Xeno-Free Approach to Manufacturing Mesenchymal Stem Cell Exosomes and Growth Factors, and their Application to Address Removal of Amyloid Plagues from the Brain.

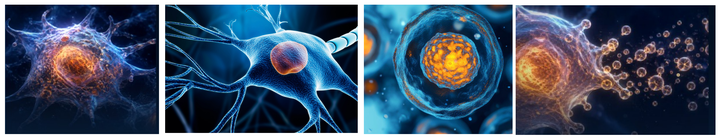
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CYTOREON

CYTOREON XXI is a biotechnology company focused on advancing next-generation stem cell–based technologies for the treatment of inflammatory diseases and associated pathological conditions.

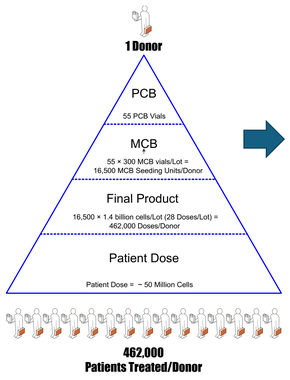
The company develops clinically relevant cellular and cell-derived therapeutic platforms designed to modulate inflammation, support tissue repair, and restore physiological function.

- Bone Marrow Derived Mesenchymal Stem Cells (MSCs)
- Human Neural Stem Cells (NSCs)
- Induce Pluripotent Stem Cells (iPSCs)
- Mesenchymal Stem Cells Exosomes and Growth Factors (Exo/GF)



MSCs NSCs iPSCs Exosomes and GF

Xeno-Free Manufacturing Capabilities and Commercial Scale





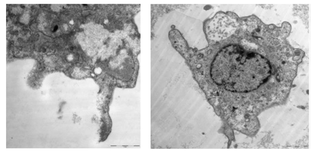
Current State
>5M Patient Treatments/Donor

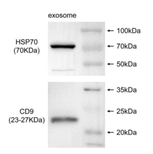
- In-house GMP manufacturing
- Unprecedented scalability with retained potency
- Single donor capable of providing millions of clinical doses
- Technological platform capable of scalability to commercial demand

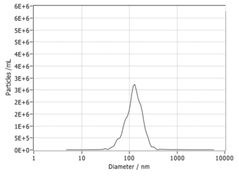
CYTOREON's xeno-free manufacturing process produces MSCs with enhanced cellular bioenergetics, characterized by increased mitochondrial respiratory and glycolytic capacity, reduced senescence, and enhanced secretory activity compared with conventional FBS-expanded MSCs[9].

Characterization of Exosomes Produced by Mesenchymal Stem Cells

- Stem cell–derived exosomes have demonstrated significant anti-inflammatory and immunomodulatory activity in preclinical studies[1,2].
- MSC-derived exosomes can suppress pro-inflammatory signaling, including the NFκB pathway, reduce production of inflammatory cytokines such as TNF-α, IL-1β, and IL-6, and promote polarization of macrophages from the pro-inflammatory M1 phenotype toward the anti-inflammatory M2 phenotype, [3,4].
- Their cargo of regulatory miRNAs, proteins, and other bioactive molecules can also modulate T cells, dendritic cells, and other immune populations, helping restore a balanced inflammatory environment and promote tissue repair.







Compositional and Functional Characterization of Growth Factor Cargo in MSC Secretome

BIOACTIVE STEM CELL FACTORS MIXTURE FACTS

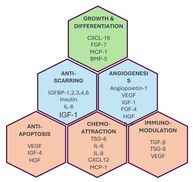
- ✓ Contains over 150 different growth factors, cytokines and proteins
- ✓ Over 40 are present in over 1 ng/ml concentration
- ✓ Minimum effective concentration is in pg/ml

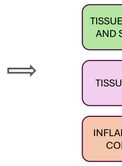
THREE CATEGORIES OF TISSUE REPAIR

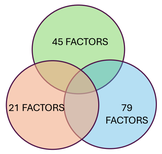
- ✓ Inflammation control and management
- ✓ Tissue repair via collagen production, reduction of fibrosis, and induction of angiogenesis.
- ✓ Tissue support by stimulating cell growth and proliferation

ANALYSIS OF COMPOSITION OF SCFS

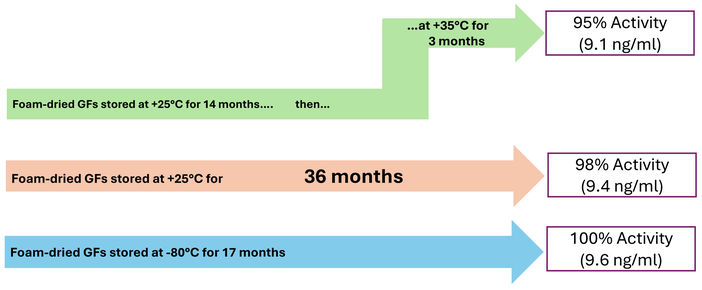
- ✓ Significant amounts of factors in each category
- ✓ Strong synergistic effect and overlapping functions
- ✓ Maximizing rejuvenating response through combinatorial action







Ambient Temperature Stability of the Exosomes and Growth Factors after Preservation by Vaporization



Preservation by Vaporization technology ***eliminates the need for cold storage*** and ***preserves activity*** of key proteins and factors throughout product shelf life.

Stabilized Exosomes and Growth Factors Enable Simple Intranasal Delivery to the Brain

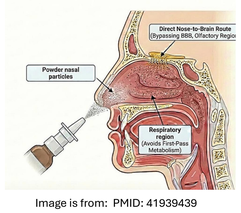


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- For intranasal delivery, stem cell–derived exosomes offer several potential advantages over conventional systemic administration.
- Their nanoscale size and natural biological membrane facilitate interaction with nasal epithelial cells and may support transport across the nasal mucosa and the nose-to-brain pathway, potentially enabling delivery of therapeutic cargo to the central nervous system while reducing dependence on the blood–brain barrier.
- Exosomes can also protect proteins, lipids, and regulatory RNAs from degradation and provide intrinsic cell-targeting and immunomodulatory properties.

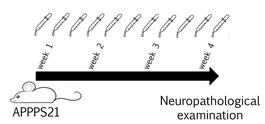
- For neuroinflammatory and neurodegenerative disorders, intranasal exosome delivery is therefore being investigated as a non-invasive, cell-free approach that may achieve localized CNS effects with lower systemic exposure.

Preclinical Studies and Evidence of Anti-inflammatory impact from MSC Secretome

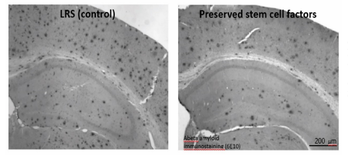
- Preclinical studies demonstrate that intranasally administered MSC-derived exosomes/extracellular vesicles can reach multiple regions of the brain and exert significant anti-inflammatory effects[6].
- In animal models of Alzheimer's disease, traumatic brain injury, epilepsy, and perinatal brain injury, intranasal MSC-EVs reduced microglial activation and pro-inflammatory mediators including TNF-α, IL-1β, IL-6 and NLRP3 inflammasome signaling, while increasing anti-inflammatory responses such as IL-10.
- These findings support the potential of intranasal MSC-derived exosomes as a non-invasive approach for delivering immunomodulatory activity directly to the CNS[7,8] .

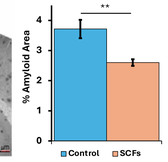
CYTOREON has conducted preclinical study (presented below) to test impact of Preserved Stem Cell Factors (SCFs), containing exosomes and growth factors produced by MSCs on amyloid-beta protein accumulation in the brain.

- Accumulation of amyloid-beta (Aβ) is a key pathological hallmark of Alzheimer's disease.
- Reduced Aβ clearance and chronic neuroinflammation contribute to disease progression.



Treatment	Mouse	Duration & frequency	Dosing	Analysis
Preserved Stem Cell Factors (SCFs)	APPPS21 transgenic (n = 137)	1 month 5 times / week	10 ul / nostril (30 ng VEGF/ml)	2 days after last application
LRS control (Lactated Ringer's Solution)	APPPS21 transgenic (n = 44)	1 month 5 times / week	10 ul / nostril	2 days after last application





30% reduction in plaque formation

N(LRS) = 44
N(SCFs) = 137
p-value: 3E-5

- Application of secretome slows Abeta amyloidosis
- Reduction in amyloid burden and neuroinflammation
- Preserved Stem Cell Factors can be used as a ***maintenance and prevention*** of neurological conditions
- Available in formulation specific for nasal applications (drops)

Human Clinical Study Design and End Points

Design: Phase 1/2a, multicenter, randomized, double-blind, placebo-controlled study evaluating repeated **intranasal administration of Exosomes and Factors** in patients with mild symptomatic Alzheimer's disease.

Part A — Dose Escalation: Provisional doses of **10 → 30 → 100 mg SMDP**, using a 3+3 design with sentinel dosing. The primary objectives are to assess safety, identify DLTs, and determine the **RP2D/RP2R**.
Part B — Expansion: Approximately **30–40 patients**, randomized 1:1 to the selected Exosomes and Factors dose/regimen or placebo.

Primary Endpoints: Safety and tolerability, including TEAEs, SAEs, AESIs, DLTs, laboratory parameters, neurological and nasal safety assessments, and MRI findings.

Exploratory Efficacy Endpoints: CDR-SB as the principal clinical efficacy measure, supported by **ADAS-Cog13, ADCS-ADL/iADL, and MMSE/MoCA**.

Biomarkers: p-tau217, Aβ42/40, GFAP, and NfL, with VEGF and other mechanistic biomarkers evaluated exploratorily.

Imaging: Brain MRI and **amyloid PET** in a subset of subjects.

Follow-up: Up to **52 weeks**.

The principal goals of this Phase 1/2a study are to **establish safety and tolerability, determine an appropriate dose/regimen for further development, and identify preliminary clinical and biomarker signals of activity**.

Citations:

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   — General background on MSC-EV/exosome-mediated immunomodulation and regenerative effects.
2. **Mokarizadeh A, Delirezh N, Morshedi A, et al.** Microvesicles derived from mesenchymal stem cells: potent organelles for immunomodulation. *Biochemical and Biophysical Research Communications.* 2012;425(4):728–733.
   — Evidence for immunomodulatory effects of MSC-derived vesicles.
3. **Phinney DG, Pittenger MF.** Concise review: MSC-derived exosomes for cell-free therapy. *Stem Cells.* 2017;35(4):851–858.
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4. **Monguió-Tortajada M, Gálvez-Montón C, Bayes-Genis A, et al.** Extracellular vesicles from cell-to-cell communication to immunotherapy. *Frontiers in Immunology.* 2019;10:1002.
   — Mechanisms of EV-mediated immune modulation.
5. **Mokarizadeh A, et al.** Mesenchymal Stem Cell-Derived Exosomes and Other Extracellular Vesicles as New Remedies in the Therapy of Inflammatory Diseases. *International Journal of Molecular Sciences.* 2020;21(1):E?
   — Reviews evidence that MSC-EVs deliver regulatory miRNAs and proteins that modulate inflammatory immune cells, including conversion of pro-inflammatory M1 macrophages toward an anti-inflammatory M2 phenotype.
6. **Losurdo M, Pedrazzoli M, D'Errico V, et al.** Intranasal delivery of mesenchymal stem cell-derived extracellular vesicles exerts immunomodulatory and neuroprotective effects in a 3xTg model of Alzheimer's disease. *Journal of Neuroinflammation.* 2020;17:181.
   — Particularly important for your application: intranasal MSC-EVs reached the brain, reduced microglial activation, and produced anti-inflammatory/neuroprotective effects in an Alzheimer's disease mouse model.
7. **Kodali M, Madhu LN, Reger RL, et al.** Intranasally Administered Human MSC-derived Extracellular Vesicles Inhibit NLRP3-p38/MAPK Signaling after TBI and Prevent Chronic Brain Dysfunction. *Cell Transplantation.* 2023;32.
   — Demonstrated that intranasal human MSC-EVs incorporated into neurons and microglia and reduced **NLRP3 inflammasome activation, IL-1β, IL-18 and pro-inflammatory signaling**, with associated improvements in behavioral outcomes in a mouse TBI model.
8. **Shen W, You T, Xu W, et al.** Rapid and Widespread Distribution of Intranasal Small Extracellular Vesicles Derived from Mesenchymal Stem Cells throughout the Brain Potentially via the Perivascular Pathway. *Pharmaceutics.* 2023;15(11):2578. DOI: 10.3390/pharmaceutics15112578.
   — Provides direct biodistribution evidence that intranasally administered MSC-sEVs rapidly reach multiple brain regions and may utilize perivascular pathways for CNS delivery.
9. **Oikonomopoulos, A., van Deen, W., Manansala, AR. *et al.*** Optimization of human mesenchymal stem cell manufacturing: the effects of animal/xeno-free media. *Sci Rep* **5**, 16570 (2015). https://doi.org/10.1038/srep16570