of contamination through 5-day bacterial culture
- Mycoplasma detection
- In vitro potency assay

Final Study Product

2.5 million hMSCs / mL

- Cells lifted from culture and washed in excipients to a minimum dilution of 1 in 200,000

Excipients:

- Final concentration 5% Human Albumin (Alburex®25, CSL Behring AG)
- PlasmaLyte A for injection, pH 7.4 (Baxter, USP grade)

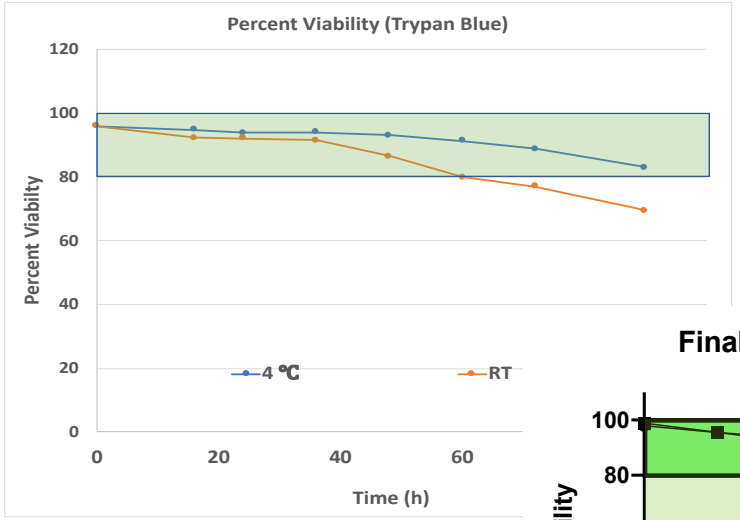
- Similar Dosing Protocol as used in CISS Phase 1 trial
- Infusion at 1ml/min
  - ➢ No cryoprotectants (DMSO) and reduced cell debris as compared to a thawed DP
  - ➢ Previously stablished stability of 8 hours has been extended to 48 hours at 4°C

Final Study Product Stability

Thaw cells and
seed in HYPERFlasks

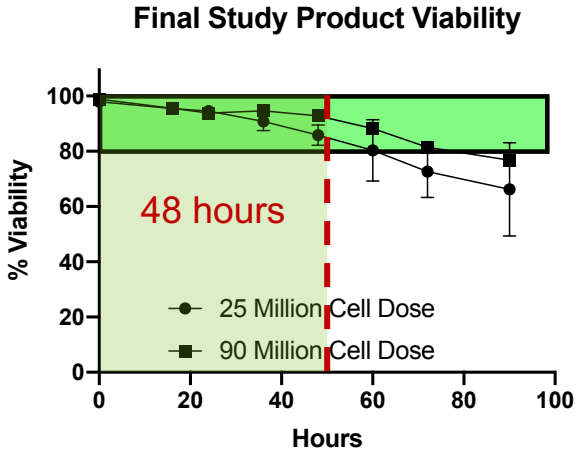
Cells were cultured for
72 to 96h

Harvested cells were
washed and loaded into
bags at 25M (10mL) or
90M (36mL)



- Final study product was maintained at Room temperature (RT) or 4 ℃ (2 – 8 ℃) for up to 90h
- Cell viability via Trypan blue exclusion or Annexin V (AV) and Propidium Iodide (PI) was measured at: 16, 24, 36, 48, 60, 72, and 90h

N = 3



Key considerations for manufacturing due to COVID-19

- Use of previously qualified bone marrow cell banks for rapid deployment
  - Delays in qualification due to vendor availability
- USP Risk Assessment of Key Ancillary reagents
  - As per next slide
- Modifying donor screening questionnaires for Umbilical Cord/Bone Marrow donations with COVID-19 related questions
- ACE-2 expression on hMSCs (receptor for SARS-CoV2)
- Tissue Factor Activity of hMSCs
  - COVID can induce a hypercoagulable state

USP Risk Assessment of Key Ancillary Materials for hMSC Processing

- **Tier 1**: Low Risk
  → Highly qualified materials; licensed biologic, approved drug or medical device
- **Tier 2**: Low Risk
  → Materials well-characterized, suited for drug, biologic, or medical device manufacturing. Excludes most animal derived products.
- **Tier 3**: Moderate Risk
  → Require a higher level of qualification; often produced for in vitro or diagnostic use
- **Tier 4**: High Risk
  → Extensive qualification is required prior to use in manufacturing (i.e. FBS)

USP Risk Assessment of Key Ancillary Materials for hMSC Processing

- CIRCA risk assessment (only for risks >Tier 1)
  - ✓ **Nutristem cell culture media** & Supplements for BM hMSCs (Biological Industries, Israel) *manufactured to GMP standards, Master File (HPB):* **Tier 2/3**
  - ✓ **DMEM culture media** for UC hMSCs (low glucose, pyruvate, no glutamine, no phenol red, GMP grade; Gibco): **Tier 2**
  - ✓ **Human Platelet Lysate** (PLTMax/PLTGold Clinical Grade; Pathogen Inactivated; Mill Creek): **Tier 2**
  - ✓ **Human Fibronectin** (Roche Custom Biotech, GMP grade): **Tier 3**
    - *51 x 5 mg lyophilized vials in house, manufactured prior to emergence of COVID-19*
  - ✓ **TrypLE Select** (GMP grade, Life Technologies): **Tier 2**
    - *microbial trypsin-like enzyme certified as animal origin free*
  - ✓ **Alburex®25** (CSL Behring AG): **Tier 2**
    - *In house LOT: YC42006, EXP: 2021-05, sufficient for CIRCA-19 clinical trials*

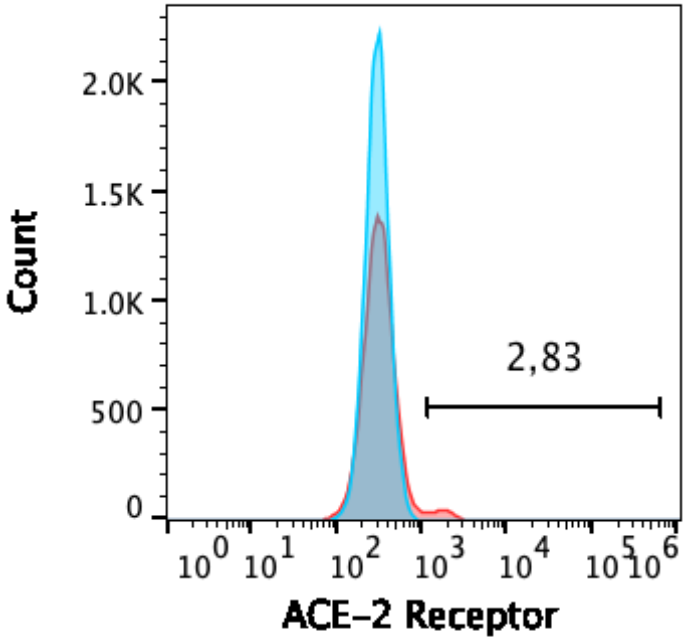
Managing the Risks of Ancillary Materials

- **Full cGMP requires complete adherence to all USP Guidance** → *earlier phase products/trials may not require this*

  - Mitigation of risk
    - ✓ Working closely with suppliers: site visits (audits), quality agreements
    - ✓ If possible, review source documents (CoAs) for all critical source reagents
    - ✓ Regular in-house testing of materials before use for sterility and stability
    - ✓ Establish rigorous storage and protocols to establish working shelf life
    - ✓ Define the potential risks, source to lowest risk (country, batch size)
    - ✓ Consider the use of alternative processing methods (sterile filtration, antibiotics) if high risk materials are unavoidable

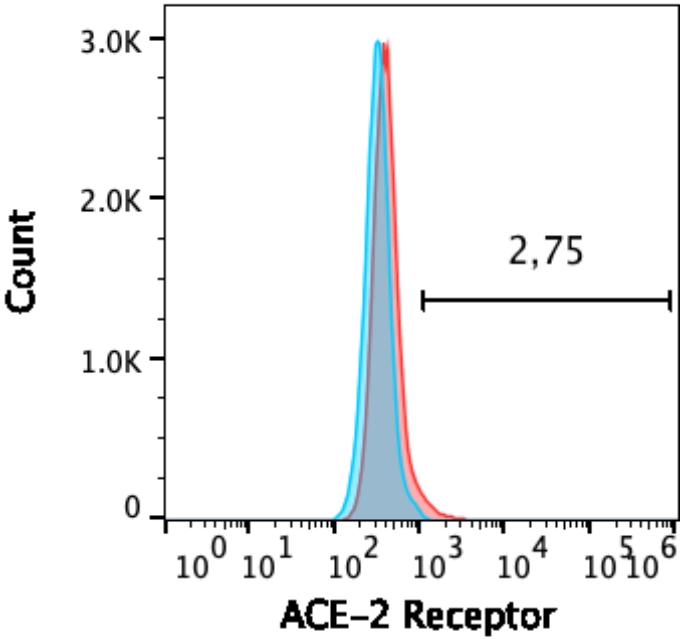
No ACE-2 Expression on hMSCs

BM-MSCs



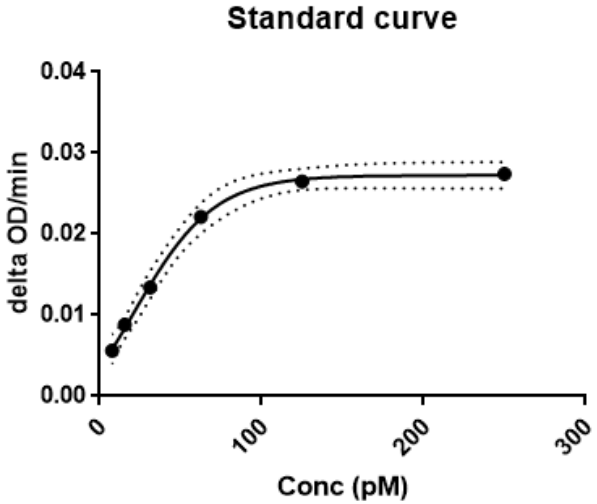
	Sample Name	Subset Name	Count
	FITC IgG1.fcs	BM-MSCs	33059
	ACE2 FITC.fcs	BM-MSCs	26529

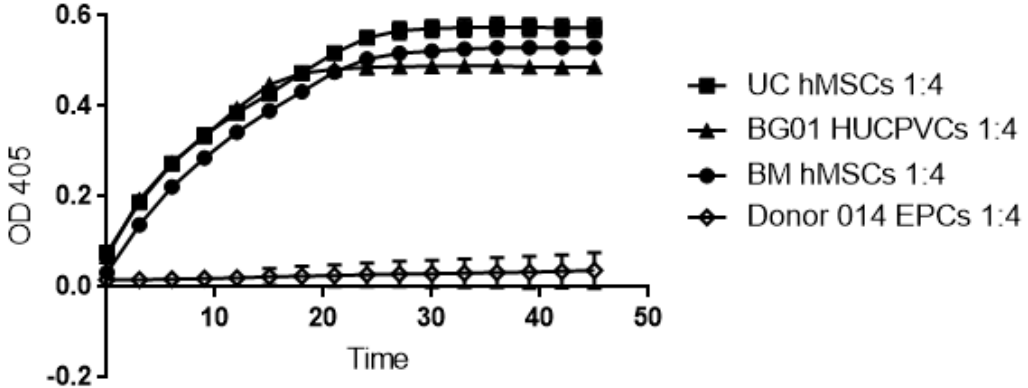
UC-MSCs



	Sample Name	Subset Name	Count
	FITC IgG.fcs	UC-MSCs	44666
	ACE2 FITC.fcs	UC-MSCs	44963

Tissue factor activity assay





	Interpolated conc. (pM)	Sample conc.(pM)
Umbilical Cord MSCs	71.9	287

Eligibility criteria ensure that patients are receiving local standard of care thromboprophylaxis

EPCs	Undetected	**Undetected**

Key milestones

- ✓ Health Canada approval
  - May 15, 2020
- ✓ Research Ethics approval
  - May 25, 2020
- ✓ First cell product ready
  - End of June

All we need are some patients!

June 16th projections for Ottawa

Thanks to the CIRCA-19 ‘Dream Team’!

Dean Fergusson
Program Director, CEP

Shane English
Scientist, ICU

Manoj Lalu
Scientist, CEP, RM

Bernard Thébaud
Scientist RM

David Courtman
SD, CMF

Michael Jamieson
Regulatory

Josee Champagne
Research Assistant

Irene Watpool
Program Manager

Saad Khan
CMF Manager

Samantha Hodgins
CMF Manager

Meaghan Serjeant
RA (Thebault lab)

Mohamad Sobh
Clinical RA, CEP

Joshua Montroy
Clinical RA, CEP

STEM CELL NETWORK RÉSEAU DE CELLULES SOUCHES

oirm Ontario Institute for Regenerative Medicine

Ontario

CIHR IRSC
Canadian Institutes of Health Research Instituts de recherche en santé du Canada