Regenerative Therapies in Neurodegenerative Disorders

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Disclosures

- TheBioBox, LLC: Senior Vice President of R&D
- RESTEM, LLC: Senior Vice President
- Gentera Med: Scientific Advisor

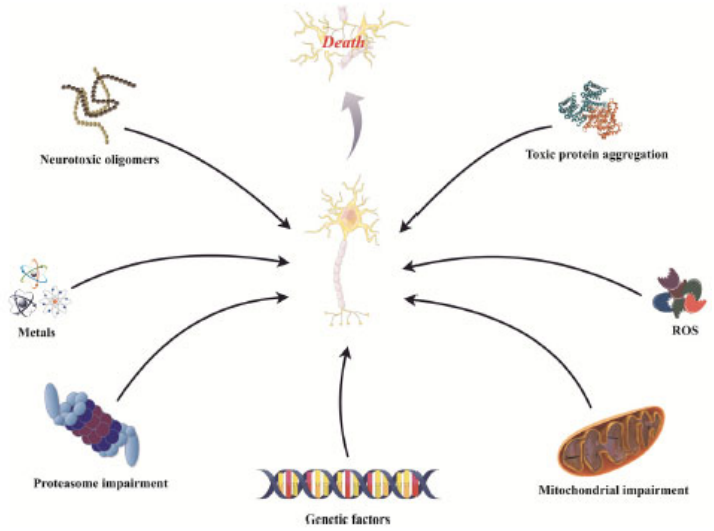
Neurodegenerative Disease

- According to WHO global study, 8 out of 10 disorders in the 3 highest disability classes are neurologic problems
- 2016: 9 million deaths
- Worldwide causes over 276 million people are disabled
- As we live longer, neurodegenerative diseases of aging will increase

www.worldhealthorganization.org

Pathological Hallmarks of Neurodegenerative Disease

- Reactive Oxygen Species (ROS) generation-causing chronic inflammation
- Proteasome complex impairment
- Mitochondrial function impairment
- Toxic protein aggregation
- Neurotoxic oligomers
- Genetic factors



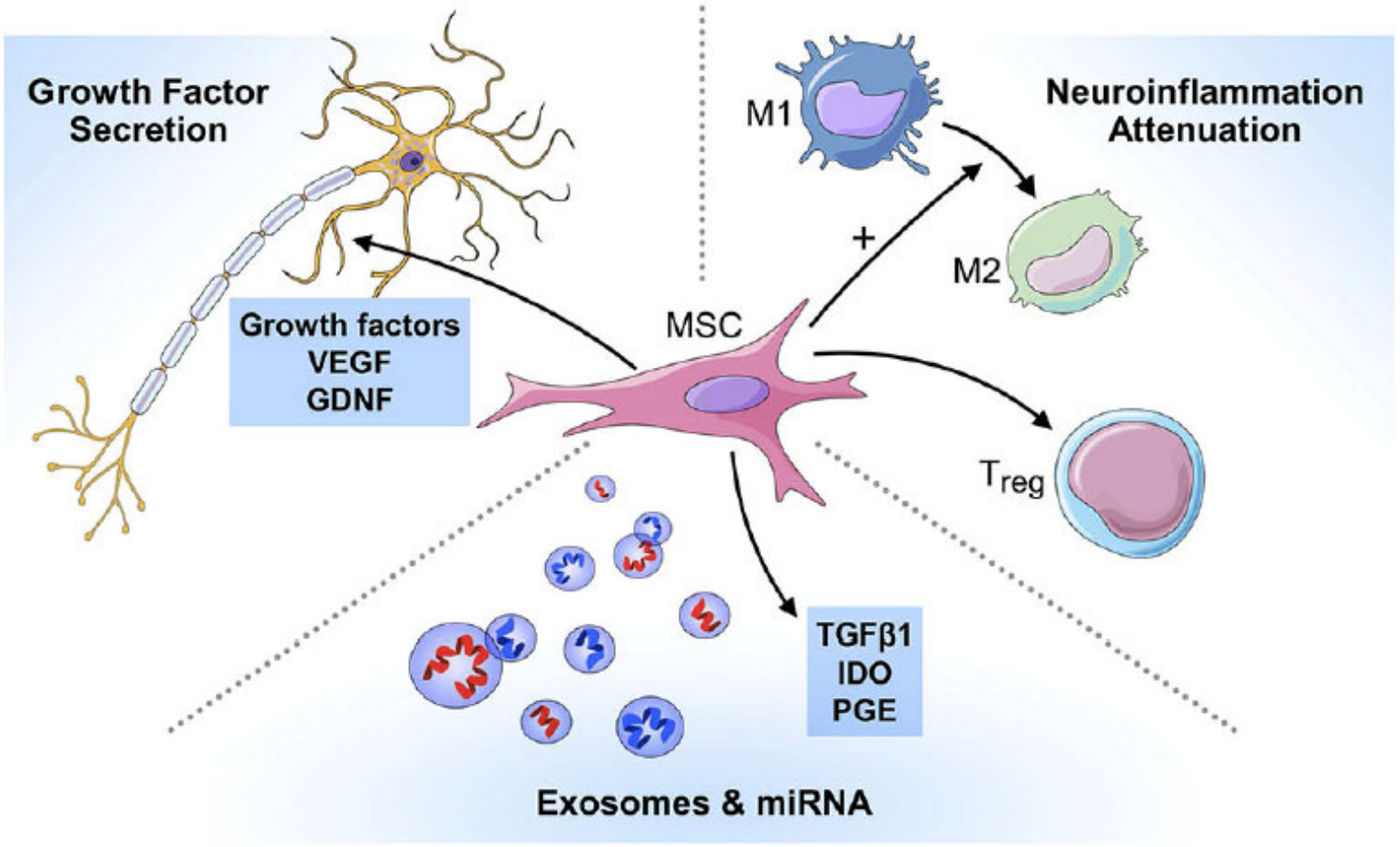
Shariati A, et al. Mesenchymal stromal cells (MSCs) for neurodegenerative disease: a promising frontier. Eur J Cell Biol 2020. doi.org/10.1016/j.ejcb.2020.151097

Mesenchymal Stem Cells

MSCs in Neurodegenerative Disease: Mechanism of Action

- Precise mechanisms still unknown
- Growth factor secretion
  - Glial cell-derived neurotrophic factor (GDNF)
  - Vascular endothelial growth factor (VEGF)
  - Brain-derived neurotrophic factor (BDNF)
- Neuroinflammation attenuation
- Exosome and miRNA secretion
- Mitochondria donation

Staff NP, et al. Mesenchymal stromal cell therapies for neurodegenerative diseases. Mayo Clin Proc 2019; 94(5): 892-905.



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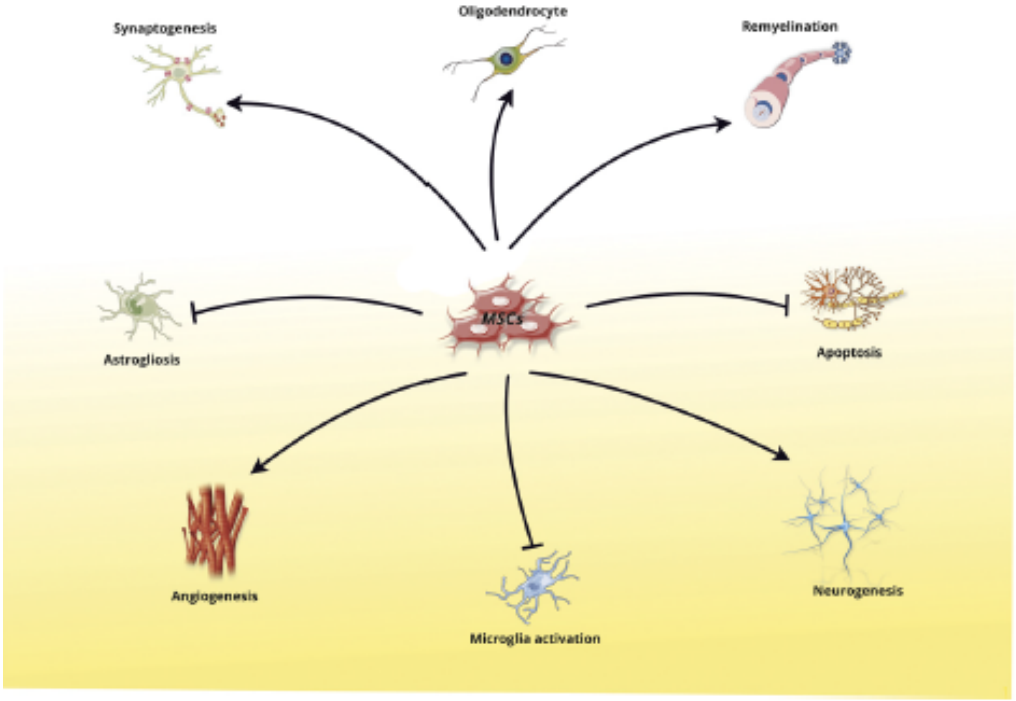
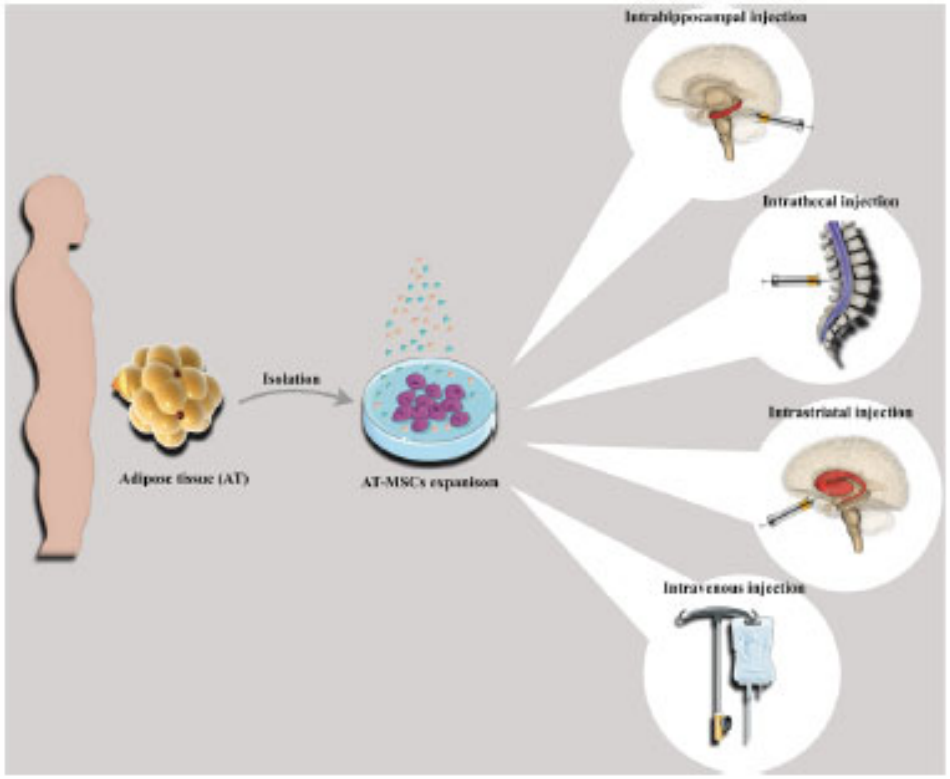


Fig. 5. Mesenchymal stromal cells (MSCs) potential to treat neurodegenerative diseases. MSCs can exert beneficial effects for neurodegenerative disorders treatment through inhibition of microglial activation, astrogliosis and neural cell apoptosis, and promotion of neurogenesis, angiogenesis, and synaptogenesis along with induction of oligodendrocytes generation and remyelination.

Shariati A, et al. Mesenchymal stromal cells (MSCs) for neurodegenerative disease: a promising frontier. Eur J Cell Biol 2020. doi.org/10.1016/j.ejcb.2020.151097

Common MSC Administration Routes in Neurodegenerative Disease

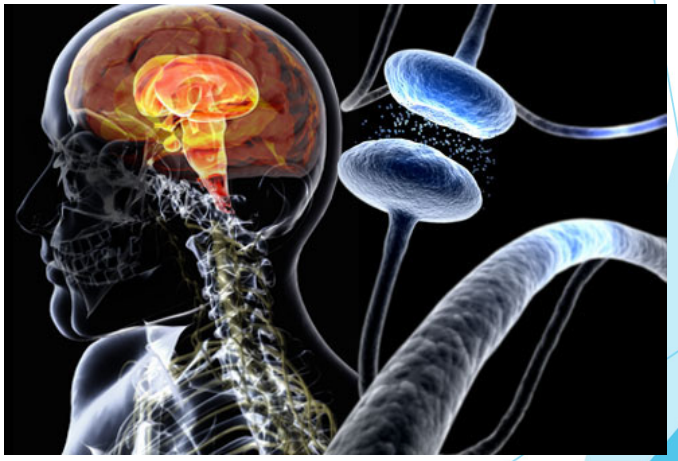


Shariati A, et al. Mesenchymal stromal cells (MSCs) for neurodegenerative disease: a promising frontier. Eur J Cell Biol 2020. doi.org/10.1016/j.ejcb.2020.151097

Parkinson’s Disease

Parkinson's Disease

- Nearly one million people live with Parkinson's Disease in US
- Approximately 60,000 Americans are diagnosed with PD each year
- More than 10 million people worldwide live with PD
- Incidence of PD increases with age; an estimated 4% of those with PD are diagnosed before age 50
- Men are 1.5 times more likely to have Parkinson's disease than women



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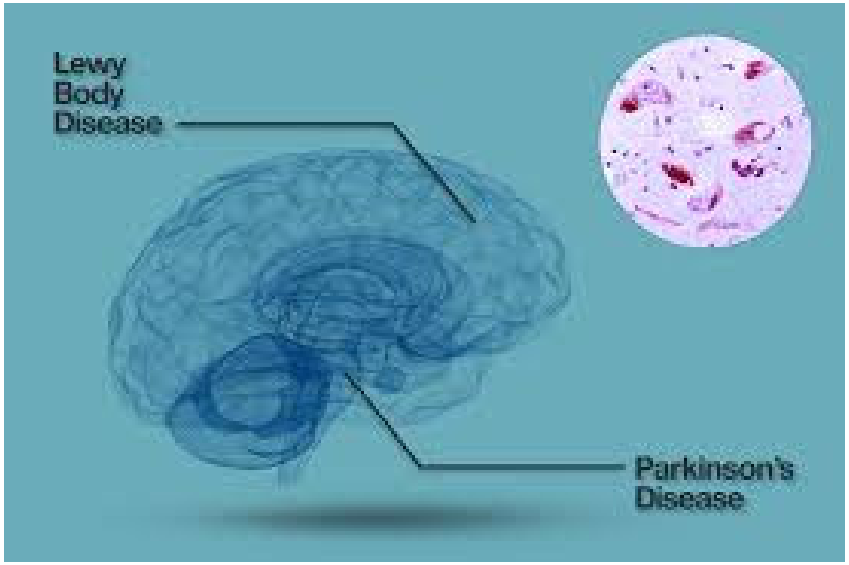
Parkinson’s Disease

- Clinically characterized by combination of tremor, bradykinesia, rigidity
- Non-motor symptoms include cognitive dysfunction, mood disorders, sleep disturbances, autonomic dysfunction

Pathophysiology of Parkinson's Disease



- Loss of dopaminergic neurons in selected areas within the brain
  - Substantia nigra
  - Locus ceruleus
  - Dorsal vagal nucleus
  - Cerebral cortex
- Lewy bodies
  - Intracytoplasmic neuronal alpha-synuclein inclusions

Factors Contributing to Neuronal Loss in Parkinson's Disease

- Genetics
- Oxidative stress
- Glial dysfunction
- Excitotoxicity
- Inflammation
- Mitochondrial dysfunction

Treatment of Parkinson's Disease

- Pharmaceuticals
  - Levodopa
  - DAergic receptor agonists
  - Monoamine oxidase B (MAO-B) inhibitors\
  - Cathecol-O-methyltransferase (COMT)
- Surgical Intervention
  - Pallidotomy surgery
  - Deep brain stimulation (DPS)
- Gene therapy

Treatment only improves symptoms and cannot control disease progression

Risk of adverse events

Mechanism of Action of MSCs in PD

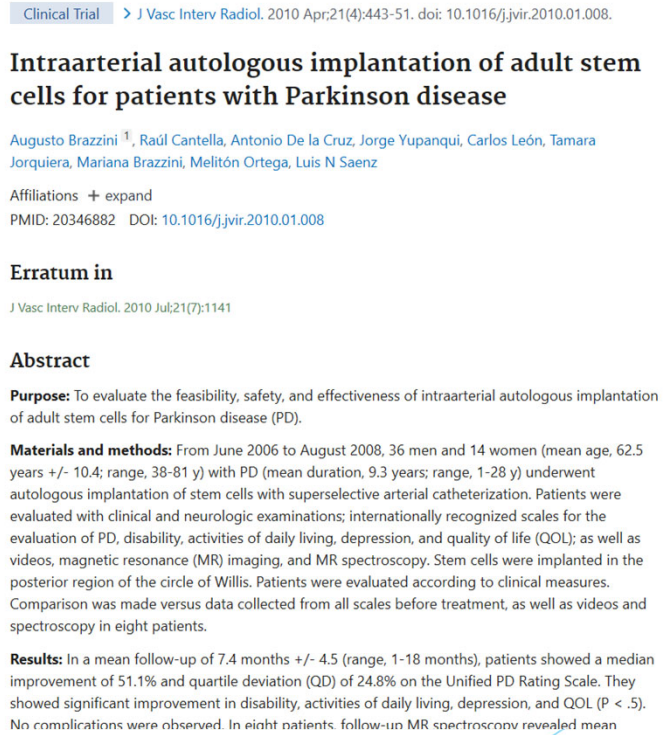
- Secretion of neurotrophic growth factors
  - VEGF, BDNF, GDNF, Interleukin-6, Glial derived proteins, Galectin-1, Pigment epitheilium-derived factor
- Modulate immune system via direct cell to cell interactions and aid wound healing
- Improve neuronal health by mitochondrial donation
- Affect DAergic neurons via prostaglandin E2 and it's signaling pathway
- Release dopamine by depolarizing potassium channels

Chen Y, et al. Clinical potential and current prognosis of MSCs for Parkinson's disease: a systematic review. Neurol Sci. 2020; 41: 1051-61.

MSC Clinical Studies for Parkinson's Disease

Parkinson's and MSCs

- 50 subjects underwent autologous stem cell implantation with super-selective arterial catheterization in posterior region of circle of Willis
- Evaluation: recognized scales of PD, disability, activities of daily living, depression, QOL, videos, MRI, MR spectroscopy
- Results after approximately 7 months:
  - Median improvement of 51.1% on Unified PD rating scale
  - Significant improvement in disability activities of daily living, depression, QOL
  - No complications
  - MR spectroscopy revealed mean improvements in n-acetylaspartate/creatine ratio in right and left basal ganglia compared to pretreatment



Clinical Trial > J Vasc Interv Radiol. 2010 Apr;21(4):443-51. doi: 10.1016/j.jvir.2010.01.008.

Intraarterial autologous implantation of adult stem cells for patients with Parkinson disease

Augusto Brazzini [1], Raúl Cantella, Antonio De la Cruz, Jorge Yupanqui, Carlos León, Tamara Jorquiera, Mariana Brazzini, Melitón Ortega, Luis N Saenz

Affiliations + expand

PMID: 20346882 DOI: 10.1016/j.jvir.2010.01.008

Erratum in

J Vasc Interv Radiol. 2010 Jul;21(7):1141

Abstract

Purpose: To evaluate the feasibility, safety, and effectiveness of intraarterial autologous implantation of adult stem cells for Parkinson disease (PD).

Materials and methods: From June 2006 to August 2008, 36 men and 14 women (mean age, 62.5 years +/- 10.4; range, 38-81 y) with PD (mean duration, 9.3 years; range, 1-28 y) underwent autologous implantation of stem cells with superselective arterial catheterization. Patients were evaluated with clinical and neurologic examinations; internationally recognized scales for the evaluation of PD, disability, activities of daily living, depression, and quality of life (QOL); as well as videos, magnetic resonance (MR) imaging, and MR spectroscopy. Stem cells were implanted in the posterior region of the circle of Willis. Patients were evaluated according to clinical measures. Comparison was made versus data collected from all scales before treatment, as well as videos and spectroscopy in eight patients.

Results: In a mean follow-up of 7.4 months +/- 4.5 (range, 1-18 months), patients showed a median improvement of 51.1% and quartile deviation (QD) of 24.8% on the Unified PD Rating Scale. They showed significant improvement in disability, activities of daily living, depression, and QOL (P < .5). No complications were observed. In eight patients, follow-up MR spectroscopy revealed mean

Parkinson's and MSCs

- Results of clinical trials conducted by Venkatarama and colleagues verified feasibility and safety of unilateral autologous BM-MSCs in 7 patients with PD after 36 months
- 3 of 7 subjects displayed stable progress in their "off/on" Unified PD Rating Scale (UPDRS)
- Second trial by this team showed safety and efficacy of adult allogeneic BM-MSC implantation into the subventricular zone (SVZ) of the brain in 12 early stage PD subjects

Venkataramana NK, et al. Bilateral transplantation of allogeneic adult human bone marrow derived mesenchymal stem cells into the subventricular zone of Parkinson's disease: a pilot clinical study. Stem Cells Int. 2012: 931902.

Venkataramana NK, et al. Open-labeled study of unilateral autologous bone marrow-derived mesenchymal stem cells transplantation in Parkinson's disease. Transl Res. 2010; 155: 62-70.

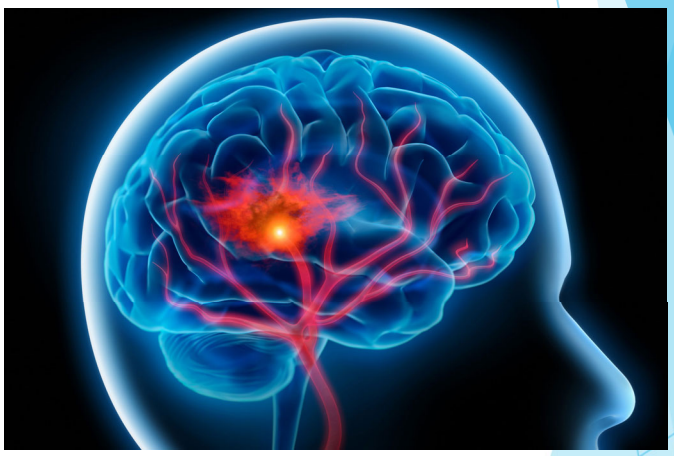
MSC Trials for PD: clinicaltrials.gov

	Title	Status	Study Results	Conditions	Interventions	Locations
1	Allogeneic Bone Marrow-Derived Mesenchymal Stem Cell Therapy for Idiopathic Parkinson's Disease	Completed	No Results Available	•Parkinson's Disease	•Biological: Allogeneic bone marrow-derived MSCs (1 x 10 6 MSC/kg) •Biological: Allogeneic bone marrow-derived MSCs (3 x 10 6 MSC/kg) •Biological: Allogeneic bone marrow-derived MSCs (6 x 10 6 MSC/kg) •Biological: Allogeneic bone marrow-derived MSCs (10 x 10 6 MSC/kg)	•The University of Texas Health Science Center at Houston, Houston, Texas, United States
2	Use of Mesenchymal Stem Cells (MSCs) Differentiated Into Neural Stem Cells (NSCs) in People With Parkinson's (PD).	Recruiting	No Results Available	•Parkinson Disease	•Biological: Injection of Umbilical cord derived MSCs	•Cell Therapy Center, University of Jordan, Amman, Jordan
3	Autologous Mesenchymal Stem Cell Transplant for Parkinson's Disease	Terminated	No Results Available	•Parkinson's Disease	•Procedure: Autologous Bone marrow derived stem cells transplant	•Jaslok Hospital And Research Centre, Mumbai, Maharashtra, India
4	Phase IIa Randomized Placebo Controlled Trial: Mesenchymal Stem Cells as a Disease-modifying Therapy for IPD	Not yet recruiting	No Results Available	•Parkinson's Disease	•Drug: MSC+placebo •Drug: MSC •Drug: Placebo	•The University of Texas Health Science Center at Houston, Houston, Texas, United States
5	Mesenchymal Stem Cells Transplantation to Patients With Parkinson's Disease	Unknown status	No Results Available	•Parkinson's Disease	•Biological: bone marrow derived mesenchymal stem cells	•Guangzhou General Hospital of Guangzhou Military Command, Guangzhou, Guangdong, China
6	Individual Patient Expanded Access IND of Hope Biosciences Autologous Adipose-derived Mesenchymal Stem Cells for Parkinson's Disease	No longer available	No Results Available	•Parkinson Disease	•Drug: HB-adMSCs	•Clinical Trial Network, Houston, Texas, United States
7	Umbilical Cord Derived Mesenchymal Stem Cells Therapy in Parkinson's Disease	Enrolling by invitation	No Results Available	•Parkinson's Disease	•Biological: mesenchymal stem cells	•Hebei Newtherapy Bio-Pharma Technology Co., Ltd, Shijiazhuang, Hebei, China
8	Parkinson's Disease Therapy Using Cell Technology	Active, not recruiting	No Results Available	•Transplantation:Mesenchymal Stem Cell Transplantation	•Biological: Autologous mesenchymal stem cells	•the Belarusian Medical Academy of Postgraduate Education, Minsk, Belarus

Stroke

Stroke

- In 2018, 1 in every 6 deaths from cardiovascular disease was due to stroke
- Someone in the US has a stroke every 40 seconds. Someone dies of a stroke every 4 minutes.
- About 87% of all strokes are ischemic strokes
- Nearly 1 in 4 strokes are patients who have had a previous stroke
- Leading cause of serious long-term disability
- Reduces mobility in more than half of stroke survivors age 65 and over



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Stroke Pathogenesis

- A stroke, also called a cerebrovascular accident (CVA) occurs when part of the brain loses its blood supply and stops working. This causes the part of the body that it controls to stop working as well.
  - The nerve cells lacking oxygen die, disrupting the neuronal circuits
  - Ischemic strokes occur when a blood vessels becomes occluded
    - due to a gradual narrowing
    - or sudden embolization
  - A hemorrhagic stroke describes brain tissue that is damaged because of bleeding, most often because of uncontrolled high blood pressure.
- A stroke is a medical emergency.
  - From onset of symptoms, there is only a 3 to 4 1/2 hour window for thrombolytics
  - A transient ischemic attack or a TIA, resolves usually within minutes but symptoms may persist up to 24h

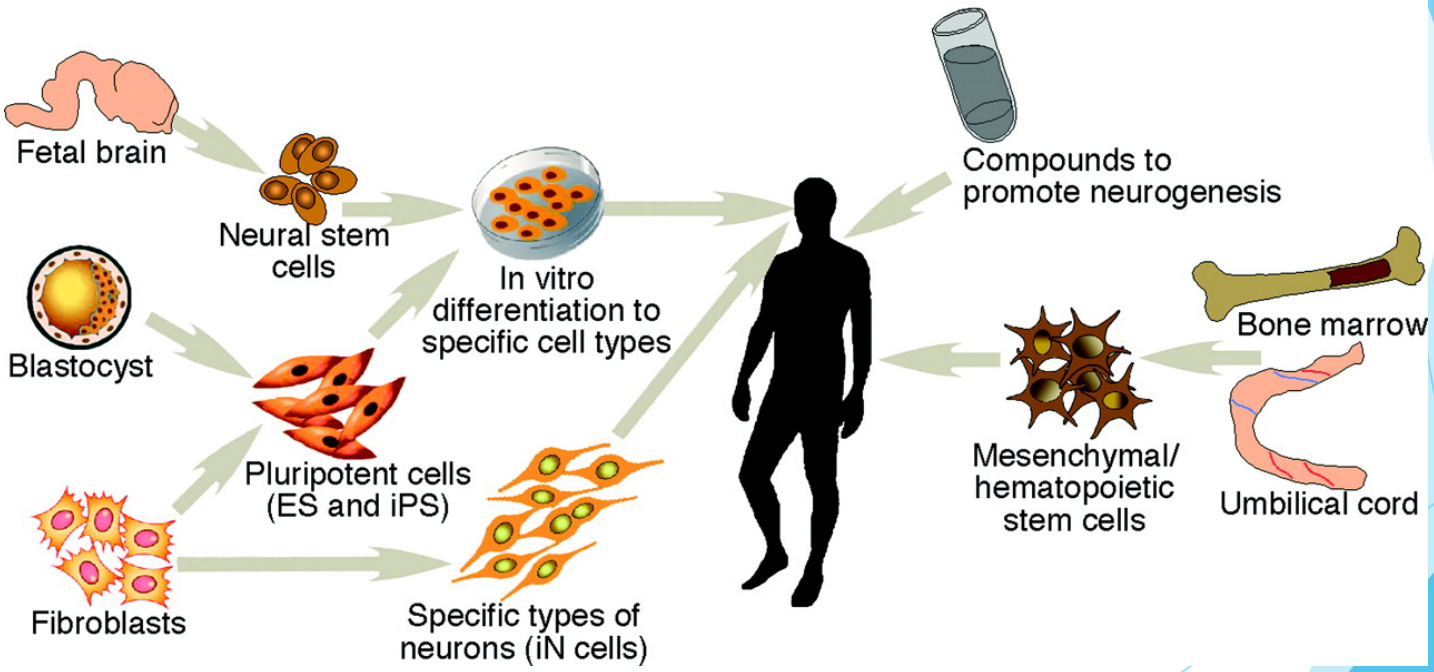
Treatment of Stroke

- A stroke is a medical emergency imposing prompt vital support: ABC, IV lines, oxygen.
- Prompt diagnostic: blood test, CT scans to distinguish between a hemorrhagic stroke and an ischemic stroke before treatment begins.
- Decision to initiate thrombolytic therapy (tPA, tissue plasminogen activator):
  - If the diagnosis of ischemic stroke has been made
  - Within the therapeutic window: 3 to 4.5 hours
  - And discussed with the patient and family as there is a 6% risk that an ischemic stroke can turn into a hemorrhagic stroke
  - In strokes involving the vertebrobasilar system and posterior circulation, the time frame may be extended beyond 4.5 hours.
  - The most common delay that prevents tPA from being administered is due to patient delay in seeking medical attention, CT scan and review
  - If tPA is given, the patient will need to be admitted to an intensive care bed for monitoring.
- For other causes of stroke (including hemorrhage or tumor), or tPA alternatives consult with an interventional radiologist or neurosurgeon for treatment options available to the patient.
- In those patients where tPA and other interventions are not possible or are not indicated, the patient is usually admitted to the hospital for observation, supportive care, and referral for rehabilitation

Prognosis

- In the U.S., 20% of stroke patients will die within a year.
- Specialized stroke centers, hospitals that have the personnel, equipment, and resources to intervene quickly and treat strokes aggressively, have shown to increase stroke survival and patient function and recovery. These hospitals are certified by The Joint Commission, the American Stroke Association, and the health departments of some states.
- There are many complications that can develop in stroke patients and some may not be able to return to full employment because of disability.
- Patients are affected physically with decreased body function, mentally with decreased cognition, and emotionally with depression and anxiety.
- The return to function depends upon the severity of the stroke, affected parts of the brain and body, and what complications develop.
- Patients who lose their ability to swallow may develop aspiration pneumonia
- Patients who have difficulty moving can develop pressure sores and infections
- Seizures may be a complication in up to 10% of patients, more frequent in more severe stroke.

Stem Cell Types Proposed For Stroke Therapy



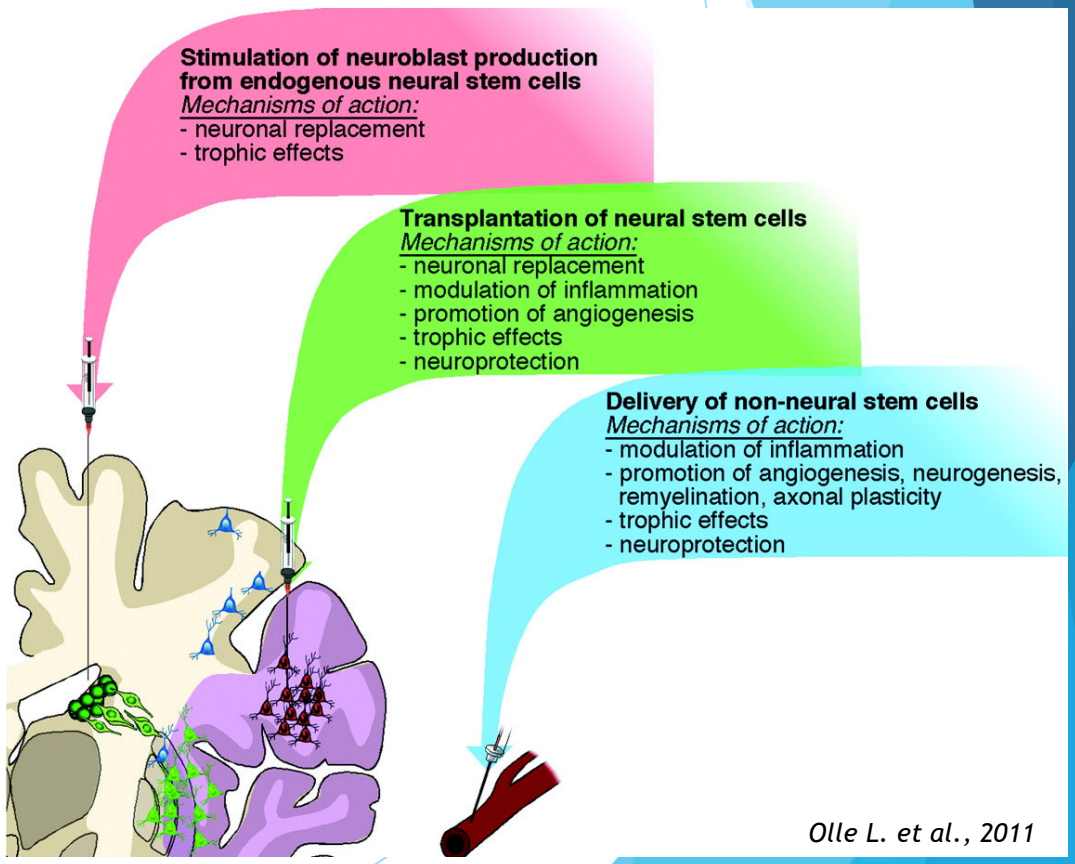
Olle L. et al., 2011

Transplant Timing In Ischemic Stroke

- **Acute stage**
- *Opposing factors*
  - Acute release of excitotoxic neurotransmitters, free radicals, and proinflammatory mediators
  - Ongoing cell apoptosis in the penumbra for several weeks after stroke.
  - Inflammation and microglial activation may suppress the growth and survival of transplanted cells.
- *Synergistic factors*
  - Release of neurotrophic factors from the intrinsic milieu and the host environment during the early recovery phase to facilitate implant growth, survival, differentiation, and/or integration.
- *Consideration of the patient diagnostic and treatment course*
- **Chronic stage**
  - Endogenous neurogenesis starts in periventricular regions and in the hippocampus.
  - Scar formation in the infarcted area starts in the same time
- *Considerations on the natural course of recovery from stroke*
  - Some recommend transplantation delay until deficits have plateaued.
  - Others suggest that transplantation might benefit most during the early synaptic plasticity in recovery
  - Two clinical trials have chosen to study disabled patients at least 6 months after a stroke.

Proposed Mechanisms of Action for Cell Therapies in Stroke

- **Interventional approaches:**
  - Stimulation of endogenous stem cell niche
  - Transplant of exogenous neural stem cells
  - Systemic delivery of non-neural stem cells
- **Classes of mechanism:**
  - Cell replacement
  - Trophic, neuroprotective effect
  - Modulation of inflammation
  - Neurogenesis and angiogenesis



Transplantation of Neural Cell Types in Stroke

- **NCT01151124 A Phase I Safety Trial of CTX0E03 Drug Product Delivered Intracranially in the Treatment of Patients With Stable Ischemic Stroke**
  - First in man study. Open-label, single-site, dose-escalation study. Sponsor: ReNeuron, Pencoed, UK (2010-2013)
  - Safety of a manufactured neural stem cell line (CTX cells) delivered by intracerebral injection of male patients 60 years of age or over who remain moderately to severely disabled 6 months to 5 years following an ischemic stroke.
  - CTX0E03 is a human NSC line, which was derived from human somatic stem cells following genetic modification with c-mycERTAM, a conditional immortalizing gene. This transgene generates a fusion protein that stimulates cell proliferation in the presence of a synthetic drug 4-hydroxy-tamoxifen (4-OHT). The cell line is clonal, expands rapidly in culture and has a normal human karyotype (46 XY). In the absence of growth factors and 4-OHT, the cells undergo growth arrest and differentiate into neurons and astrocytes.
  - Men aged 60 years or older with stable disability, 6-60 months after ischemic stroke were implanted with single doses of 2 million, 5 million, 10 million, or 20 million cells by stereotactic ipsilateral putamen injection.
  - Clinical and brain imaging data were collected over 2 years. The primary endpoint was safety (adverse events and neurological change).

Kalladka D. et al., Human neural stem cells in patients with chronic ischaemic stroke (PISCES): a phase 1, first-in-man study. Lancet. 2016 Aug 20;388(10046):787-96. doi: 10.1016/S0140-6736(16)30513-X. Epub 2016 Aug 3.

Transplantation of Neural Cell Types in Stroke

- FINDINGS:
  - 13 men were recruited between September, 2010, and January, 2013, of whom 11 received CTX-DP.
  - Median NIHSS score before implantation was 7 (IQR 6-8) and the mean time from stroke was 29 (SD 14) months.
  - No immunological or cell-related adverse events were seen.
  - At 2 years, improvement in NIHSS score ranged from 0 to 5 (median 2) points.
  - Single intracerebral doses of CTX-DP up to 20 million cells induced no adverse events and were associated with improved neurological function, supporting further investigation of CTX-DP in stroke patients.
  - Follow up study: NCT02117635: Pilot Investigation of Stem Cells in Stroke Phase II Efficacy (PISCES-II) – Active, not recruiting

MSCs For Stroke

- **Adult stem cells such as MSCs may be a better choice for stroke therapy:**
  - Overcome tumorigenesis of neural stem cells, hESC or IPSC derived progenitors
  - Secrete a variety of bioactive substances, trophic factors, extracellular vesicles (EVs, 0.1–1 μm sized circular membrane fragments shed from the cell surface)
  - Associated with enhanced neurogenesis, angiogenesis, and synaptogenesis
  - Inflammation attenuating
  - Attenuated neuronal death by suppressing activated microglia
  - Scar thickness reduction
  - Autophagy enhancing
  - Microenvironmental/metabolic profiles normalization
- **Routes of administration**
  - Systemic IV
  - Arterial – to avoid filter organs
  - Intranasal, in vicinity of cribriform plate of the ethmoid bone
  - Intracerebral

Differential sources of stem cells

- **Bone marrow-derived MSCs are most commonly used**
- **Adipose tissue-derived stem cells (ADSCs)**
  - Relatively large number of ADSCs can be separated from subcutaneous fat tissue with minimally invasive procedures.
  - ADSCs were reportedly superior to bone marrow MSCs in their paracrine functions and angiogenic potential
  - ADSCs were more refractory to the effects of advanced donor age in a mouse model of stroke
- **Umbilical cord and Wharton's jelly MSCs**
  - expressed preferentially secreted factors related to neuroprotection, neurogenesis, and angiogenesis
  - Umbilical cord MSCs also showed favorable differentiation capabilities and low immunity.
  - improved functional recovery in animal models of stroke even when administered after a delayed time

Bang OY, et al. Adult Stem Cell Therapy for Stroke: Challenges and Progress. J Stroke. 2016 Sep;18(3):256-266.

- **Allogeneic mesenchymal stem cells**
  - Safety: short-term existence in the host after the application.
  - Scalability: scalable from a manufacturing perspective, with standardized procedures.
  - MSCs from younger healthy donors or iPSC- or ESC-derived adult stem cells may differ in terms of their proliferation and neurorestorative capacity, from those of cells obtained from elderly patients with chronic illness.
  - The use of allogeneic MSCs reduces the time required to obtain a sufficient number of cells (the “off the shelf” approach).
  - After contact with serum, allogeneic MSCs can be injured by complement,
  - The viability of allogeneic MSCs after infusion is greatly reduced compared with autologous MSCs

Mesenchymal Stem Cells For Stroke

The effects of mesenchymal stem cells (MSCs) therapy on ischemic stroke damage

Treatment regimens	Main results	References no.
Neonatal stroke rats or mice received intranasal or intracerebral injection of MSCs	Decreasing cerebral damage by reducing both overproduction of IL-6 and TNF-α and microgliosis, but stimulating neurogenesis (e.g., increased production of HGF, VEGF, IGF, EGF, 6FGF, IL-10, GDNF, BDNF, NF3, angiopoietin, TGF, and I-CAM 1	van Velthoven et al. (2010), Yasuhara et al. (2008), van Velthoven et al. (2012, 2013), Wei et al. (2009)
Adult stroke rats received intravenous or intracerebral injection of MSCs	Decreasing cerebral damage by stimulating synaptogenesis and vessel density, reducing apoptosis in the ischemic boundary zone, and increasing proliferation of progenitor cells in the subventricular zone.	Wakabayashi et al. (2010), Xu et al. (2010); Bao et al. (2011), Lin et al. (2011); Walker et al. (2010), Wei et al. (2012), Ma et al. (2013), Tang et al. (2014), Cheng et al. (2015)
Adult stroke monkeys received intracerebral injection of MSCs	Reducing cerebral damage by stimulating production of IL-10	Li et al. (2010)
Adult stroke patients received intravenous injection of MSCs	Reducing cerebral damage by promoting nerve cell proliferation	Weimann et al. (2003) Bang et al. (2005) Lee et al. (2010)

Hsuan YC, et al. , 2016

Intravenous Autologous Bone Marrow Mononuclear Cells For Ischemic Stroke

- **NCT00859014: Safety/Feasibility of Autologous Mononuclear Bone Marrow Cells in Stroke Patients**
  - Sponsor: The University of Texas Health Science Center, Houston, January 2009 - November 2013
  - Open-label prospective study of a bone marrow harvest followed by rea-dministration of autologous MNCs in 10 patients, 18 to 80 years old, with acute middle cerebral artery ischemic stroke.
  - Bone marrow harvested from the iliac crest, MNCs were separated and administered IV up to a maximum of 10 million cells/kg.
  - The harvest and infusion had to occur between 24 and 72 hours after stroke.
  - Patients were monitored for 6 months.
- **RESULTS:**
  - Bone marrow aspiration was successfully completed in all patients, no significant adverse events related to harvest or infusion.
  - Eight received 10 million cells/kg, and 2 received ≥7 million cells/kg.
  - Two patients had infarct expansion between enrollment and harvest and underwent hemicraniectomy after cell infusion.
  - One patient died at 40 days due to a pulmonary embolism related to the stroke.
  - There were no study-related severe adverse events.
  - Median NIH Stroke Scale score was 13 before harvest, 8 at 7 days, and 3 at 6 months.
  - At 6 months, all surviving patients had shifted down by at least 1 point on the modified Rankin Scale compared to day 7.
  - Seven of 10 patients achieved a Barthel Index ≥90.
- **INTERPRETATION:**
  - This study suggests that a bone marrow harvest and reinfusion of autologous MNCs were safe and feasible in acute stroke patients.

Study to Examine the Effects of Multipotent Adherent Progenitor Cells in Ischemic Stroke

- **NCT01436487: Double-Blind, Randomized, Placebo-Controlled Phase 2 Safety and Efficacy Trial of MultiStem® in Adults With Ischemic Stroke**
- Sponsor: Athersys Inc, October 2011 - December 2015
  - Multipotent adherent progenitor cells (MultiStem) are derived from mononuclear bone marrow cells that are depleted of CD45(+)/glycophorin-A (GlyA)(+) cells, using micromagnetic beads. Cells are grown in CD45−GlyA−cell expansion medium supplemented with EGF (10 ng/mL), PDGF-BB (10 ng/mL) and FBS (18%), on plates coated with fibronectin (10 ng/mL), laminin, or type IV collagen
- Arms:
  - Cohort 1. Low dose MultiStem or Placebo, single infusion 1-2 days following ischemic stroke
  - Cohort 2. High dose MultiStem or Placebo, single infusion 1-2 days following ischemic stroke
  - Highest, safe MultiStem dose (from Cohorts 1 and 2) or Placebo, single infusion 1-2 days following ischemic stroke
- Primary Outcome Measures:
  - Frequency of dose limiting adverse events
  - Stroke recovery based on global test analysis including modified Rankin Scale (mRS), NIHSS, and Barthel Index (BI)
- Patients:
  - Male or female subjects between 18 and 83 years of age (inclusive)
  - Clinical diagnosis of cortical cerebral ischemic stroke
  - Occurrence of a moderate to moderately severe stroke

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- **NCT01436487: Double-Blind, Randomized, Placebo-Controlled Phase 2 Safety and Efficacy Trial of MultiStem® in Adults With Ischemic Stroke**
- **Results:**
- The 90 day interim data:
  - It failed to demonstrate any significant difference compared to placebo on the primary endpoint
  - It failed secondary goals of statistical significant improvement on two other measures of stroke rehabilitation.
  - Patients who got MultiStem earlier in the treatment window did better against the study's endpoints than those who got it later.
- The one year follow up data:
  - MultiStem-treated subjects had a significantly higher rate of "Excellent Outcome" (defined clinically as attaining mRS 0-1, NIHSS 0-1 and BI ≥95) compared to placebo subjects when evaluating all subjects enrolled in the study ($p=0.02$), i.e., the intent-to-treat population.
  - The relative improvement in Excellent Outcomes was even more pronounced in the patients who received MultiStem treatment within 36 hours of the stroke ($p < 0.01$).
  - The treatment continued to be well tolerated through 365 days;

http://www.athersys.com/releasedetail.cfm?ReleaseID=955434

Study to Examine the Effects of Multipotent Adherent Progenitor Cells in Ischemic Stroke

- **NCT01436487: Double-Blind, Randomized, Placebo-Controlled Phase 2 Safety and Efficacy Trial of MultiStem® in Adults With Ischemic Stroke**
- **Results:**

Subjects with Excellent Outcome	Day 90	Day 365
All MultiStem (n=65)	15.4%	23.1%
All Placebo (n=61)	6.6%	8.2%
Difference with all placebo	8.8%	14.9% (p=0.02)
Early Treatment with MultiStem (n=31)	16.1%	29.0%
Difference with all placebo	9.5%	20.8% (p<0.01)

Outcomes	Placebo	MultiStem
Barthel Index (BI) ≥95	44.3%	61.5% (p=0.05)
Patients treated within 36 hours, BI>95	44.3%	67.7% (p=0.03)
Excellent mRS index (≤1)	13.1%	27.7% (p=0.04)
Patients treated within 36 hours, mRS≤1		32.3% (p=0.03)
Difference with all placebo	9.5%	20.8% (p<0.01)

Atheresys is preparing for a confirmatory clinical study in Japan (with HEALIOS K.K.) and a separate trial in North America and Europe.

http://www.athersys.com/releasedetail.cfm?ReleaseID=955434

Active Adult Stem Cell Clinical Trials for Stroke

https://clinicaltrials.gov

	Title	Status	Study Results	Conditions	Interventions	Locations
1	Autologous Bone Marrow Mesenchymal Stem Cell Transplantation for Chronic Ischemic Stroke	Unknown status	No Results Available	•Stroke	•Genetic: Intravenous stem cell transplantation	•Xiaodan Jiang, Guangzhou, Guangdong, China
2	Autologous Bone Marrow Mesenchymal Stem Cell Transplantation for Chronic Stroke	Unknown status	No Results Available	•Stroke	•Genetic: intracerebral stem cell transplantation	•The First Affiliated Hospital of Wenzhou Medical College, Wenzhou, Zhejiang, China
3	Umbilical Cord Derived Mesenchymal Stem Cells Treatment in Ischemic Stroke	Unknown status	No Results Available	•Stroke	•Biological: Human umbilical cord mesenchymal stem cells	•Department of Neurosurgery,Affiliated Hospital of Academy of Military Medical Sciences(307 Hospital), Beijing, Beijing, China
4	Evaluate the Safety and Explore Efficacy of Umbilical Cord Mesenchymal Stem Cells in Acute Ischemic Stroke	Recruiting	No Results Available	•Acute Stroke	•Biological: UMSC01	•China Medical University Hospital, Taichung, Non-US, Taiwan
5	Safety of Escalating Doses of Intravenous Bone Marrow-Derived Mesenchymal Stem Cells in Patients With a New Ischemic Stroke	Withdrawn	No Results Available	•Ischemic Stroke	•Biological: bone marrow-derived mesenchymal stem cells •Drug: Placebo	•University of California Irvine Medical Center, Orange, California, United States
6	Allogenic Adipose Tissue-derived Mesenchymal Stem Cells in Ischemic Stroke	Not yet recruiting	No Results Available	•Ischemic Stroke •Adipose Tissue-derived Stem Cell •Functional Status	•Other: Alogenic adipose tissue-derived stem cells •Drug: Placebo solution	
7	Reparative Therapy in Acute Ischemic Stroke With Allogenic Mesenchymal Stem Cells From Adipose Tissue. Safety Assessment. a Randomised, Double Blind Placebo Controlled Single Center Pilot Clinical Trial	Completed	No Results Available	•Ischemic Stroke	•Drug: Allogenic mesenchymal stem cells from adipose tissue •Drug: Placebo	•University Hospital La Paz, Madrid, Spain
8	Mesenchymal Stem Cells for The Treatment of Acute Ischemic Stroke	Recruiting	No Results Available	•Acute Ischemic Stroke	•Biological: UMC119-06	•Taipei Medical University - Shuang Ho Hospital, Ministry of Health and Welfare., New Taipei City, Taiwan
9	The STem Cell Application Researches and Trials in NeuroloGy-2 (STARTING-2) Study	Unknown status	No Results Available	•Stroke, Ischemic	•Other: Mesenchymal stem cell	•Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic of
10	Mesenchymal Stem Cells Therapy in Patients With Recent Intracerebral Hemorrhage	Recruiting	No Results Available	•Hemorrhagic Stroke •Intracerebral Hemorrhage	•Biological: MSC	•Mayo Clinic in Florida, Jacksonville, Florida, United States

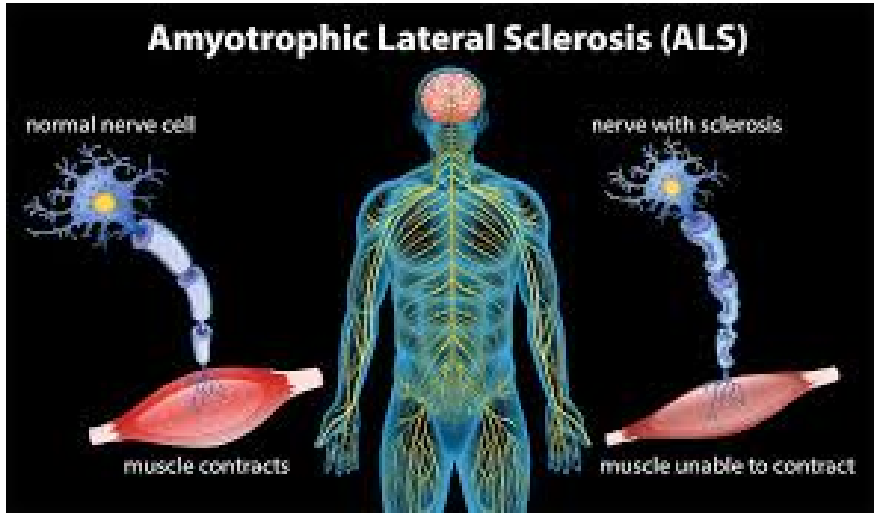
Completed Adult Stem Cell Clinical Trials for Stroke

https://clinicaltrials.gov

Study	Interventions	Sponsor	Location	Identification
Study to Examine the Effects of MultiStem in Ischemic Stroke	MultiStem; Placebo	Athersys, Inc	USA	NCT01436487
Intravenous Autologous Bone Marrow-derived Stem Cells Therapy for Patients With Acute Ischemic Stroke	Autologous bone marrow stem cell	Manipal Acunova Ltd.	India	NCT01501773
Efficacy Study of CD34 Stem Cell in Chronic Stroke Patients	Intracerebral implantation of Autologous Stem Cells; Convention therapy	China Medical University Hospital	China	NCT00950521
Autologous Bone Marrow Stem Cells in Middle Cerebral Artery Acute Stroke Treatment.	Infusion on autologous CD34+ stem cells into middle cerebral artery	Hospital Universitario Central de Asturias	Spain	NCT00761982
Safety/Feasibility of Autologous Mononuclear Bone Marrow Cells in Stroke Patients	Autologous Bone Marrow Mononuclear Cells	The University of Texas Health Science Center, Houston	USA	NCT00859014
Stem Cell Therapy For Acute Ischemic Stroke Patients (InVeST)	BMSCs	All India Institute of Medical Sciences, New Delhi	India	NCT02425670
Safety and Efficacy of Autologous Stem Cell Therapy in Chronic Stroke	Autologous bone marrow mononuclear cell transplantation	Neurogen Brain and Spine Institute	India	NCT02065778
Autologous Bone Marrow Stem Cells in Ischemic Stroke.	Autologous CD34+ stem cells into middle cerebral artery	Imperial College London	UK	NCT00535197
Study of Autologous Stem Cell Transplantation for Patients With Ischemic Stroke	Autologous Bone Marrow Cell Transplantation	Universidade Federal do Rio de Janeiro	Brazil	NCT00473057

Amyotrophic Lateral Sclerosis (ALS)

Amyotrophic Lateral Sclerosis (ALS)
Lou Gehrig's Disease



- Most people with ALS are between ages 40-70
- Most common among persons over age 60
- Affects as many as 30,000 in US, with 5000 new cases diagnosed every year
- Incidence of ALS is 5 times higher than Huntington's disease and about equal to MS

www.hopkinsmedicine.org

Amyotrophic lateral sclerosis (ALS)

- Amyotrophic lateral sclerosis (ALS), also known as Charcot disease, Lou Gehrig's disease and Motor Neuron disease (MND)
- Progressive loss of the upper and lower motor neurons (LMNs) at the spinal or bulbar level.
- **Forms:**
  - Sporadic form (90–95%) which has no obvious genetically inherited component.
  - Familial-type ALS (FALS), 5-10% due to their associated genetic dominant inheritance factor.
- **Epidemiology**
  - Incidence 1.5-2.7/100,000 in Europe and North America,
  - Geographic pockets with much higher 50-100X incidence (Guam, Kii Peninsula of Japan, and South West New Guinea)
  - Male: female ratio =1.5:1
  - Mean age of onset of ALS varies from 50 to 65 years
  - Increased association with presence of β-methylamino-L-alanine (BMAA) in environment, and performance sport participation prone to head injuries (football players, odd ratio = 3.2)
  - Associated with increased history of depression (22.8% vs. 11.6%), dementia (5.8% vs. 1.3%), Parkinson's (1.8% versus 0.1–0.2%), and epilepsy (1.6% versus 0.45–1%), common underlying mechanism?

ALS – Risk Factors

- **Risk Factors**
- Smoking
  - It is thought that cigarette smoking is the most consistent nongenetic risk factor for ALS
  - A beneficial effect of quitting smoking on ALS patient has not been examined so far.
- Physical activity
  - Athletes have higher ALS risk compared to the general population;
  - The physical activity itself is not proven to be a cause of ALS, associated but not the cause
  - A genetic profile altered by exogenous factors promoting physical fitness increases ALS susceptibility
- Chemical exposure and metals
  - ALS has shown an association with exposure to agricultural chemicals such as pesticides, fertilizers, herbicides, insecticides, and formaldehyde, lead
- Radiation/electromagnetic fields
  - None of the current studies found a conclusive connection between electromagnetic field exposure, oxidative stress in neurons, and/or ALS development.
- Diet
  - High level of glutamate and fat can have adverse effects on ALS patients
  - Omega 3 in conjunction with Vitamin E has been reported to reduce ALS risks up to 60%.

ALS – Mechanism

- **Molecular Mechanism**
- The most common cause of ALS is a mutation of the gene encoding the antioxidant enzyme superoxide dismutase 1 (SOD1)
- Mutant SOD1 has a structural instability that causes a misfold in the mutated enzyme, which can lead to aggregation in the motor neurons within the central nervous system (CNS).
- The most important proposed hypothesis for the pathogenesis of ALS includes
  - Glutamate excitotoxicity: alteration of EAAT2 gene leading to increased extracellular glutamate, over-stimulation of glutamate receptors, leading to neuronal death by increasing the influx of Na+ and Ca2+ or by the generation of free radicals
  - Structural and functional abnormalities of mitochondria, related to cytosolic ability of Ca2+ buffering due to presence of aberrant SOD1
  - Impaired axonal structure or transport defects, both antero- and retrograde, causing accumulation of neurofilaments, mitochondria, and autophagosomes in degenerated motor neurons
  - Free radical-mediated oxidative stress. SOD1 is a major antioxidant protein, thus a mutation in this gene could cause cytotoxicity
- Even though these mechanisms play a critical role in neurodegeneration, they all are considered as secondary events in the causes behind ALS onset.

ALS - Symptoms

- **Symptoms**
  - Primary symptoms include muscle weakness and atrophy, spasticity, speech disturbances, poor management of oral secretions, difficulty swallowing, and respiratory complications that result in death.
  - Secondary symptoms usually accompany primary symptoms, and they can significantly reduce the quality of life of patients, such as pain or difficulty performing daily tasks related to muscle cramping and spasticity.
- The main clinical feature in ALS is a combination of UMN and LMN damage involving brainstem and multiple spinal cord innervation regions.
  - Localized muscle weakness that begins distally or proximally in their upper and lower limb starting asymmetric and develop in progressive generalized weakness
  - The majorityof the patients develop bulbar and respiratory symptoms and spasticity, which affects manual dexterity and gait.
  - Pseudobulbar symptoms including emotional lability and excessive yawning.
  - About 5% of patients present type 2 respiratory failure or nocturnal hypoventilation including dyspnea, orthopnea, disturbed sleep, excessive somnolence in daytime, morning headaches, anorexia, decreased concentration, and irritability or mood changes
  - Muscle atrophy, including muscles of the hands, forearms or shoulders, and proximal thigh or distal foot muscle in lower limbs, is usually discovered early in the development of limb-onset
  - Speech disturbances tend to appear before the development of dysphagia for solids and liquids

ALS - Treatment

- **Treatment**
  - Antiapoptotic, addressing mitochondrial impairment and calcium handling
    - Minocycline, Pentoxifyline, Caspase inhibitors
  - Anti-inflammatory, targeting reactive astrocytes, microglia, infiltrating lymphocytes and macrophages
    - Celastrol, Minocycline, Arundic acid, Thalidomide, Pioglitazone, Rofecoxib etc
  - Anti-excitoxicity, anti-glutamatergic
    - Cobalamin, Ceftriaxone, Gabapentin, L-Arginine, Memantine, etc
  - Antioxidant, to compensate the SOD activity
    - Creatine, Celastrol, Coenzime Q10, N-Acetyl cysteine, Tamoxifen, Vit E etc
  - Anti-aggregation, to address cellular aggregation propensity (Bunina bodies) related to SOD1 mutation
    - Ariclomol, Valproate, Na phenilbutirate, Celastrol
  - Neuroprotective and neurotrophic to stimulate neurogenesis and preventing neurodegeneration.
    - BDNF, GDNF, HGF CNTF, IGF-1, VEGF, EPO etc
  - Dietary supplements
    - Vit. A, Vit. E, Creatine, Pu-erh tea extract (PTE)
- Riluzole, 50 mg twice daily, is currently the only FDA-approved drug identified to have beneficial use in the survival of patients with ALS

Cell Sources for Therapies in ALS

- **Originating tissue**
  - Neural stem cells (fetal, embryonic or IPS)
    - Motor neuron progenitors
    - Astrocytes (glial progenitors)
    - Mixed neural progenitors
  - Mesenchymal stem cells
    - Umbilical cord stem cells
    - Hematopoietic stem cells
    - Bone marrow stem cells
    - Adipose derived mesenchymal stem cells
  - Engineered cells to produce growth factors (BDNF, IGF-1, etc)
- **Allogeneic or Autologous:**
  - Allogeneic does not carry the mutations associated with ALS
  - IPSC, gene therapy for autologous cells
- **Routes of administration**
  - IV, Intramuscular
  - Intrathecal (IT)
  - Intra-arterial

Active Clinical Trials Using Cell Therapy in ALS

	Title	Status	Study Results	Conditions	Interventions	Locations
1	Mesenchymal Stem Cell Injection in Amyotrophic Lateral Sclerosis	Withdrawn	No Results Available	•Amyotrophic Lateral Sclerosis	•Biological: mesenchymal stem cell	•Neurosciences Research Center, Isfahan, Iran, Islamic Republic of
2	The Clinical Trial on the Use of Umbilical Cord Mesenchymal Stem Cells in Amyotrophic Lateral Sclerosis	Unknown status	No Results Available	•Amyotrophic Lateral Sclerosis	•Procedure: stem cell transplantation	•Yihua An, Beijing, China
3	Autologous Bone Marrow Mesenchymal Stem Cells in the Treatment of Patients With Amyotrophic Lateral Sclerosis	Unknown status	No Results Available	•Amyotrophic Lateral Sclerosis	•Other: Biological: Cell-based therapy	
4	Mesenchymal Stem Cells for Treatment of Amyotrophic Lateral Sclerosis (ALS)	Completed	No Results Available	•Amyotrophic Lateral Sclerosis	•Biological: autologous mesenchymal stem cells	•Mayo Clinic, Rochester, Minnesota, United States
5	A Dose-escalation Safety Trial for Intrathecal Autologous Mesenchymal Stem Cell Therapy in Amyotrophic Lateral Sclerosis	Completed	No Results Available	•Amyotrophic Lateral Sclerosis	•Biological: autologous mesenchymal stem cells	•Mayo Clinic, Rochester, Minnesota, United States
6	Intraventricular Transplantation of Mesenchymal Stem Cell in Patients With ALS	Withdrawn	No Results Available	•Amyotrophic Lateral Sclerosis	•Biological: Intraventricular injection	•Royan Institute, Tehran, Iran, Islamic Republic of
7	Intravenous Injection of Adipose Derived Mesenchymal Stem Cell for ALS	Completed	No Results Available	•Amyotrophic Lateral Sclerosis	•Biological: mesenchymal stem cells	•Royan Institute, Tehran, Iran, Islamic Republic of
8	Intravenous Transplantation of Mesenchymal Stem Cell in Patients With ALS	Completed	No Results Available	•Amyotrophic Lateral Sclerosis	•Biological: intra venous injection of stem cell	•Royan Institute, Tehran, Iran, Islamic Republic of
9	Intrathecal Transplantation of Mesenchymal Stem Cell in Patients With ALS	Completed	No Results Available	•Amyotrophic Lateral Sclerosis	•Biological: intrathecal injection	•Royan Institute, Tehran, Iran, Islamic Republic of
10	Escalated Application of Mesenchymal Stem Cells in Amyotrophic Lateral Sclerosis Patients	Completed	No Results Available	•Motor Neuron Disease	•Biological: Autologous Mesenchymal stem cells (MSCs)	•Hospital e Maternidade Dr Christovao da Gama, Santo Andre, Sao Paulo, Brazil •Instituto de Ensino e Pesquisas - IEP-São Lucas, Sao Paulo, SP, Brazil
11	Study of Two Intrathecal Doses of Autologous Mesenchymal Stem Cells for Amyotrophic Lateral Sclerosis	Unknown status	No Results Available	•AMYOTROPHIC LATERAL SCLEROSIS	•Other: Two intrathecal MSC injections	•University of Sao Paulo School of Medicine Clinics Hospital, Sao Paulo, SP, Brazil
12	Therapeutic Treatment of Amyotrophic Lateral Sclerosis	Unknown status	No Results Available	•Amyotrophic Lateral Sclerosis	•Other: Biological: Cell-based therapy	
13	A Multicenter Phase I/II Clinical Trial to Evaluate Safety of Mesenchymal Stem Cell in Patients With Amyotrophic Sclerosis Lateral	Active, not recruiting	No Results Available	•Amyotrophic Lateral Sclerosis	•Other: Intravenous administration of placebo •Drug: Intravenous administration of 1 million of MSC •Drug: Intravenous administration of 2 million of MSC •Drug: Intravenous administration of 4 million of MSC	•Hospital Regional Universitario Reina Sofia, Cordoba, Spain •Hospital Regional Universitario de Málaga, Málaga, Spain •Hospital Universitario Virgen Macarena, Servicio de Neurología, Seville, Spain •Hospital Universitario Virgen del Rocío, Seville, Spain
14	Phase 2, Randomized, Double Blind, Placebo Controlled Multicenter Study of Autologous MSC-NTF Cells in Patients With ALS	Completed	No Results Available	•Amyotrophic Lateral Sclerosis (ALS)	•Biological: Autologous MSC-NTF cells •Biological: Placebo	•Massachusetts General Hospital, Boston, Massachusetts, United States •UMass Medical School, Worcester, Massachusetts, United States •Mayo Clinic, Rochester, Minnesota, United States

Completed Clinical Trials Using Cell Therapy in ALS

Trial		Phase	Sponsor	Location
Cell Therapy for Motor Neuron Disease/ ALS	Autologous BM Mononuclear Cell	I	Neurogen Brain and Spine Institute	India
Effect of Intrathecal Administration of Hematopoietic Stem Cells in Patients With ALS	Intrathecal autologous stem cell (BMSC)	II, III	Hospital Universitario Dr. Jose E. Gonzalez	Mexico
Stem Cell Therapy for Amyotrophic Lateral Sclerosis	Autologous BM mononuclear cells intrathecal and intramuscular transplantation	II	Neurogen Brain and Spine Institute	India
Clinical Trial on the Use of Autologous Bone Marrow Stem Cells in Amyotrophic Lateral Sclerosis (CMN/ELA)	Laminectomy and autologous bone marrow stem cells transplantation	I, II	Fundacion para la Formacion e Investigacion Sanitarias de la Region de Murcia	Spain
Human Neural Stem Cell Transplantation in Amyotrophic Lateral Sclerosis (ALS) (hNSCALS)	Intra-spinal Cord Delivery of Human Neural Stem Cells (fetal)	I	Azienda Ospedaliera Santa Maria, Terni, Italy	Italy
Mesenchymal Stem Cells for Treatment of ALS	Autologous mesenchymal stem cell	I	Mayo Clinic	US
Autologous Cultured Mesenchymal Bone Marrow Stromal Cells Secreting Neurotrophic Factors (MSC-NTF), in ALS Patients.	Autologous cultured BMSC secreting neurotrophic factors	I, II	Hadassah Medical Organization	Israel
Phase 2, Randomized, Double Blind, Placebo Controlled Multicenter Study of Autologous MSC-NTF Cells in Patients With ALS (NurOwn)	Autologous Mesenchymal Stem Cells Secreting Neurotrophic Factors (MSC-NTF)	II	Brainstorm-Cell Therapeutics	US, Israel
Intravenous Transplantation of Mesenchymal Stem Cell in Patients With ALS	Intravenous Transplantation of Bone Marrow Derived Mesenchymal Stem Cell (autologous)	I	Royan Institute	Iran
Safety and Efficacy Study of Autologous Bone Marrow Derived Stem Cell Treatment in ALS	autologous bone marrow-derived stem cells("HYNR-CS inj"), through IT delivery	I, II	Corestem, Inc.	US
Intrathecal Transplantation of Mesenchymal Stem Cell in Patients With ALS	Intrathecal Transplantation of Autologous Bone Marrow Derived Mesenchymal Stem Cell	I	Royan Institute	Iran

Human Cell Therapies in ALS

- ***Glass, J. D., Boulis, N. M., et al. Lumbar Intraspinal Injection of Neural Stem Cells in Patients with Amyotrophic Lateral Sclerosis: Results of a Phase I Trial in 12 Patients.*** *STEM CELLS, 2012, 30: 1144–1151.*
  - NSI-566RSC, isolated from the spinal cord of a single 8-week-old fetus, expanded under cGMP for about 25 passages in a MCB and WCB, >99% nestin+, tested free of human, porcine bovine or rodent pathogens
  - **Patient selection and pre-surgical assessment**
    - hand-held dynamometry (HHD), forced vital capacity (FVC), ALS functional rating scale-revised (ALSFRS-R), and MRI scans of brain and entire spinal cord
  - **Surgical procedure**
    - standard anesthetic and monitoring, including lower extremity somatosensory evoked potentials and motor evoked potentials
    - A two-level, bilateral laminectomy at el T11 and T12, the surgical apparatus was attached to the vertebrae
    - Five unilateral or five bilateral (10 total) injections, containing 100,000 cells in an 8.5–10 µl volume (10,000 cells /µL).
    - Injected over more than a 2-minute period, needle kept in place for an additional minute to prevent reflux during needle exit.
  - **Immunosuppression**
    - 125 mg methylprednisolone i.v. immediately prior to surgery and oral prednisone 60 mg tapering to 0 over 1 month.
    - Two doses of basiliximab (20 mg i.v.), one during surgery and another on postoperative day 4.
    - Tacrolimus was given in b.i.d. oral dosing to maintain a trough level of 4–8 ng/ml for the duration of trial
    - Mycophenolate mofetil was given at 1,000 mg orally b.i.d. for the duration of the trial.

Human Cell Therapies in ALS

- **Objectives:**
  - 384 people manifested interest, 15 patients screened, 12 enrolled
- **Adverse events**
  - In the opinion of the site PI and the study team, there were no adverse events or toxicities that could, with any certainty, be attributed to the test article
  - Dural fistula in one patient was related to the surgery, extradural drains were discontinued
  - Two deaths, one suddenly at 8 month (ALS progressed, congenital bicuspid aortic valve), another patient to progressive respiratory failure at 13 months
  - Majority of nonserious adverse events attributed to immunosupression
  - Immunorejection monitored by HLA antibodies, consistently negative
- **Functional outcomes**
  - No obvious or consistent acceleration of disease progression for any patient
  - No indication of a positive change in the slope of progression in majority of patients
  - One patient improved over the trial period

Glass JD, et al. Lumbar intraspinal injection of neural stem cells in patients with ALS: results of a phase I trial in 12 patients. Stem Cells. 2012; 30: 1144-1151.

Human Cell Therapies in ALS

- ***Feldman E.L., Boulis, N. M., et al., Intraspinal neural stem cell transplantation in amyotrophic lateral sclerosis: Phase 1 trial outcomes.*** *Ann Neurol., 75: 363–373.*
  - **Objectives**: to assess the safety and feasibility of stem cell transplantation into lumbar and/or cervical spinal cord regions in amyotrophic lateral sclerosis (ALS) subjects
    - 6 ALS subjects to receive 5 unilateral cervical intraspinal neural stem cell injections
    - 3 subjects received in the previous trial 10 total bilateral lumbar injections
    - 100,000 cells per injection, total dose (including previous) – up to 1.5 million cells
  - **Modifications for the cervical approach:**
    - the injection rate was lowered to 5 µL/2 minutes
  - Results
    - Additional total 5/12 deaths caused by respiratory complications associated with ALS disease progression, 2/6 from the cervical cohort at 20 months and 200 days
    - pathological analysis: no evidence of hemorrhage, cyst formation, or inflammatory reaction
    - Subjects continued to demonstrate outcomes consistent with disease progression
    - No acceleration of the disease course.
  - Conclusions
    - Functional and histological data allows commencement of phase II (NCT01730716)

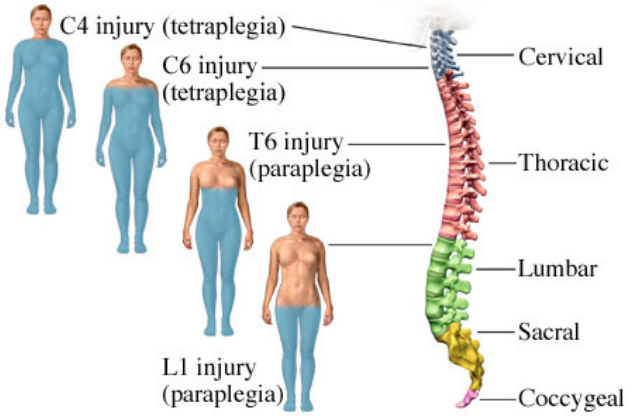
Spinal Cord Injury

Spinal Cord Injury

- ~ 2 Million people living with spinal cord injury (SCI) worldwide
- ~ 11,000 acute cases and 250,000 chronic in US
- Standard of care for acute SCI:

  Methylprednisolone –many side effects and no/minimal improvements in SCI trials (Bracken 1985, 1990, 1995)

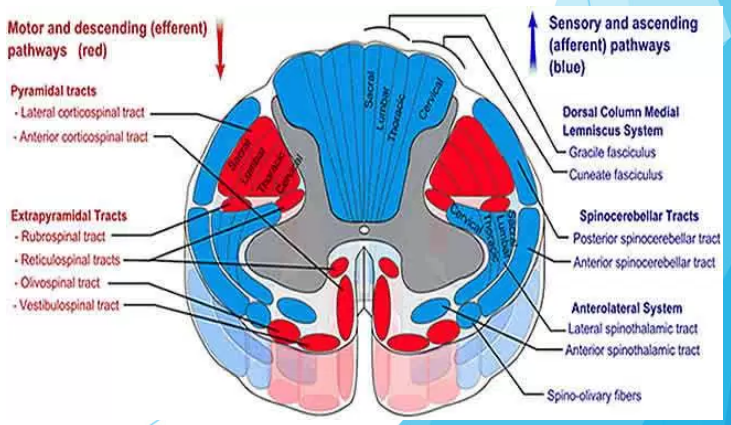
  Decompression—no significant differences in clinical trials (Vaccaro 1997)
- Yearly cost: 1st year up to $776,000; subsequent years $138,000



Spinal Cord Injury

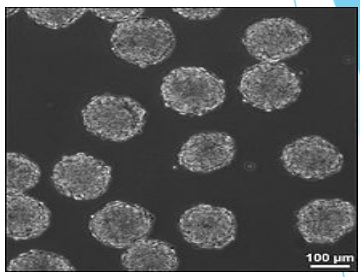
- Problem with SCI

1. Various injury types: contusion, laceration, burn, resection
2. CNS limited regenerative capacity
3. Neuronal and glial destruction
4. Secondary degeneration



Spinal Cord Injury

- ESC studies in rats lead to first US clinical work



"Human embryonic stem cells differentiate into oligodendrocytes in high purity and myelinate after spinal cord transplantation." (Nistor et al., 2005)

"Human Embryonic Stem Cell-Derived Oligodendrocyte Progenitor Cell Transplants Remyelinate and Restore Locomotion after Spinal Cord Injury." (Keirstead et al., 2005)

- Differentiation of ESC into oligodendrocyte precursors
- Remyelination of spinal cord
- Functional improvement in rats

Spinal Cord Injury

- ESC studies lead to first US clinical work
  - Human clinical studies sponsored by Geron Corp (2010).
  - 10 patient study: only 4 were completed
  - Study was discontinued 11/2011
- Asterias Biotherapeutics: Original founders of the study from Geron form new company and get funding
- Early efficacy data 6 months ago now running higher dose studies

Spinal Cord Injury

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Administration of Autologous Bone Marrow Stem Cells Into Spinal Cord Injury Patients Via Multiple Routes Is Safe and Improves Their Quality of Life: Comprehensive Case Studies

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Presently, there is no cure or effective treatment for spinal cord injury (SCI). Studies in SCI patients have shown that for a treatment to be effective it must primarily improve their quality of life. Numerous studies have shown that stem cells represent an alternative treatment for various disorders and have shown promise in several disease/trauma states. For instance, the use of autologous CD34+ stem cells has been shown to ameliorate symptoms of several disorders such as leukemia, cardiomyopathy, diabetes, and several autoimmune diseases, including multiple sclerosis. For the first time, we report eight case studies of SCI (four acute, four chronic) with approximately 2 years of follow-up that were administered bone marrow stem cells (BMSCs) via multiple routes: directly into the spinal cord, directly into the spinal canal, and intravenous. Magnetic resonance imaging illustrated morphological changes in the spinal cord of some of the patients following BMSCs administration. Comprehensive evaluations demonstrate improvements in ASIA, Barthel (quality of life), Frankel, and Ashworth scoring. Moreover, in order to assess bladder function, we designed a simple numerical clinical scoring system that demonstrates significant changes in bladder function following BMSCs administration. To date, we have administration BMSCs into 52 patients with SCI and have had no tumor formations, no cases of infection or increased pain, and few instances of minor adverse events. These studies demonstrate that BMSCs administration via multiple routes is feasible, safe, and may improve the quality of life for patients living with SCI.

Key words: Spinal cord injury; Bone marrow stem cells (BMSC's); Quality of life

INTRODUCTION

Spinal cord injury (SCI) is a devastating disorder afflicting millions across the world (18). Presently, there is no cure or effective standard of care for SCI. Many treatments have been tested in clinical trials, including the use of methylprednisolone (9,10), GM1 ganglioside (24), decompression (51), and 4-aminopyridine (14), all of which have only produced marginal benefits with adverse side effects. The present standard of care for SCI is methylprednisolone and/or decompression. However, neither of these treatments has prevented the pathological cascade triggered by SCI and the efficacy of methylprednisolone is questionable (29).

Tissue loss from primary trauma to the spinal cord and the complexity of cell types required for functional recovery exemplifies a need for cellular replacement strategies (23,35,46). Scientists are compelled to find a cure or effective treatment for SCI; however, the heterogeneity of human SCI represents an enormous challenge for finding a standard of care. With our present technology, realistically the SCI community would greatly benefit with a treatment that promotes partial functional recovery leading to an improved quality of life (2,3).

Stem cells have been identified in various adult organs where they are thought to contribute to tissue repair. The expanding field of adult stem cell research has demonstrated numerous results concerning the broad differentiation potential of adult stem cells. Compared to embryonic stem cells, adult tissue-specific stem cells have a limited self-renewal ability and plasticity, but yet they have been proven to be multipotent. For example, neural stem cells were found to repopulate the hematopoietic system (5), as well as differentiate into all three

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Spinal Cord Injury

- All studies were conducted at Luis Vernaza Hospital, Guayaquil, Ecuador under IRB approval
- Recruit using inclusion/exclusion criteria
- Acute SCI: Injured < 1 year (ICCP 2006)
- Chronic SCI: Injured >1 year where preceeding 6 months there were no changes (ICCP 2006)
- MRI imaging of spinal cord
- Neurological assessment using ASIA, Ashworth, and Frankel scale
- Barthel Index (quality of life)
- Newly developed Bladder function scale (GGSF)

Study Design

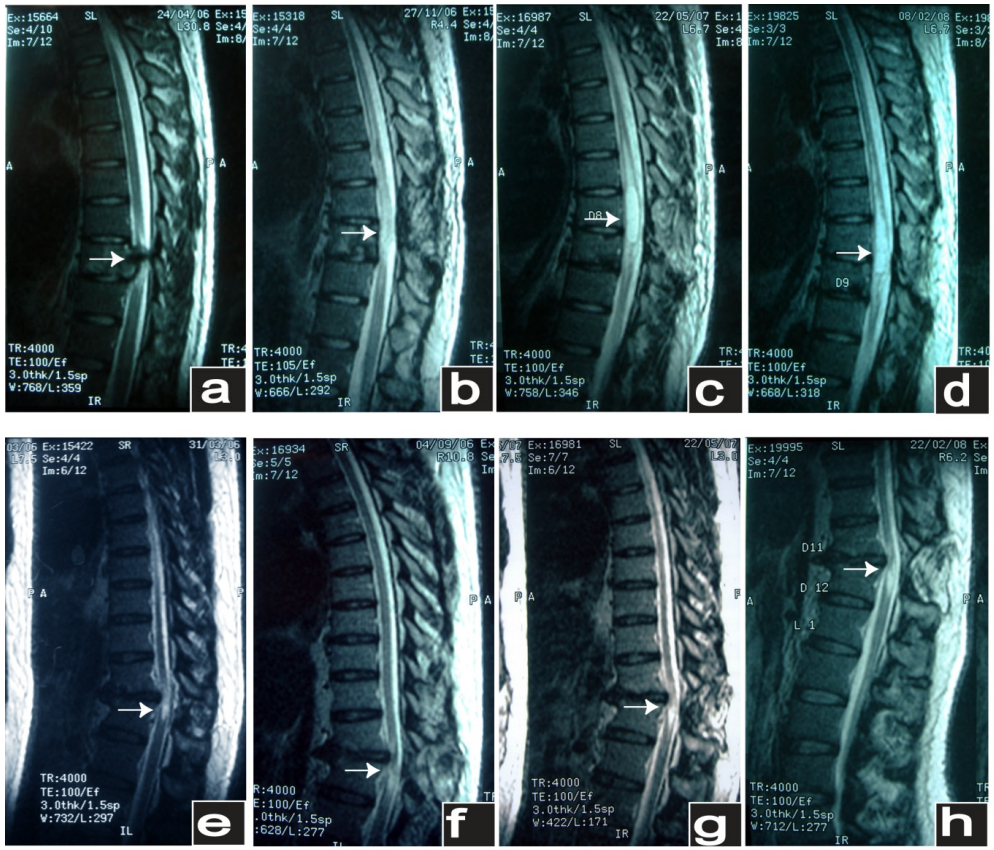
- Harvest bone marrow—isolate mononuclear cells (bone marrow stem cells (BMSCs))
- Analysis of bone marrow stem cells (FACS)
- Administration of bone marrow stem cells via multiple routes
    a. into spinal cord injury site
    b. into spinal canal
    c. intravenous
- Follow up for 2 years

Case Studies Demographics

- 8 cases: 4 acute 4 chronic

Case #	Sex	Age	Weight (kg)	Injury Level	Injury Type	CD34/kg Cell/10e6	Viability CD34+ (%)	Time of BMSCs Administration After SCI
1	M	28	80	T 9	Gunshot	1.43	89.62	1.5 Months
2	F	33	75	T 4	Gunshot	1.1	82.22	7 Months
3	M	28	79	T 5-6	Fall	1.5	77.62	13 Days
4	M	31	67	T 12-L1	Fall	0.94	96.27	5 Days
5	M	37	86	T 12	Car Accident	1.2	91.22	6 Years 3 Months
6	M	42	72	T 4	Gunshot	1.3	91.93	21 Years 10 Months
7	M	27	80	T 11	Gunshot	0.88	91.15	5 Years 10 Months
8	M	44	68	T 12	Fall	1.43	89.62	6 Years 9 Months

Patient MRIs



acute case study (a-d); prior to administration (a), at 6 months (b) at 1 year (c) and approximately 2 years (d) after administration of BMSCs. Chronic case study (e-h);); prior to administration (e), at 6 months (f) at 1 year (g) and approximately 2 years (h) after administration of BMSCs

Neurological Evaluations

Case Study	Prior to Administration	6 Months after Administration	1 Year after Administration	2 Years after Administration
ASIA IMPAIRMENT GRADE/FRANKEL GRADE/ASHWORTH SCORE				
Acute				
Case 1	A/B/0	C/C/2	C/C/3	C/C/1
Case 2	A/A/3	A/C/1	A/C/1	C/C/2**
Case 3	A/A/0	ND	A/C/1	A/C/1
Case 4	A/A/0	C/C/1	C/C/1	C/C/1
Chronic				
Case 5	B/C/1	B/C/0	C/D/1	C/D/1
Case 6	C/D/3.5	C/D/3	D/D/3.5	D/D/ND
Case 7	A/A/0	C/C/1	C/C/1	C/C/1+*
Case 8	C/C/2	C/C/2	C/D/1	C/D/0
ND- Not done		*1 year 3 months		**1 year 6 months

ASIA Impairment Scale

A - complete: no preservation of function below level of injury, and no sacral sparing (S4-S5). **B** - incomplete: sensory but no motor function is preserved below the neurological level and includes the sacral segments S4-S5. **C** - incomplete: motor function is preserved below the neurological level, and more than half of key muscles below the neurological level have a muscle grade less than 3. **D** - incomplete: motor function is preserved below the neurological level, and at least half of key muscles below the neurological level have a grade of 3 or more. **E** - normal: motor and sensory function are normal

Frankel Scale

A -complete paralysis **B** -sensory function only below the injury level **C** -incomplete motor function below injury level **D** -fair to good motor function below injury level **E** -normal function

Modified Ashworth

0 no increase in tone

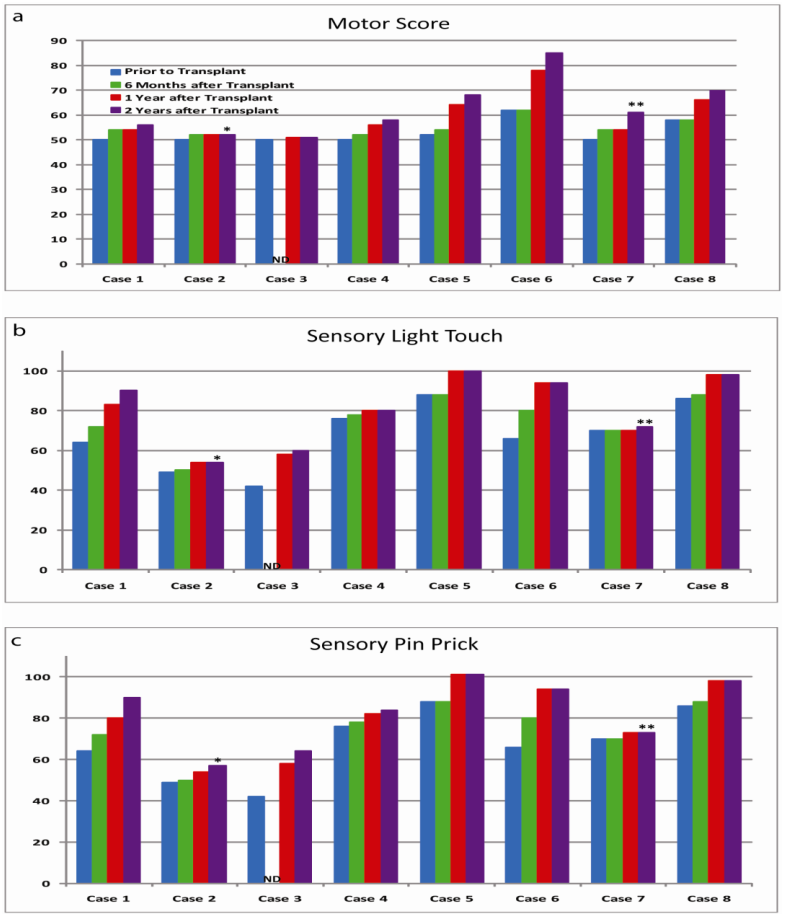
1 slight increase in muscle tone, manifested by a catch and release or minimal resistance at the end of the ROM when the affected part(s) is moved in flexion or extension

1+ slight increase in muscle tone, manifested by a catch, followed by minimal resistance throughout the remainder (less than half) of the ROM

2 more marked increase in muscle tone through most of the ROM, but affected part(s) easily moved

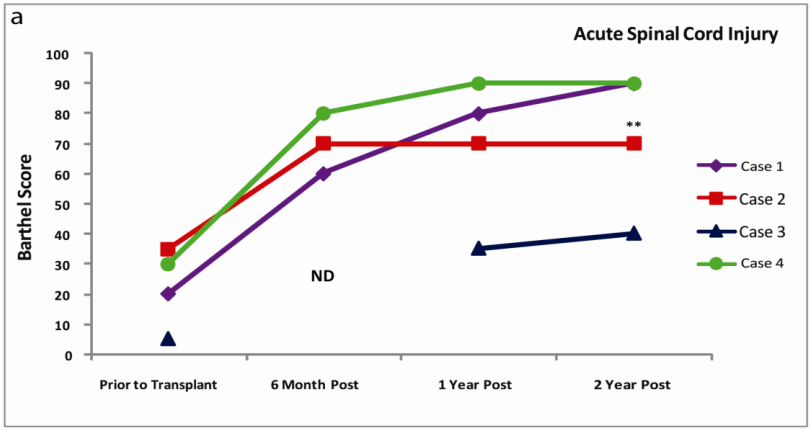
3 considerable increase in muscle tone, passive movement difficult

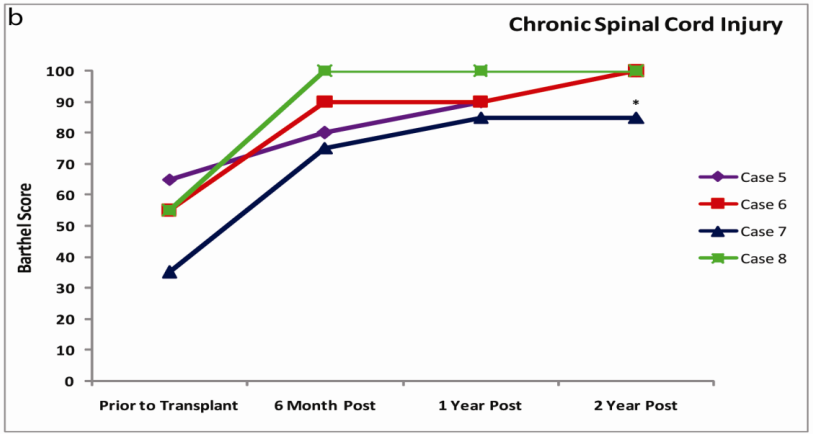
4 affected part(s) rigid in flexion or extension



Detailed ASIA motor and sensory scores for all 8 cases. Motor scoring: 0 = total paralysis, 1 = palpable or visible contraction, 2 = active movement, gravity eliminated, 3 = active movement against gravity, 4 = active movement against some resistance, and 5 = active movement against full resistance. Scores are accumulated from right and left sides and are based upon evaluating a total of 5 arm and 5 leg muscle groups (total of 100 points maximum). The ASIA sensory scoring is for light touch and pin prick; 0 = absent, 1 = impaired, and 2 = normal. There are 28 dermatomes assessed for a total of 112 possible points.

Quality of Life Score



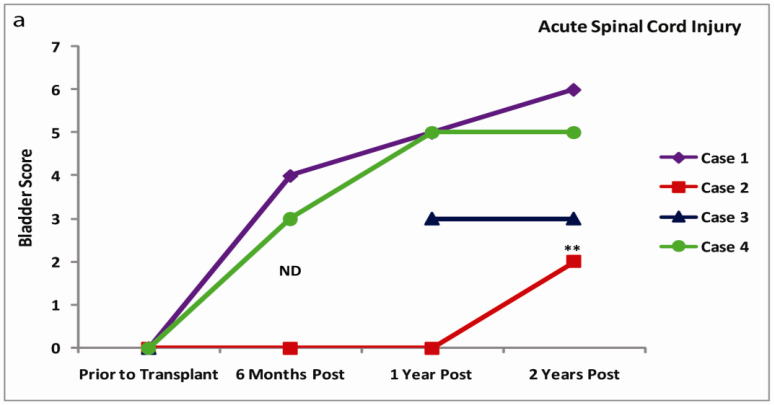


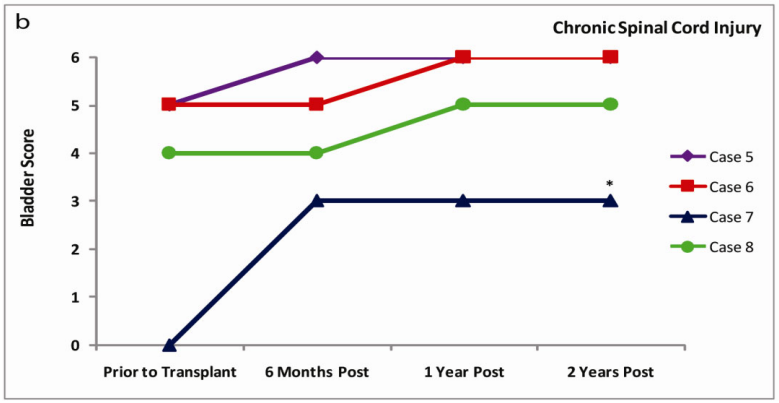
Barthel Score

Feeding - 0, 5, 10
Bathing - 0, 5
Grooming - 0, 5
Dressing - 0, 5, 10
Bowel - 0, 5, 10
Bladder - 0, 5, 10
Toilet use - 0, 5, 10
Transfers, bed to chair and back - 0, 5, 10, 15
Mobility on level surfaces - 0, 5, 10, 15
Stairs - 0, 5, 10

Maximum score of **100**

Bladder Function





Geffner, Gonzalez, Santacruz, and Flor (GGSF) Bladder Function Scale
SCALE

0. No urinary bladder sensation or function [a, b, c, d]

1. Patients with cystostomies that when are closed may involuntarily void through the urethra [a]

2. Bladder sensation or autonomic symptoms and inability to void [a, b, c, d]

3. Bladder sensation or autonomic symptoms and passive voiding (spontaneous release of urine) [a, b, c]

3.5. Patients with open cystostomies that have bladder sensation or autonomic symptoms and passively void through the urethra (spontaneous release of urine) [a]

4. Bladder sensation with incomplete voiding (needs catheterization to complete voiding) [b, c, d]

5. Bladder sensation with active ability to void; however no control while voiding

6. Complete bladder control

a. Patients use a suprapubic cystostomies in order to void their urinary bladder.
b. Patients use a catheter in order to void their urinary bladder
c. Patients with a urine collector
d. Patients that manually compress (massage) the hypogastric region in order to void their urinary bladder

Summary

- Transplantation of autologous BMSCs is safe
  1. Few adverse reactions in over 52 cases to date
  2. No tumor formations
  3. No increased pain
  4. No deterioration of function
- Transplantation of autologous BMSCs improves the quality of life for SCI patients
  1. Improves mobility & sensitivity
  2. Improves bladder function
  3. Improves overall quality of life

SCI and Stem cells Clinical Studies

- Presently 57 clinical studies (clinicaltrials.gov)
- Many completed but still lack results
- Many in early stages

Closing Thoughts

- Regenerative medicine holds great promise for neurodegenerative conditions
- Very complicated as many different cell types assessed and used
- Still in very early stages for most treatments
- More studies are needed
- Current regulatory environment does not permit these treatments without an IND government sanctioned trial

Questions?

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