

# BSUITE Red Team

## Cell Gene Therapy



**Remestemcel-L (Ryoncil)**



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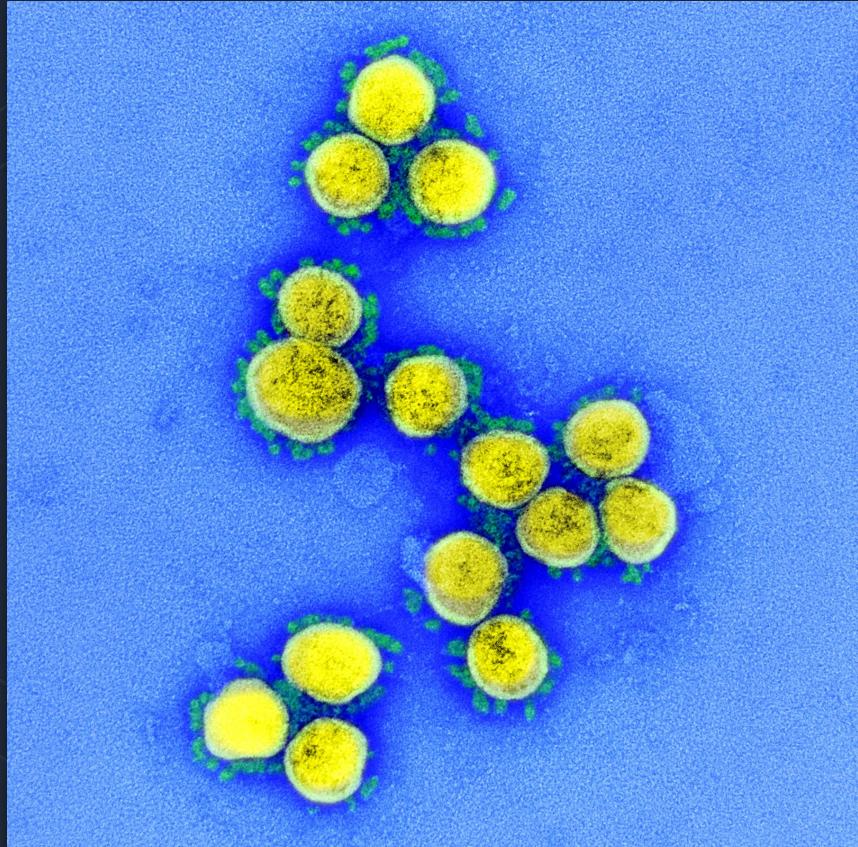
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# What is COVID-19?

- Infectious disease caused by the SARS-CoV-2 virus
- Targets: Respiratory Tract
- Origins unknown. Possibly transmitted at the Wuhan wet market



**Figure 1:** Image taken of SARS-CoV-2 virus under a scanning electron microscope. Shown: Viral envelope (yellow) Spike proteins (green) Image courtesy of the NIAID<sup>1</sup>.

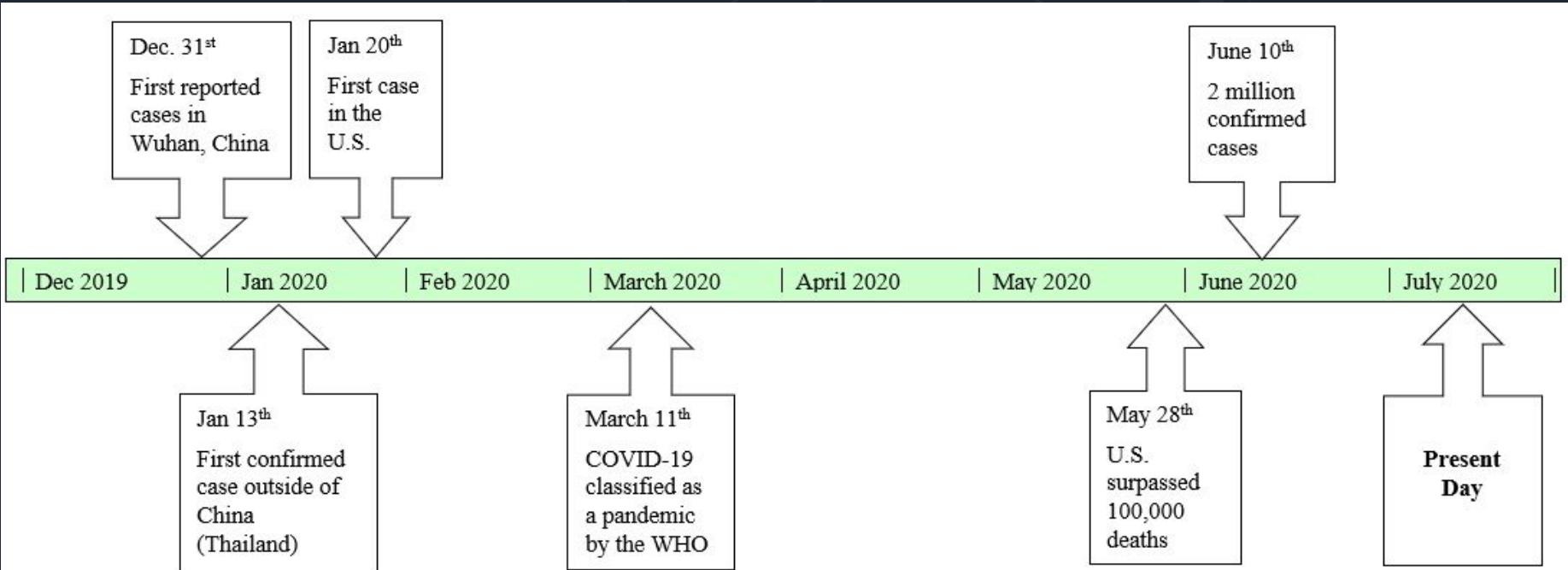
# Progression of COVID-19

- Transmitted through the inhalation or ingestion of viral droplets (coughs or sneezes)
- Targets ACE-2 cell surface receptors in the throat<sup>1</sup>
- Progresses to the lungs where cells with more ACE-2 receptors are present
- Immune response may cause Acute Respiratory Distress Syndrome (ARDS)
- Fluid buildup in alveoli due to inflammation. Can cause blood clots, scarring, collapsed lungs.



1. Krishna Sriram Postdoctoral Fellow, et al. "What Is the ACE2 Receptor, How Is It Connected to Coronavirus and Why Might It Be Key to Treating COVID-19? The Experts Explain." *The Conversation*, 23 June 2020, [theconversation.com/what-is-the-ace2-receptor-how-is-it-connected-to-coronavirus-and-why-might-it-be-key-to-treating-covid-19-the-experts-explain-136928](https://theconversation.com/what-is-the-ace2-receptor-how-is-it-connected-to-coronavirus-and-why-might-it-be-key-to-treating-covid-19-the-experts-explain-136928).

# Timeline of COVID-19



# Current State of COVID-19 in the United States



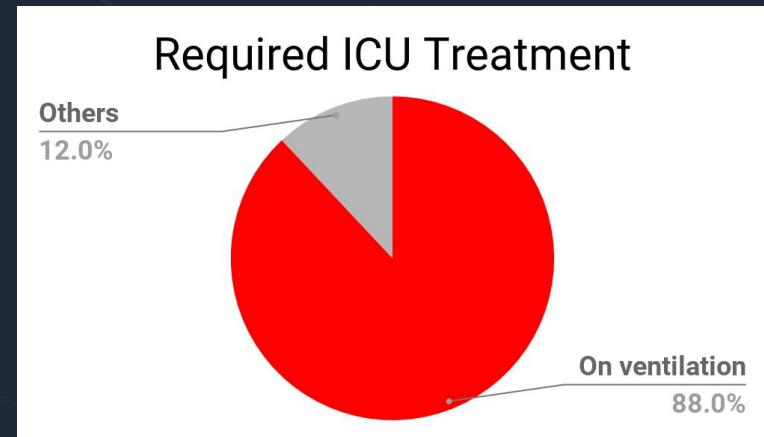
3.3 M

Confirmed cases



135,000

Deaths



64,000  
Required ICU treatment

56,320  
On ventilation  
Acute Respiratory Distress  
Syndrome (ARDS)

Data up to July 15th, 2020

# Cell Therapies!

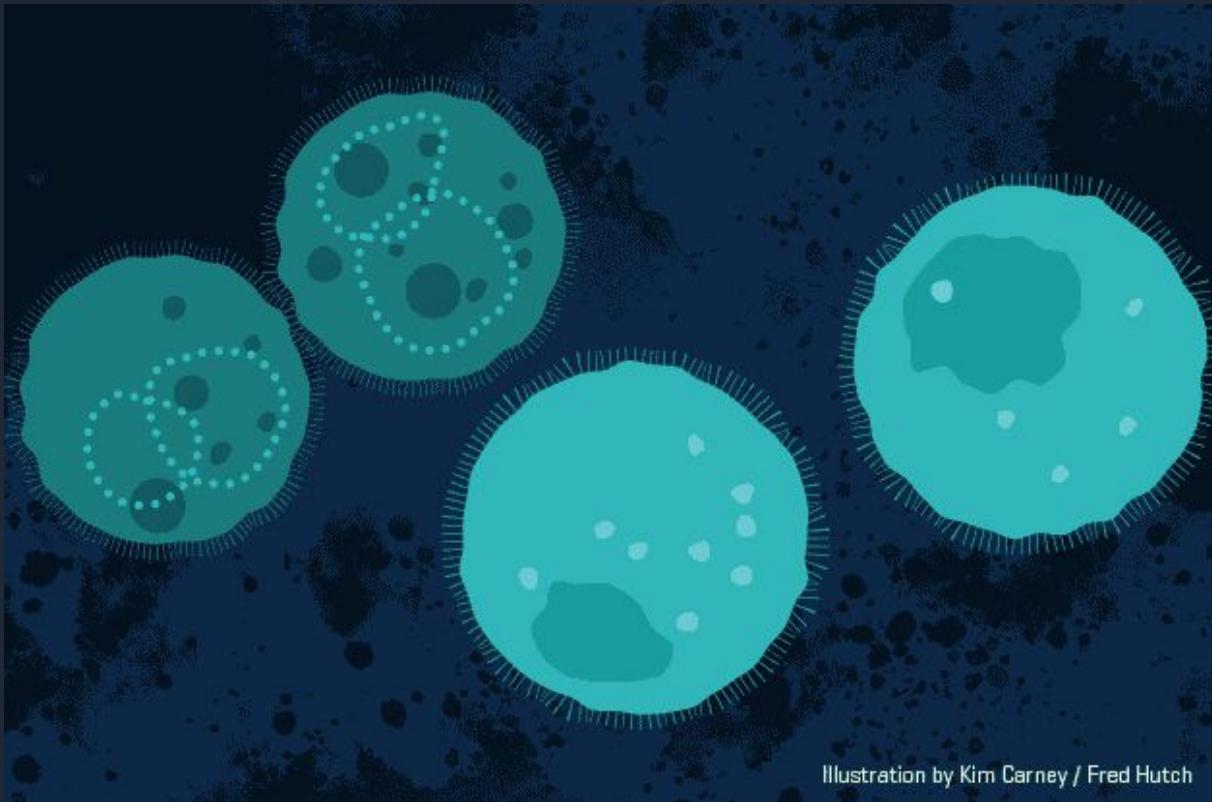
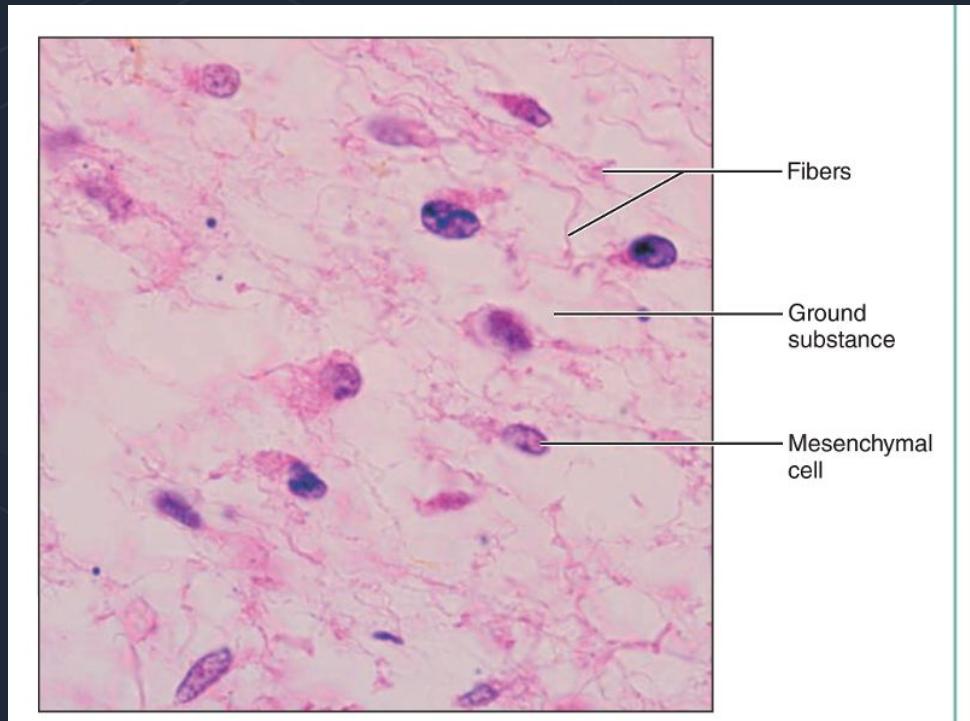


Illustration by Kim Carney / Fred Hutch

# Human Mesenchymal Stem Cells Are Optimal Candidates

- Our focus: Human Mesenchymal Stem Cells (hMSCs)
- Stem Cells are “special” human cells that can be used as a way to give rise to many different types of cells.
  - Ex: Mesenchymal stem cells, hematopoietic stem cells, etc.
- Why hMSCs?
  - Differentiation properties
  - Express low levels of surface Human Leukocyte Antigen-I (HLA), and no HLA-II
  - Produce immunomodulatory cytokines



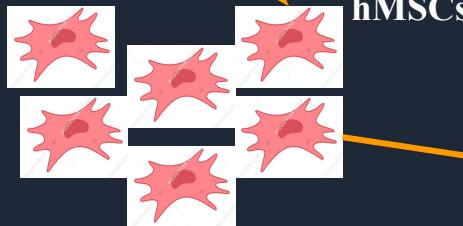
# Manufacturing of Allogeneic hMSCs



Healthy  
donor



Bone  
Marrow

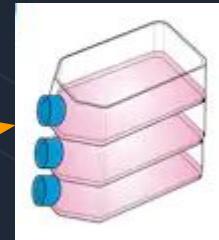


hMSCs

Seed  
train



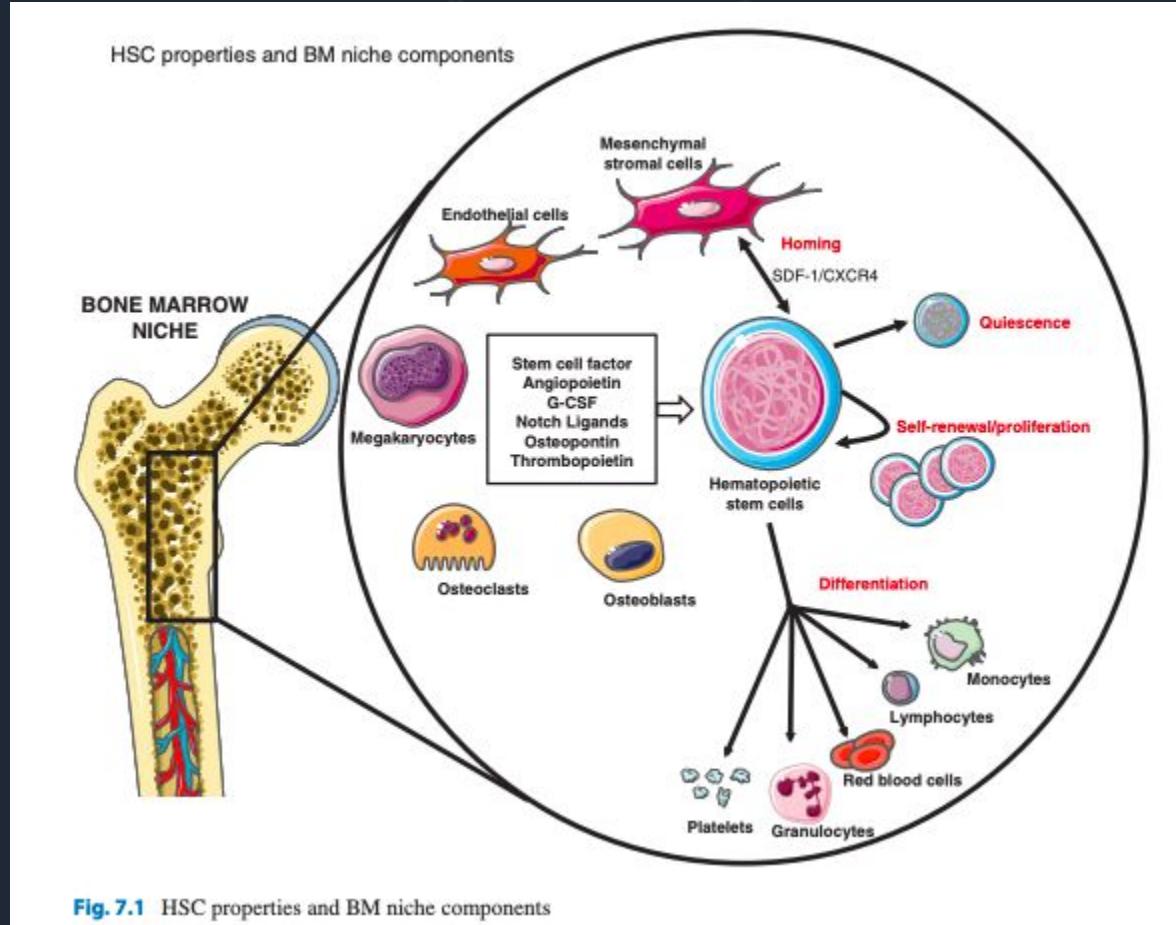
Scale  
out



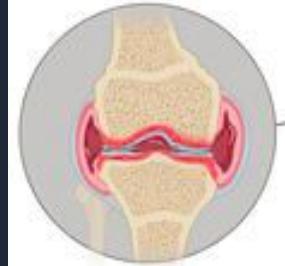
Patients



Large scale  
expansion with wave  
bag bioreactors



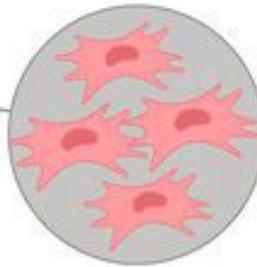
# MSC



TSG-6, STC1,  
IL-4/-10, CCL20 ...



secretome e.g.  
cytokines, chemokines,  
growth factors and  
extracellular vesicles

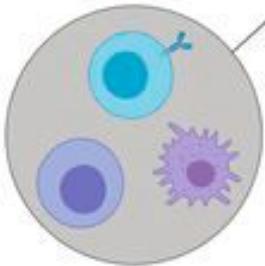


specific differentiation  
cocktails

**Self Renewal**  
(*in vivo*)

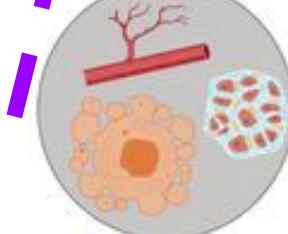
**Reduce Inflammation**

IDO, IL-6, PGE2 ...

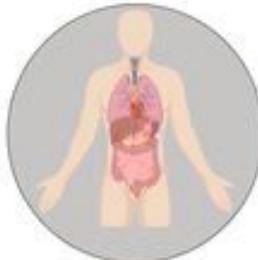


**Modulate Immune Cells**

VEGF, FGF-2, HGF, IGF-1,  
CXCL12 ...



*in vivo* - stimulate local stem  
cells to regenerate new tissue



**Fight Apoptosis and Fibrosis**  
**Improve Angiogenesis**

**Regenerate Tissue**



bone



cartilage



fat

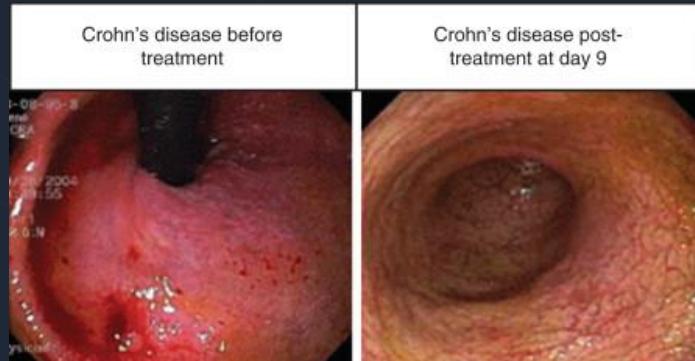
# Previous Work of hMSCs

- Used for treatment of children with steroid refractory acute graft vs. host disease (SR-aGvHD)
  - Occurs when the donated stem cells view the recipient's body as foreign, and attack the organs and tissues.
- hMSCs migrate to the damaged tissue sites that exhibit inflammation.
- MSCs prevent Th cells from differentiation into Th17 cells which produce proinflammatory cytokine
  - Also used as treatment for Crohn's Disease (inflammatory bowel disease (IBD) )
  - **Our objective:** repurpose hMSCs to combat inflammatory response to COVID

# Remestemcel-L (Ryoncil)

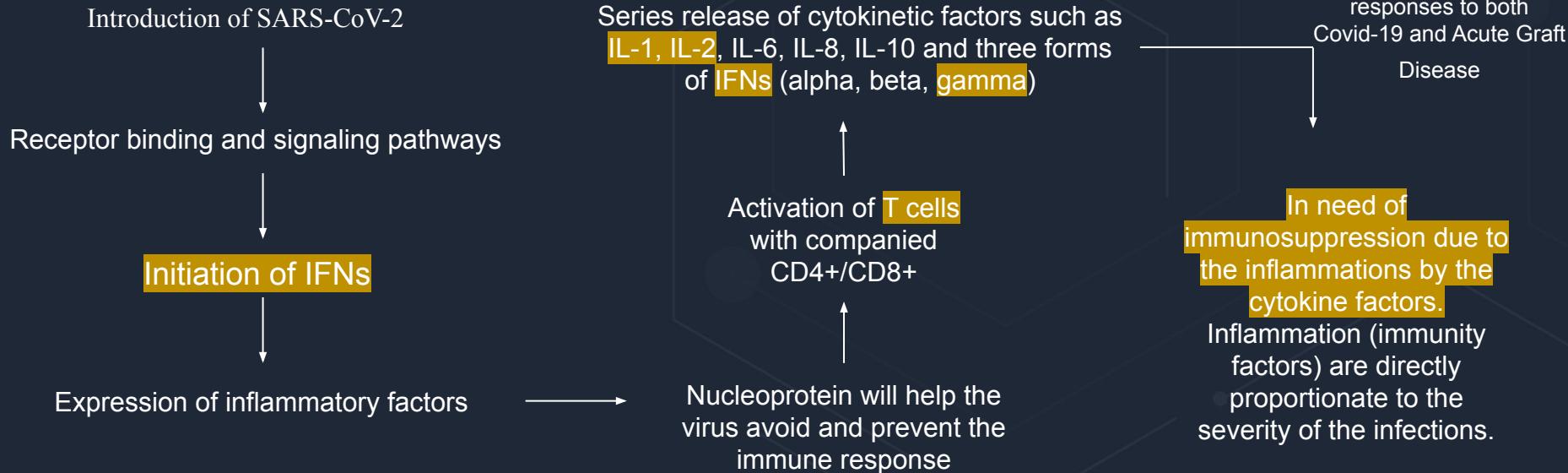
- **Commercial Name:** Ryoncil
- **Clinical Trials:** Phase III Clinical
- **Pharmacology:** Culture-expanded mesenchymal stem cells derived from the bone marrow of an unrelated healthy donor (allogeneic). It is administered to patients in a series of intravenous infusions.

Prochymal treatment for acute graft-versus-host disease. **Comprehensive Biotechnology (Second Edition)** Volume 1, 2011, Pages 341-365



# Similarities of COVID-19 and aGvHD

- There are many overlapping factors that get produced with both pathogens. These factors are the following: INFs gamma type, IL-1, IL-2, and TNF alpha type.
- Basic Diagram to show the process of which a biological system will fight SARS-CoV-2



# MOA of Remestemcel-L on COVID-19 Pathogenesis

Introduction of SARS-CoV-2



Receptor binding and signaling pathways



Initiation of IFNs



Expression of inflammatory factors



Nucleoprotein will help the virus avoid and prevent the immune response



Series release of cytokinetic factors such as **IL-1, IL-2, IL-6, IL-8, IL-10** and three forms of **IFNs** (alpha, beta, **gamma**)

hMSCs release PGE2 and block TNF-alpha [2]



hMSCs bind IFN gamma and release IDO [2]

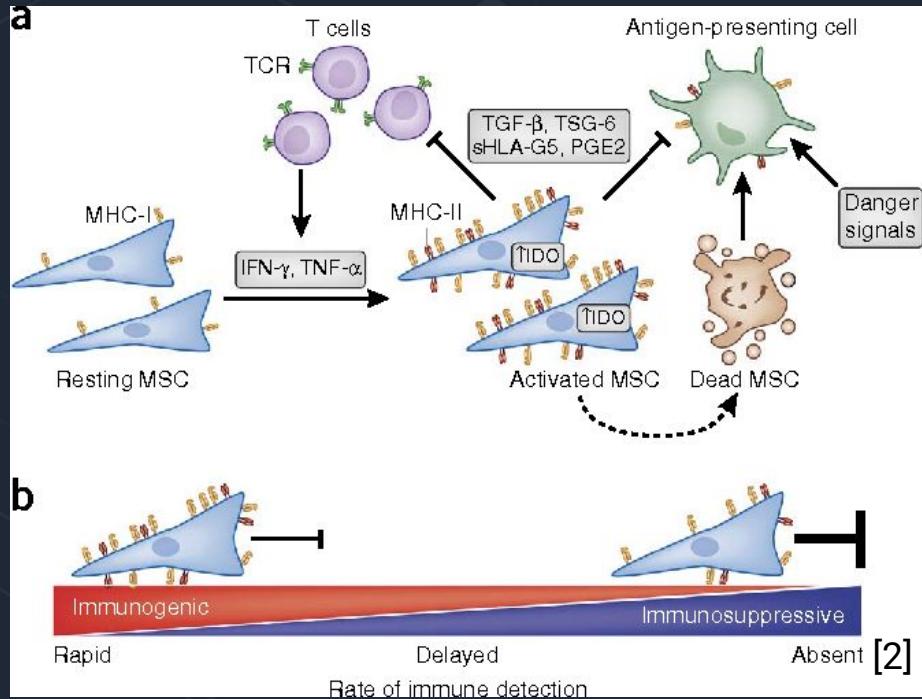
In need of immunosuppression due to the inflammations by the cytokine factors.

Inflammation (immunity factors) are directly proportionate to the severity of the infections.

Increase of anti-inflammatory Cytokines (IL-4, IL-10) [2]

# Prevention of Immune Rejection

- hMSCs extracted from the donors are:
  - Genetically unmodified
  - Undifferentiated
  - Hypo-immunogenic
- Evasion of immune system → none to minimal immune response
  - Supported by toxicology reports from mice and human models [1]
- Antihistamines prior to injection

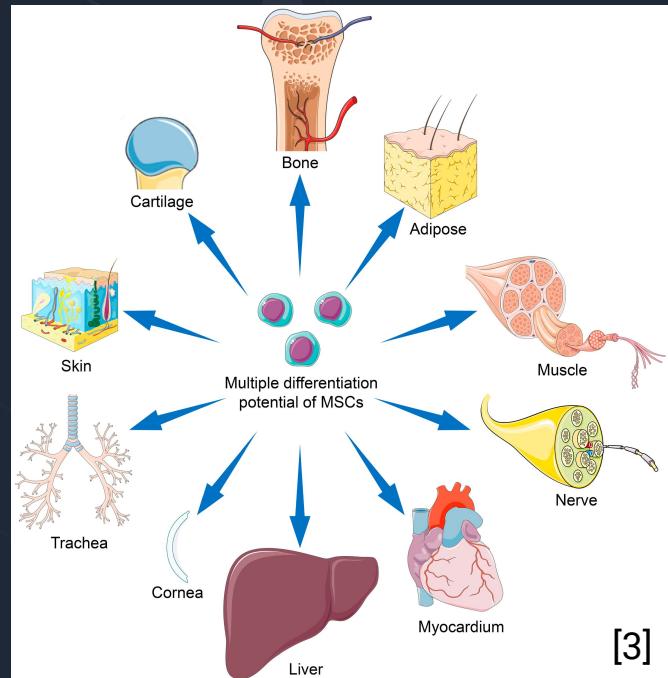


[1]: Health Canada Product Monograph: Remestemcel-L adult mesenchymal stem cells

[2]: Mesenchymal Stem Cells: Immune evasive

# Percentage of Cells to Target (Lungs)

- 4 hours to activate in lungs
- Cells accumulate in lungs first, then distribute to body
- Migration to inflammation sites and tissue damage [2]
  - Chemokine production
  - Activation by toll-like receptors and viral CpG-DNA
  - TGF-B1 factor
- Percentage of cells to lungs is variable on time [1]



[3]

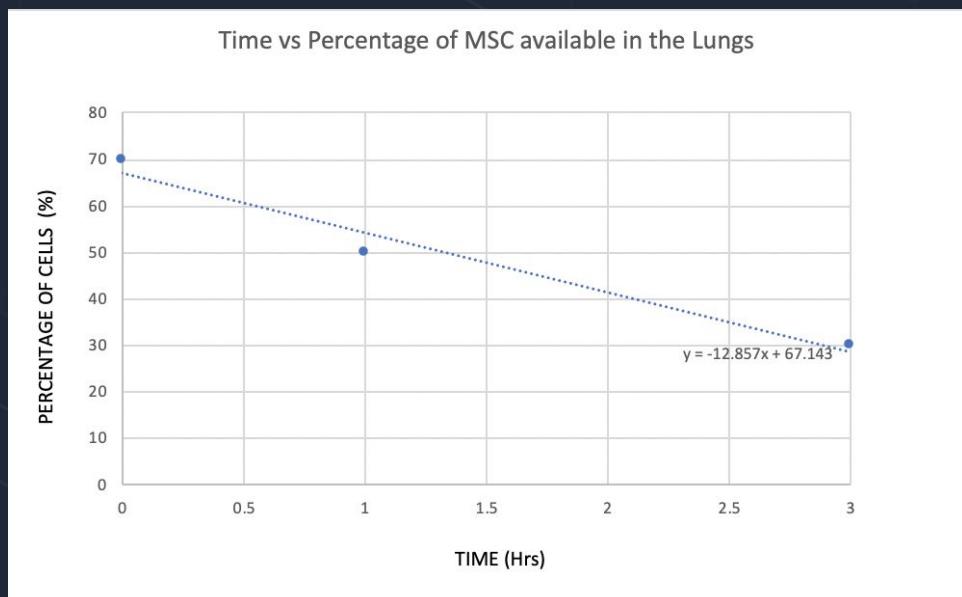
[1]: Mesenchymal Stem Cell-Based Therapy of Inflammatory Lung Diseases: Current Understanding and Future Perspectives

[2]: Health Canada Product Monograph: Remestemcel-L adult mesenchymal stem cells

[3]: What are mesenchymal stem cells (MSCs)

# Cell Availability in Lungs 4 Hours After Injection

Time after injection	Percentage of MSCs in lungs
0 hour [1]	70% [1]
1 hour [1]	50% [1]
3 hours [1]	30% [1]
<b>4 hours</b>	<b>~16%</b>



- Used a linear function method to find out the # of cells available in the lungs

# Drug Product Profile

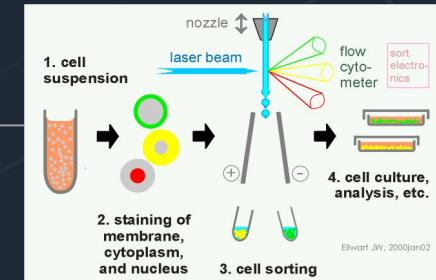
Product Attribute:	Target:
Dosage form	Liquid [1]
Dose	2x10 <sup>6</sup> hMSC/kg of body weight administered twice during the first week of treatment (4 days between doses) [2]
Mode of administration	Intravenous infusion [1]
Container	Cryogenic cell storage bags (15 mL) [1]
Concentration and drug product constituents	1.40x10 <sup>8</sup> MSCs per 15 ml frozen bag; cell density: ~ 9.33x10 <sup>6</sup> MSC/mL - in Plasma-Lyte A medium, 5% Human Serum Albumin (HSA) and 10% DMSO
Shelf-life	2 months at ≤ -135°C in the vapor phase of a liquid nitrogen freezer [1]
Biocompatibility	No acute infusional toxicities [1]
Macroscopic appearance	Opaque; no cell aggregation or particulate matter [1]

[1]: Health Canada Product Monograph: Remestemcel-L adult mesenchymal stem cells

[2]: Mesenchymal Stem Cells for the Treatment of Moderate to Severe COVID-19 Acute Respiratory Distress Syndrome (NCT04371393)

# Critical Quality Attributes

Identity	Phenotype characterization based on three cluster differentiation (CD) markers: CD45-, CD105+ and CD166+ (cell surface proteins)
Potency	A potency assay is the most accepted and recommended tool to confirm that the product expresses the TNF Receptor and suppression of the IL-2-RA.
Safety	<ul style="list-style-type: none"> <li>- Container safety</li> <li>- Bacterial endotoxin safety</li> <li>- Sterility safety</li> <li>- Pathogen safety</li> </ul> <p>Current manufacturing process raises no concerns with respect to the above parameters</p>
Impurities	Heparin, and trypsin present at levels estimated to be undetectable by the most sensitive enzyme-linked immunoassay



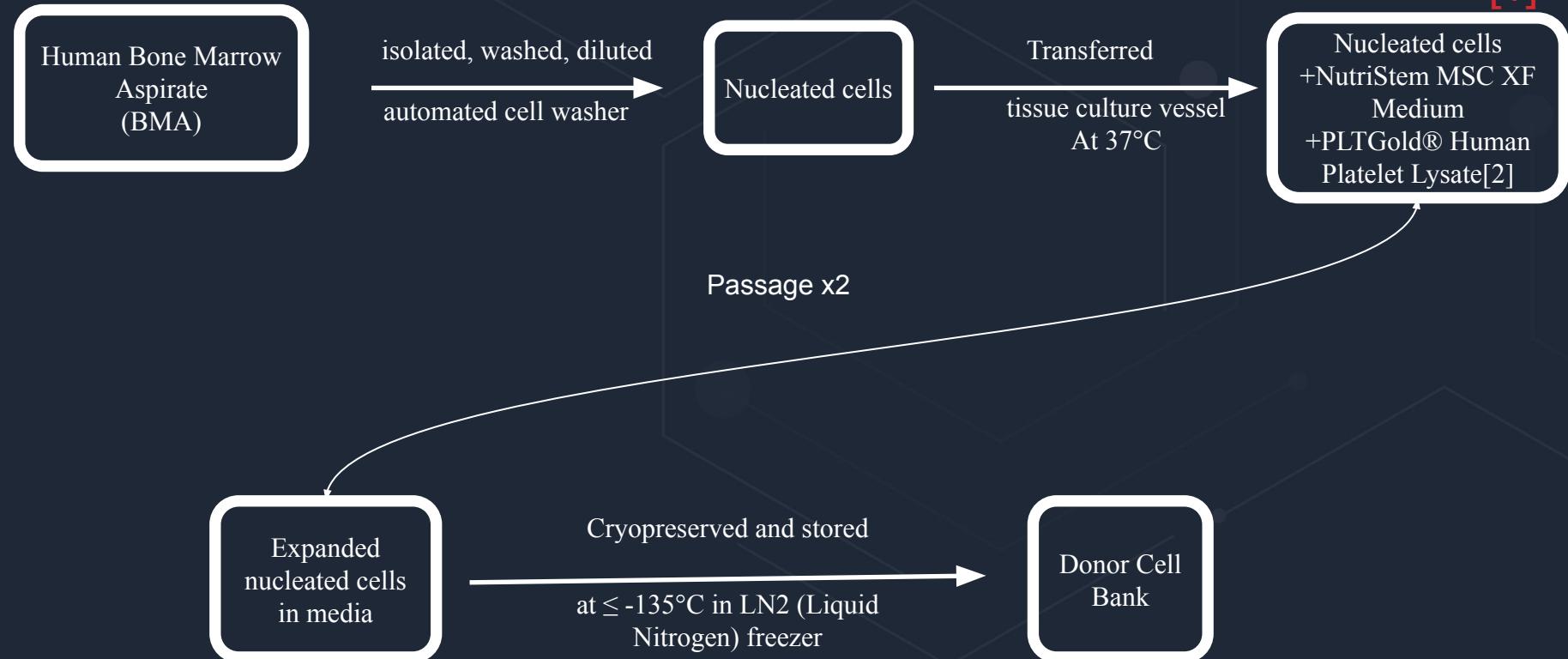
# Product and Raw Material Calculations

Patient Market (US)	~ 56,500
Average Body Weight	70 kg
1 dose (current clinical trial)	2 million MSCs/kg
Total doses per patient	2
Product concentration	140 million MSCs/bag
Total product losses	~40% (thaw cycles, manufacturing losses, etc.)
Total MSCs required (accounting for losses)	$2.21 \times 10^{13}$ MSCs
<b>Total final product goal based on patient market (15 ml)</b>	<b>~ 113,000 final product bags</b>

Average volume of bone marrow aspirate per donor	20 mL
Average number of MSCs per donor	$1.20 \times 10^7$ MSCs
After 5 passages	$2.92 \times 10^9$ MSCs
<b>Required number of donors</b>	<b>12,700 donors</b>

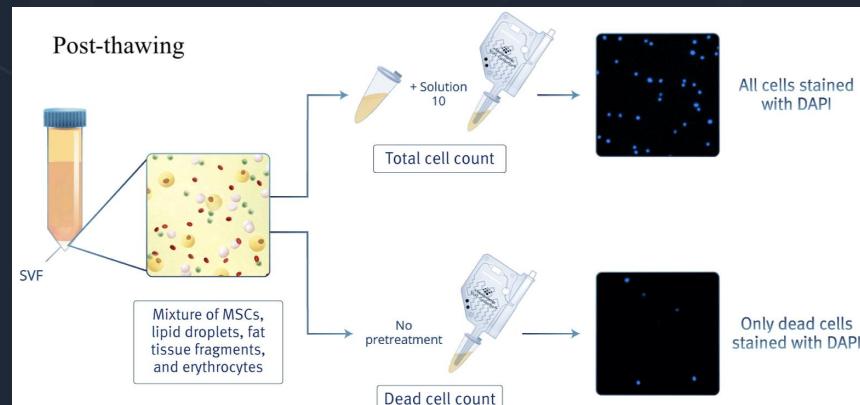
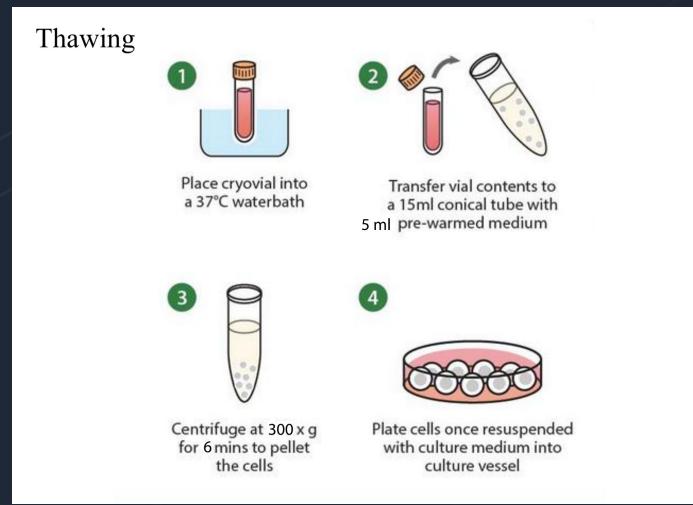
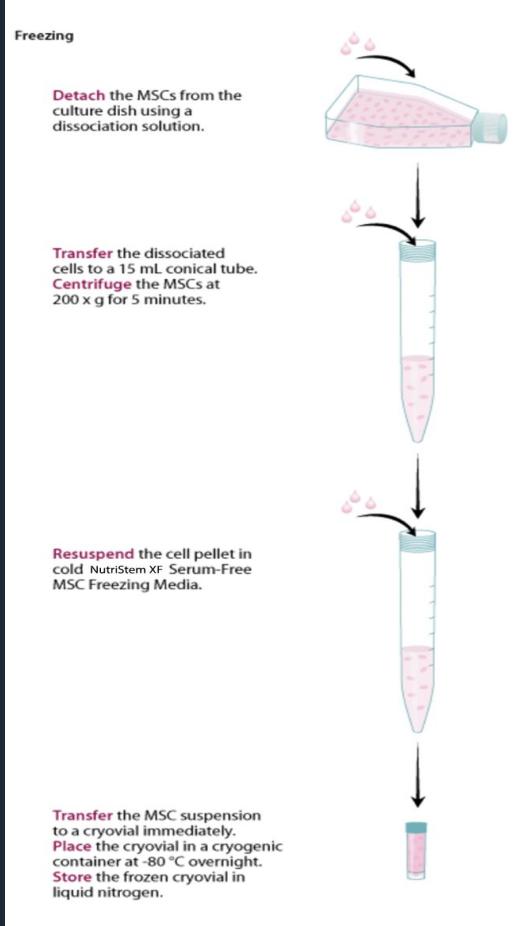
1 Donor ≈ Treatment for 5 Patients

# Upstream Processing: Formation of a Donor Cell Bank<sup>[1]</sup>

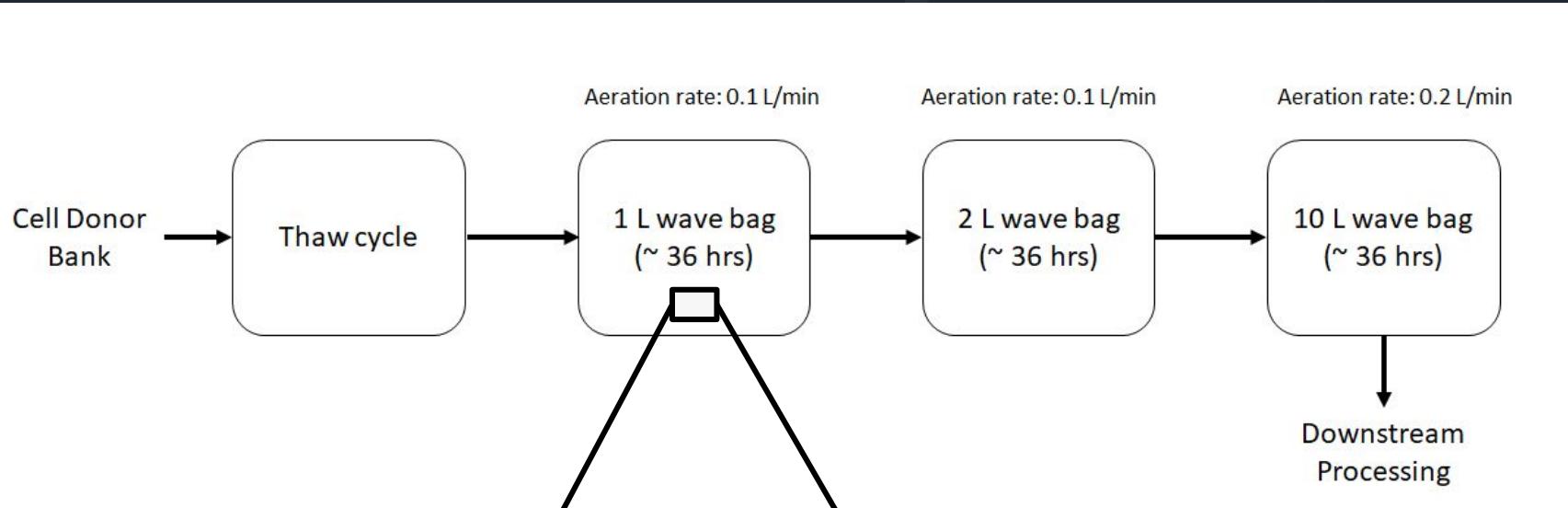


[1]: Australian Public Assessment Report for Remestemcel-L, ex vivo adult human mesenchymal stem cells (Prochymal)

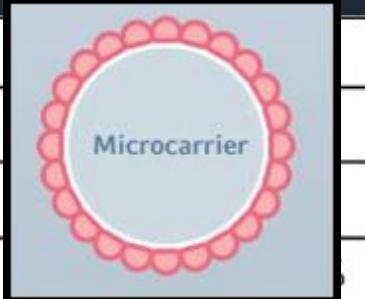
# Preservation and Recovery of hMSCs



# Upstream Processing: Seed Train Design

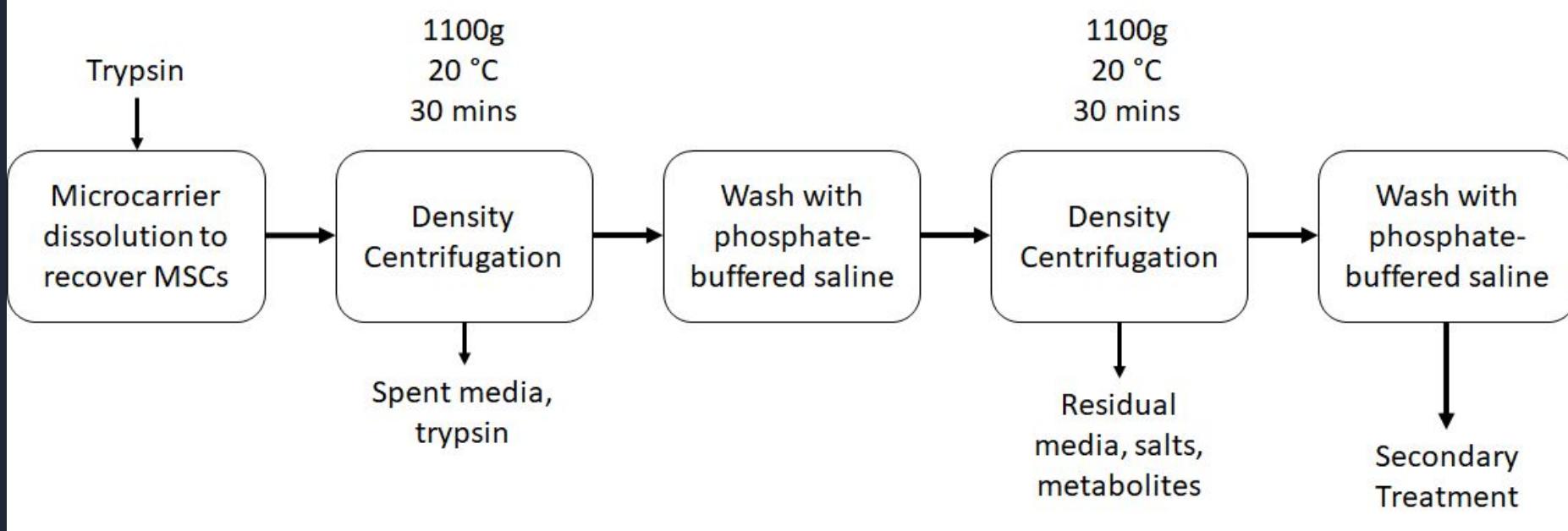


Control Variables	
Temperature	37 °C
pH	6.8-7.8
O <sub>2</sub> level	21 %

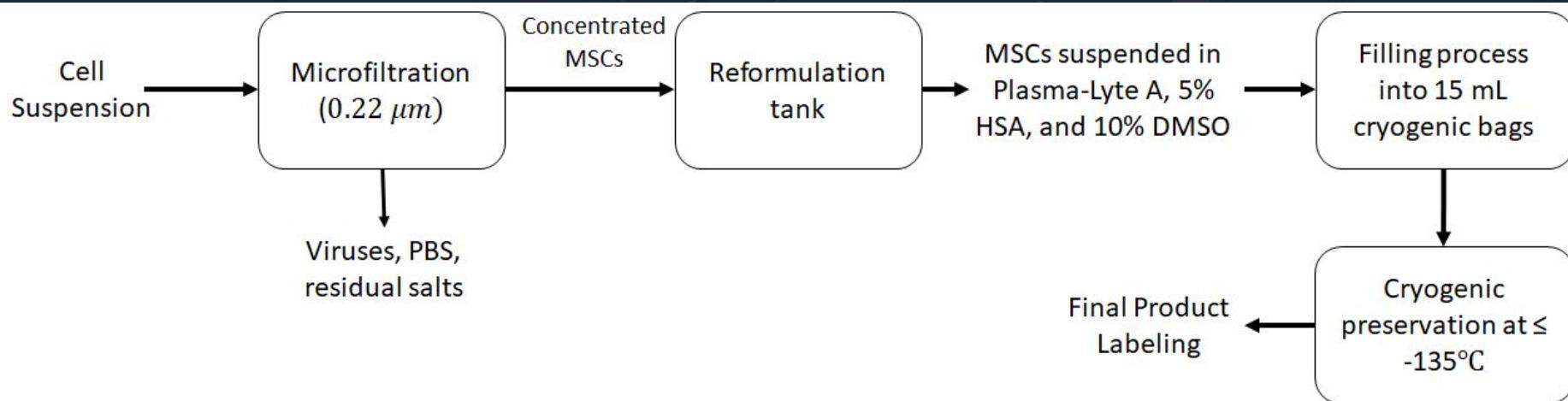


Australian Public Assessment Report for Remestemcel-L, ex vivo adult human mesenchymal stem cells (Prochymal)  
 GE Healthcare. WAVE Bioreactor systems. Cell culture procedures.  
 GE Healthcare. Disposable Cellbag bioreactors for WAVE bioreactor systems.  
<http://publications.lib.chalmers.se/records/fulltext/211540/211540.pdf>

# Downstream Processing: Clarification



# Downstream Processing: Purification



# Production Facility Timescale

Upstream processing (days)	7.5
Downstream processing; fill and finish (days)	0.5
1 process run (days)	8
Total days of plant operation per year	300
Total process runs per year	~ 37
Minimum number of parallel processing rooms to achieve annual production goal*	~610

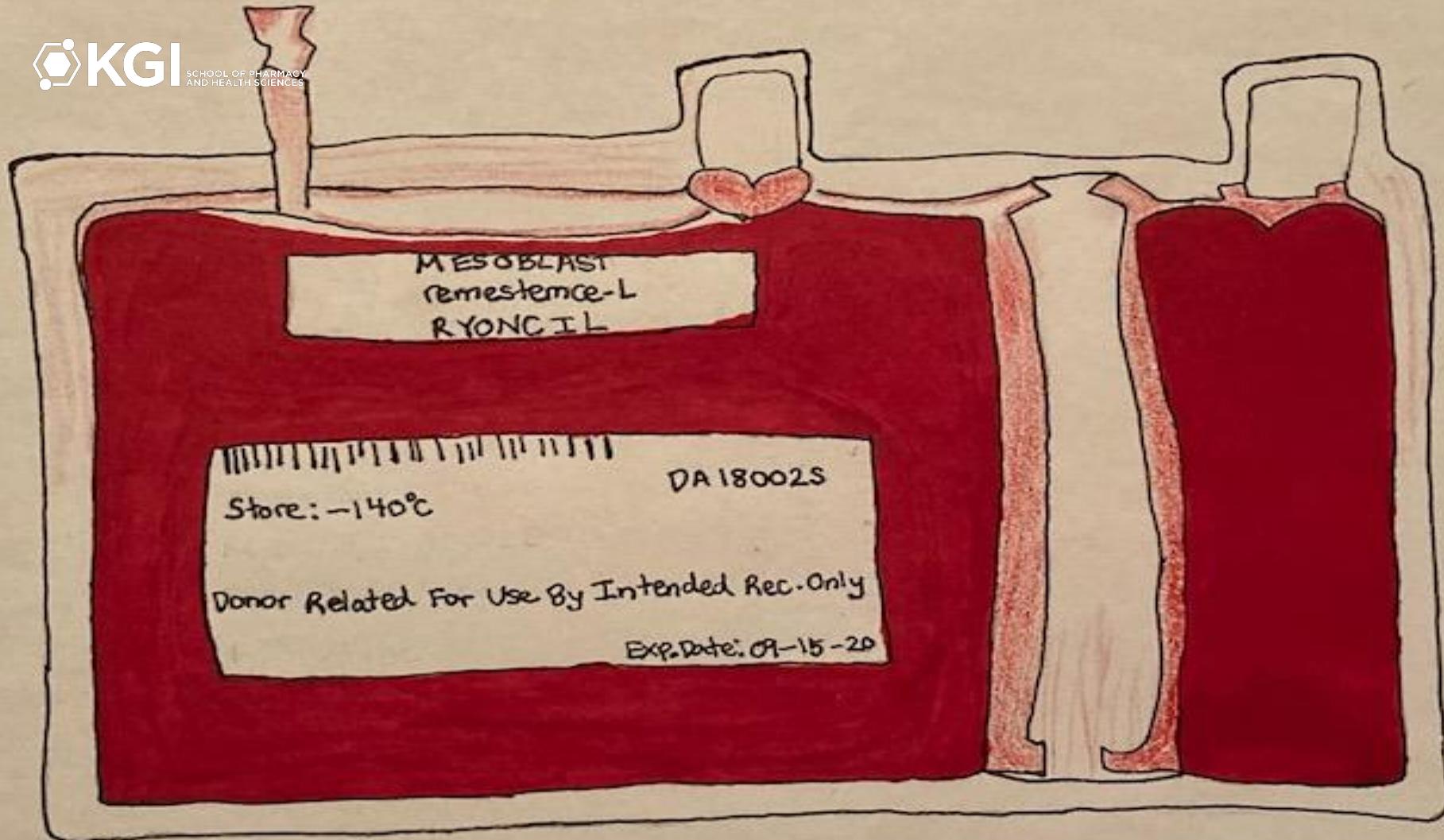
\*Each processing room would operate under cGMP's

# Final Product Manufacturing

Mode of Administration	Intravenous Infusion
Container	Cryogenic bags
Number of batches	1 cell donor bank → 5 final products
Storage Method	Frozen for 2 months at <-135°C
pH	6.8-7.8
Labeling criteria	<p>Date administered: 07-15-20            Exp: 09-15-20            Batch number: DA18002S            Storage: -140°C</p> <p>Adverse effect: Based on mesenchymal cells infusion, no serious adverse effects were shown before or after injection.</p>



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<https://www.sciencedirect.com/science/article/pii/S2352177515000047>



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- <https://ghr.nlm.nih.gov/primer/therapy/genetherapy#~:text=Gene%20therapy%20is%20an%20experimental%20using%20drugs%20or%20surgery>
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- [https://www.google.com/search?q=cryogenic+storage+bags&lz=1C1CHZL\\_enUS710US710&sxsrf=ALEKk03bWbaSN9CGd4NF406zGz--HD3jaQ:1594748300236&source=lnms&tbo=isch&sa=X&ved=2aUKEwi38cOvpM3AhVMpZ4KHdJ8DHUQ\\_AUoAoSACAwQBA&biw=610&bih=610#imgrc=7MfbrVqGiSzNM](https://www.google.com/search?q=cryogenic+storage+bags&lz=1C1CHZL_enUS710US710&sxsrf=ALEKk03bWbaSN9CGd4NF406zGz--HD3jaQ:1594748300236&source=lnms&tbo=isch&sa=X&ved=2aUKEwi38cOvpM3AhVMpZ4KHdJ8DHUQ_AUoAoSACAwQBA&biw=610&bih=610#imgrc=7MfbrVqGiSzNM)
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# Q&A



# Thanks for Listening