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Alzheimer's Disease Treated with Autologous and Allogeneic Telomerase-Positive Stem Cells

Henry E. Young[1-3]* and Mark O. Speight[4-6]

[1]*Dragonfly Foundation for Research & Development, Macon, GA 31210 USA.*

[2]*Henry E Young PhD Regeneration Technologies LLC.*

[3]*Mercer University School of Medicine, Macon, GA 31207 USA.*

[4]*Research Designs, Charlotte, NC 28105 USA.*

[5]*The Charlotte Foundation for Molecular Medicine, Charlotte, NC 28105 USA.*

[6]*Center for Wellness, Charlotte, NC 28105 USA.*

***Correspondence:**

Henry E. Young PhD, Chief Science Officer, Dragonfly Foundation for Research & Development, 101 Preston Ct, Suite 101, (Corporate Office), Macon, GA 31210 USA, Tel: 1+478-319-1983, Fax: 1+478-743-2080.

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ABSTRACT

Alzheimer's disease is an insidious and progressive loss of balance and cognitive memories from present time to distant past that occurs in reverse chronological order. While the cause for AD is poorly understood, the presence of tau, aggregation of beta-amyloid protein, activated microglia, and massive losses of neurons and their synaptic processes have been associated with the disease. Genetics appear to play a major role in AD, but comorbidities intervene as well. There are five drug treatments approved to control the symptoms of the disease, but none were clinically proven to alter the course or decrease the risk for AD. Death eventually occurs, usually 3-9 years after initial diagnosis. Stem cells, e.g., embryonic stem cells, induced pluripotent stem cells; mesenchymal stem cells, medicinal signaling cells, and neural stem cells have been suggested as potential treatments for AD. While clinical trials demonstrated safety of administering some of these stem cells, none demonstrated any efficacy for reversing the symptoms of AD. We report the use of adult telomerase positive stem cells as a treatment modality for reversing the symptoms of AD. In a small cohort clinical trial (n=4), there were no adverse reactions reported for any individual treated. In addition, efficacy for telomerase positive stem cells approximated 50% for reduction in symptoms of Alzheimer's disease up to four months after their last telomerase positive stem cell treatment.

Keywords

Alzheimer's disease, Adult Stem Cells, Telomerase-positive, Totipotent Stem Cells, Pluripotent Stem Cells, Mesodermal Stem Cells, ESCs, iPSCs, MSCs, NSCs, Regenerative Medicine, Neurodegenerative diseases.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease predominantly affecting individuals age 65 and older. It was initially described by a German psychiatrist and pathologist, Alois Alzheimer in 1906. In 2015, AD resulted in almost 2 million deaths. By 2020 it was predicted that approximately 50 million people world-wide would have AD. Due to the aging world population, if treatments are not discovered to alleviate the disease, it is predicted that more than 150 million people will have AD by 2050. Alzheimer's disease continues to be one of the most-costly diseases in developed countries [1-7].

Alzheimer's disease is an insidious and progressive loss of cognitive memories from present time to distant past that occurs in reverse chronological order. Losing in reverse order what was learned from birth to onset of the disease. It usually begins with difficulty in remembering current events, then locations of

misplaced items, directions, recent friends, and relatives, learned behaviors, past friends and relatives, spouses, parents, taking care of oneself, language, ambulation, balance, etc. Throughout this reverse progressional loss of acquired memories, the patient with Alzheimer's disease demonstrates mood swings and behavioral issues, loss of balance, finally withdrawing from relatives and society. Eventually, death occurs, usually 3-9 years after initial diagnosis [8-10].

Alzheimer's disease is the cause of 60-70% of all diagnosed cases of dementia [10]. Usually, other potential causes of dementia should be assessed before final diagnosis of Alzheimer's disease is made. Other causes of dementia include systemic lupus erythematosus, vascular disease, Parkinson's disease, Syphilis, HIV, dementia with Lewy bodies, and Creutzfeldt-Jakob disease [10]. Potentially reversible causes of dementia include hypothyroidism, Vitamin B-12 deficiency, neurosyphilis, metabolic problems (kidney function, electrolyte levels and type-2 diabetes) and levels of heavy metals (e.g., lead, aluminum and mercury), hearing loss, Lyme disease, and anemia [11-18]. Methods used to diagnose AD include HPL, e.g., History of illness, Physical examination, and Laboratory tests, including cognitive testing (Mini Mental Status Examination, MMSE), blood tests, and medical imaging (Fig. 1) [8,19-23]. Proactive efforts to decrease risk factors include managing obesity, high blood pressure, diabetes Type-2, and smoking [17,18].

Although the cause of Alzheimer's diseases is poorly understood, four core features have been associated with the disease, e.g., (a) Tau becomes hyperphosphorylated, which leads to aggregation and formation of neurofibrillary tangles. b) There is sequential cleavage of the APP protein by two enzymes, b-secretase and g-secretase, that lead to beta-amyloid protein accumulation into plaques. c) The presence of activated microglia found in close association with the beta-amyloid protein plaques to produce cytokines, such as interleukin-1-beta (IL-1b), nitric oxide (NO), and tumor necrosis factor-alpha (TNF-a), all of which influence neuroinflammation. And d) which is followed by a massive loss of neurons and their synaptic connections [24-28].

Genetics appear to play a major role [29,30], but other factors intervene as well, such as comorbidities (head injuries, hypertension, hypotension, depression, heart failure, stroke, coronary artery disease), drugs, medications, supplements, diet, lifestyle, etc. [8,31]. Avoiding obesity and engaging in physical and mental exercise are thought by some to decrease the risk of AD [32]. There are only five pharmacological treatments approved to control the symptoms of the disease [7,25-28]. However, there are no known pharmacological treatments clinically proven to alter the course or decrease the risk for AD, although some pharmacological treatments may temporarily forestall the eventual outcome of the disease [7,25-28].

Stem cells in general have been suggested as a potential treatment for Alzheimer's disease [34-45]. More specifically, stem cells such as embryonic stem cells (ESCs) [46-50], induced pluripotent stem cells (iPSCs) [51-61], mesenchymal stem cells/medicinal signaling cells (MSCs) [62-95], and neuronal stem cells (NSCs) [96-105], have been proposed as potential agents to alleviate the signs and symptoms of Alzheimer's disease.

Embryonic stem cells (ESCs) can be derived from blastomeres of either the morula or the inner cell mass of the developing blastocyst. If ESCs are derived from blastomere of the morula they are classified as totipotent for their ability to form all cells of the embryo, derived from the embryonic layers of ectoderm, mesoderm, and endoderm, as well as the gametes and the nucleus pulposus of the intervertebral disc (the only adult derivative of the notochord). If ESCs are derived from blastomeres of the inner cell mass, they are classified as pluripotent for their ability to form all cells of the embryo, except the gametes and the nucleus pulposus. Induced pluripotent stem cells (iPSCs) are derived from mature somatic cells in vitro with the insertion of the Yamanaka factors, to become pluripotent and inner cell mass ESC-like in phenotypic expression and differentiation capabilities. Mesenchymal stem cells (MSCs) have been isolated from bone marrow, adipose tissue, umbilical cord blood, and Wharton's Jelly. They express CD105, CD117, CD123, and CD166 on their cell surface. They have been shown to form cartilage, fat, and bone, i.e., tissues of mesodermal origin. Medicinal signaling cells (MSCs) have been isolated from bone marrow, adipose tissue, Wharton's Jelly, and umbilical cord blood. They express CD73, CD90, and CD105 on their cell surface. They have shown the ability to secrete exosomes that influence immunomodulatory activity. Neural stem cells (NSCs) are responsible for the generation of neuronal cell types during development, e.g., neurons and glial cells. They are also present in discrete niches in the adult brain [46-105].

We offer an alternative stem cell to ESCs, iPSCs, MSCs/MSCs, and NSCs. This alternative stem cell is the use of naturally occurring adult-derived autologous and/or allogeneic telomerase-positive stem cells, e.g., totipotent stem cells (TSCs), pluripotent stem cells (PSCs), and mesodermal stem cells (MesoSCs), as potential treatment options for decreasing symptoms of Alzheimer's disease relating to the restoration of functional loss of cognitive memories [106-123,126-130,133,134]. In culture [106-109], in animal models [110-112], and in human models [111-114], TSCs and PSCs were shown to differentiate into neuronal cell types, replace damaged neuronal tissues, and restore function in the individual. MesoSCs have been shown to act in a supporting role to revascularize damaged tissues in cerebral cortex [111], cardiovascular disorders [114,115], and pulmonary disorders [116-118], and affect repair in osteoarthritis [119], and autoimmune disorders [120,121]. We therefore hypothesize that adult-derived telomerase positive stem cells would reverse the decline in MMSE scores for cognitive symptoms seen clinically in Alzheimer's disease.

Materials and Methods

Adult-derived autologous (TSCs, PSCS, and MesoSCs) or allogeneic (TSCs and PSCs) telomerase-positive stem cells were

utilized in an IRB-approved study protocol for neurodegenerative diseases. Inclusion criteria were any male or female, age 18 to 120, with diagnosed Alzheimer's dementia. Two females (ages 72 and 92) and two males (ages 58 and 75) were assessed in this ongoing clinical study. Cognitive decline was measured by the Mini-Mental Status Exam (MMSE, with 30 as the maximal score), with participants having an initial range of MMSE scores ranging from zero to 25 (Table 1). Neither female could walk without assistance, while the males were not tested for ambulation (Table 1). The 72-year-old female and the 58- and 75-year-old males received their own autologous telomerase-positive stem cells, while the 92-year-old female received one autologous and six allogeneic stem cell transplants (Table 1). Allogeneic stem cells for the 92-year-old's treatments were harvested from her identical twin daughters, both having the same blood type as their mother. Telomerase positive stem cell donors are routinely screened for gender (in this case, female), ABO-blood group (in this case, A-positive), and infectious diseases. Donors (the twin daughters) were also given the option to have their activated mesodermal stem cells returned to them [118].

Recipients of telomerase positive stem cell transplants and their respective donors were mandated to follow the informed consent guidelines for telomerase-positive stem cells for clinical therapy [122]. These guidelines consisted of a defined protocol to maximize the number of telomerase-positive stem cells for harvest and subsequent repair of the tissues, and included avoidance of alcohol, tobacco products, vaping, recreational drugs, lidocaine, and chemotherapeutic agents because they kill telomerase-positive stem cells; limit use of caffeine because it prevents differentiation of telomerase-positive stem cells; limit the use of corticosteroids because they prematurely induce a commitment of TSCs and PSCs into the mesodermal lineage. They were instructed to ingest combinatorial nutraceuticals (CN) (DFRD, Macon, GA) daily for a minimum of 30 days prior to initial harvest and then throughout subsequent treatments of recipient (and donor harvests) to increase proliferation of telomerase-positive stem cells within the person's own connective tissues, thus making the person their own bioreactor for stem cell proliferation. They were to drink plenty of fluids two weeks before stem cell harvest to remain hydrated to ease blood removal at harvest. Moderate to excessive exercising was excluded during a two-week window around stem cell harvest/treatment to maximize directed repair responses [122]. And 18 hours before stem cell harvest two glacial caps (GC, DFRD) were ingested to induce mobilization (reverse diapedesis) of the telomerase-positive stem cells into the blood stream.

Harvesting of telomerase-positive stem cells occurred using venipuncture, withdrawing 210 to 420cc's of blood, based on body weight of the individual. The telomerase-positive stem cells were separated from the blood cells utilizing 'FDA-mandated minimal manipulative procedures', utilizing zeta potential, gravity, and differential gradient centrifugation with serum, sterile saline, and sterile distilled water gradients. The stem cells were segregated into individual populations of TSCs, PSCs, and MesoSCs, and activated [112-121]. Time period for entire treatment period varied from ~24 (males) to ~72 hours (females) (Table 1). Allogeneic mesodermal stem cells from donors were not used due to their expression of self-recognition MHC Class-I molecules on their cell surface [123,124]. Since MHC Class-I molecules might induce a graft versus host disease (GvHD) response [125,126], it was felt that it was too great a risk for potential detriment to the recipient. Neither TSCs nor PSCs display either MHC Class-I or HLA-DR molecules on their cell surface [123,124] and therefore

Table 1: Treatment of Alzheimer's disease Participants with Telomerase Positive Stem Cells

Description	Male	Female	Male	Female
Age	58	72	75	92
Diagnosis	Alzheimer's Disease	Alzheimer's Disease	Alzheimer's Disease	Alzheimer's Disease
Initial Neuro: Mini Mental Status Exam, MMSE = 30 max	Cognitive Decline 0	Cognitive Decline 0	Cognitive Decline 25	Cognitive Decline 0
Initial Balance	ND	Walk with Help only	ND	Walk with Help only
Hours Needed for entire Treatment	~23	~72	~23	~72
Telomerase Positive Stem Cell Treatment(s)	Autologous	Autologous	Autologous	Autologous & Allogeneic
Months Between Treatments	2	NA	4	4-Auto 4-Allo
Number of Treatments	6	1	3	7 (1-Auto; 6-Allo)
Post Treatment(s) Neuro MMSE = 30 max	0, 1, 3, 0, 0, dropped	30, dropped	28,29, dropped	28,30,30,30,30 30,30, dropped
Balance	ND	Walk & Jog Without Help	ND	Walk & Jog Without Help
Adverse Events	None Reported	None Reported	None Reported	None Reported
Other Events	Increased heart function	None Reported	None Reported	None Reported

were utilized in the treatment protocol from the allogeneic donors [115,117,118,120,121].

The participants received one (autologous, 72-year-old female), three (autologous, 75-year-old male), six (autologous, 58-year-old male), and seven (one autologous and six allogeneic, 92-year-old) telomerase positive stem cell transplants (Table 1). The TSCs, either autologous or allogeneic, were delivered by intranasal infusion for neurogenic treatment. The cells were concentrated in 0.5cc's of 0.9% sterile saline and split into two equal populations of 0.25cc's each. The recipient was instructed to wash the mucus from their nostrils with 0.65% sterile saline, after which they were placed into the reversed Trendelenburg position. Each nostril received an aliquot of 0.25cc's concentrated TSCs, placed dropwise onto the olfactory epithelium in the superior meatus of the nose. The recipient remained in the reverse Trendelenburg position for five minutes and then placed in the upright position. Pooled autologous MesoSCs and PSCs or allogeneic PSCs only were diluted in 250cc's of normal sterile heparin/saline for regular intravenous infusion into an accessible vein, preferably the median cubital vein [111-121].

The Mini-Mental Status Examination, MMSE [20-23] was used to measure the severity and progression of cognitive impairment and to track changes over time. The MMSE uses a 30-point scale ($0 \leq 9$, severe impairment; 10 – 18, moderate impairment; 19 – 23, mild impairment; 24 – 30, normal cognition), tests for complex commands (drawing a clock face), attention (spelling "world" backwards), calculation (serial "sevens"), orientation to place, orientation to time, repeating name prompts, recall, naming an object (pencil or watch), and repeating a phrase to measure cognition [19-23]. MMSE was used to measure improvement and/or deterioration of cognitive impairment in participants before, during, and after cessation of treatment with telomerase positive adult stem cells.

Results

All participants demonstrated cognitive impairment before the study began. Their initial MMSE scores were 0/30 (58-year-old male, 72- and 92-year-old females) and 25/30 (75-year-old male) (Table 1). Prior to their respective stem cell treatments, the two females (age 72 and age 92) displayed balance problems, i.e., difficulty in walking without assistance. They could not draw a clockface; they could not calculate serial "sevens"; they did not know where they were (stem cell clinic) or why they were there (stem cell treatment), they did not recognize who they were with (72-year-old could not recognize her husband, her stepson, or her stepson's wife; the 92-year-old could not recognize her identical twin daughters); they did not know the year, the month, or the day (name or date); they did not know what had happened within the last day, last week, last month, last year, or the current President of the United States.

All participants demonstrated some increase in cognitive function, as assessed by their respective MMSE scores, e.g., 3/30 for 58-year-old male, 29/30 for 75-year-old male, and 30/30 for the 72-year-old female and 30/30 for 92-year-old females (Table 1), at least through their first three adult telomerase positive stem cell treatments (both males). The females demonstrated a more striking response to their adult telomerase positive stem cell treatment(s). The day after treatment and for the next 4 months following treatment, both females could walk (and jog) without assistance (regain of balance). They could draw a recognizable clockface. They could calculate serial "sevens" (72-year-old) and serial "ones", "threes", "fives", "sevens", "nines", "11's", and "13's" (92-year-old). They knew they were visiting a stem cell clinic and they knew they were there for either assessment and/or continued treatments. They recognized who they were with (72-year-old, her husband, stepson, and stepson's wife; and 92-year-old, her identical twin daughters by name, stem cell physician, and stem cell isolator). They knew the date, day of the week, month, and year. They knew current events for the past week, month, and year. They knew the current President of the United States. Unfortunately, once stem cell treatments ceased, and approximately four months after their last treatment, they reverted to displaying similar Alzheimer's symptoms that they had had before their respective treatments began. No adverse events were reported during the time course of the study for any participant, although there was an increase in heart function reported for one of the participants (Table 1).

Discussion

Alzheimer's disease is one of the most common causes of dementia. It is characterized by the progressive loss of cognitive memories from present time to distant past that occurs in reverse chronological order of acquisition. Current pharmacological therapies for Alzheimer's disease only alleviate the symptoms for a short period of time, but do not forestall the eventual outcome of the disease. There is still a lack of an effective treatment for the disease [34].

Due to multiple chronic and terminal diseases with no known cure and only palliative treatments at best, stem cells have become the "holy grail" for regenerative medicine [35-45]. With respect to which stem cell type is preferred for treating neurodegenerative diseases, such as Alzheimer's disease, five stem cell types have been proposed, e.g., embryonic stem cells, induced pluripotent stem cells, mesenchymal stem cells, medicinal signaling cells, and neural stem cells. Models of Alzheimer's disease using embryonic stem cells, induced pluripotent stem cells, mesenchymal stem cells, medicinal signaling cells, or neural stem cells have demonstrated enhanced self-renewal capabilities, multi-directional differentiation capabilities, neurotrophic properties, immunoregulation, immunomodulatory effects, and no immunological rejection. However, efficacy for reversal of Alzheimer's disease symptoms have not been realized [46-105].

Embryonic Stem Cells (ESCs)

While use of embryonic stem cells in rodent models of brain injury has shown a restoration of functional activity, their use in humans has been limited. When undifferentiated ESCs are

transplanted into an individual there is the formation of teratomas. To circumvent teratoma formation, ESCs are pre-induced in culture into NSCs and then into cholinergic neurons that showed improvements in spatial memory when transplanted into a rodent model of AD and humans with AD. However, besides the moral and ethical issues surrounding the use of embryonic stem cells for human transplantation therapies, there are still potential problems associated with immunosuppressive regimens and graft versus host disease response when utilizing allogeneic donor cells [46-50].

Induced Pluripotent Stem Cells (iPSCs)

Induced pluripotent stem cell technologies allow for the generation of autologous pluripotent stem cells, avoiding moral and ethical issues of using ESC-like stem cells as well as immune related issues of immunosuppressant use and graft versus host disease response when utilizing allogeneic donor cells. However, similar problems when ESC transplantation occurs, i.e., teratoma formation and potential for GvHD response, when allogeneic iPSCs are implanted into an individual in the naïve state. The potential for using autologous iPSCs induced before transplantation has been investigated and found to be promising for production of numerous tissues in vitro and in vivo. IPSC-generated and induced neurons are structurally and functionally mature and capable forming active synaptic networks. Using additional inductive agents in culture it is possible to direct iPSCs into selective neuronal subtypes. However, human iPSCs-induced neurons generated from patients with Alzheimer's disease display multiple neuropathies, such as elevated tau phosphorylation, abnormal amyloid-beta levels, reduced neurite length, and altered electrophysiology, making them of limited value for use as neuronal cell replacements in AD [51-61].

Mesenchymal Stem Cells/Medicinal Signaling Cells (MSCs)

In 2015, US-FDA granted IND status for a phase-2A clinical trial of MSCs to treat AD in USA. Two phase I clinical trials of moderate AD using mesenchymal stem cell transplantation have been completed. Although the studies revealed no adverse severe acute or long-term side effects to mesenchymal stem cell transplantation therapy, no significant clinical efficacy was observed [56]. The exact mechanism for MSC activity on neuronal tissue has not been elucidated thus far. It has been theorized that the resulting effects, e.g., anti-inflammatory, angiogenic stimulation, antiapoptotic, neurogenesis, neuronal cell apoptosis, immunomodulation, and immunoregulation to prevent immunological rejection would be delivered through their paracrine cytokines and neurotrophic factors within secreted exosome vesicles. Adipose-derived MSCs from the stromal vascular fraction has gained interest due to its ability to secrete paracrine factors via exosomes that accelerate endogenous repair, their ease of accessibility, and lack of identified major adverse events. Recent studies indicate that MSC's paracrine effect could prove beneficial to patients with neurological diseases, including those with Alzheimer's disease. MSC exosomes are 30-120 nm membrane bound packets of microRNAs and protein, which play an important role in their paracrine effects. MicroRNA (miR) controls the translation and stability of mRNAs after transcription and is an integral component of an exosome. A specific microRNA, designated a miR-223, found in MSC exosomes, has been suggested as a potential predictor of neuronal protection, by limiting inflammation surrounding neurons. To test that and other hypotheses, an AD culture model system was developed in which various disease pathways and treatment regimens could be examined. The AD culture model demonstrated an increase in HIF-1a expression, impaired cell migration, a reduction in miR-223, and increased cell apoptosis. Addition of serum exosomes isolated from AD patients induced neuronal cell apoptosis in the AD culture model system. Utilizing miR-223 derived MSC exosomes in the AD culture model system, a protection of neuronal cells from apoptosis was demonstrated, suggesting a potential route of treatment. MSC exosomes containing miR-223 could thus mediate neuronal protection and nerve regeneration, potentially slowing down or reversing the effects of AD [62-95].

Neural Stem Cells (NSCs)

Transplantation of neural stem cells to both repair and protect upper and lower motor neurons from inflammation and degeneration are being investigated. The transplanted neural stem cells exert their paracrine effect on the surrounding tissues by releasing neurotrophic factors, immunomodulatory molecules, and replacement of damaged cells. Transplantation of human NSCs over-expressing choline acetyltransferase into a cholinergic rodent model resulted in reversal of learning deficits and spatial memory. Transplantation of growth factor secreting NSCs increased neurogenesis and cognitive function in an aged primate brain and a rodent model of Alzheimer's disease. Other rodent model studies demonstrated that NSC transplantation decreased tau neuropathology, beta-amyloid neuropathy, neuro inflammation; promoted synaptogenesis and neurogenesis; and caused a reversal of cognitive deficits. Unfortunately, their limited engraftment success and survivability is due to the hostile environment in which these stem cells are being transplanted. To circumvent these problems, new technologies are being devised, including genetic engineering, biopolymer scaffolds, and environmental stress preconditioning [96-105].

Telomerase-Positive Stem Cells

Naturally occurring adult endogenous telomerase positive stem cells have been located throughout the connective tissue niches of an individual from newborn to geriatric-aged individuals (94 years-of-age, thus far). These stem cells include totipotent stem cells, pluripotent stem cells, ectodermal stem cells, mesodermal stem cells and endodermal stem cells. These telomerase positive stem cells were isolated and cloned from single cells from multiple species of animals using a process termed repetitive single cell clonogenic analysis. A series of characterization studies in animals and humans were performed to determine unique qualities associated with each telomerase positive stem cell type utilizing both single cell-derived clones and cells derived by cell sorting of unique cell surface markers [109].

Totipotent stem cells (TSCs) are 0.1-2 microns in size. TSCs are Trypan blue positive. TSCs express carcinoembryonic antigen-cell adhesion molecule-1 (CEA-CAM-1) and CD66e cell surface

markers. Optimal freezing and storage temperature for TSCs is -80°C. TSCs either grow in cell suspension or require a type-I collagen substratum for attachment. Their default state in culture is quiescence without the presence of an anti-differentiation factor, such as leukemia inhibitory factor (LIF). They are unresponsive to a progression agent, such as insulin. They will respond to a proliferation inducing agent, with a cell doubling rate of 12-14 hours and form multiple confluent layers of cells. They have been propagated past 300 population doublings while maintaining their karyotype and differentiation potential. They will respond to inductive factors, either as human recombinant proteins or tissue-specific exosomes. Three separate clones demonstrated capabilities to form 66 separate and distinct cell types, e.g., downstream telomerase positive stem cells (HLSCs, CLSCs, ELSCs, GLSCs, EctoSCs, MesoSCs, EndoSCs) and differentiated cell types from ectoderm, mesoderm, and endoderm, spermatogonia, and nucleus pulposus of the intervertebral disc [108,109,129,130].

Pluripotent stem cells (PSCs) are >2 and <10 microns in size. They can be divided into four separate subgroups based on size, cell surface markers, and differentiation potential. Halo-like stem cells (HLSCs) are >2 to 4 microns in size. They demonstrate a solid halo of Trypan blue staining along their periphery, with absence of staining in the center portion of the cell. They express low levels of stage-specific embryonic antigen-4 (SSEA-4) and neutral endopeptidase (CD10) on their cell surface. An optimal freezing and storage temperature for HLSCs is -80°C. HLSCs require a type-I collagen substratum for cell attachment and growth. Their default state in culture is quiescence without the presence of an anti-differentiation factor, such as leukemia inhibitory factor (LIF). They are unresponsive to a progression agent, such as insulin. They will respond to a proliferation inducing agent, with a cell doubling rate of 12-14 hours and form multiple confluent layers of cells. They will respond to inductive factors, either as human recombinant proteins or tissue-specific exosomes. A clone of HLSCs demonstrated capabilities of forming 65 separate and distinct cell types, e.g., downstream telomerase positive stem cells (CLSCs, ELSCs, GLSCs, EctoSCs, MesoSCs and EndoSCs) and differentiated cell types from ectoderm, mesoderm, and endoderm, but would not form either spermatogonia or the nucleus pulposus of the intervertebral disc [106-110,124,127-130].

Corona-like stem cells (CLSCs) are >4 to <6 microns in size. They demonstrate a crown of Trypan blue staining along one side of their periphery, with absence of staining in the center portion of the cell. They express moderate levels of SSEA-4 and CD10 on their cell surface. An optimal freezing and storage temperature for CLSCs is -80°C. CLSCs require a type-I collagen substratum for cell attachment and growth. Their default state in culture is quiescence without the presence of an anti-differentiation factor, such as leukemia inhibitory factor (LIF). They are unresponsive to a progression agent, such as insulin. They will respond to a proliferation inducing agent, with a cell doubling rate of 12-14 hours and form multiple confluent layers of cells. They will respond to inductive factors, either as human recombinant proteins or tissue-specific exosomes. A clone of CLSCs demonstrated capabilities of forming 64 separate and distinct cell types, e.g., downstream telomerase positive stem cells (ELSCs, GLSCs, EctoSCs, MesoSCs and EndoSCs) and differentiated cell types from ectoderm, mesoderm, and endoderm, but would not form either spermatogonia or the nucleus pulposus of the intervertebral disc [109].

Epiblast-like stem cells (ELSCs) are 6 to 8 microns in size. They demonstrate complete absence of Trypan blue staining. They express high levels of SSEA-4 and CD10 on their cell surface. An optimal freezing and storage temperature for ELSCs is -80°C. ELSCs require a type-I collagen substratum for cell attachment and growth. Their default state in culture is quiescence without the presence of an anti-differentiation factor, such as leukemia inhibitory factor (LIF). They are unresponsive to a progression agent, such as insulin. They will respond to a proliferation inducing agent, with a cell doubling rate of 12-14 hours and form multiple confluent layers of cells. They have been propagated past 400 population doublings while maintaining their karyotype and differentiation potential. They will respond to inductive factors, either as human recombinant proteins or tissue-specific exosomes. A clone of ELSCs demonstrated capabilities of forming 63 separate and distinct cell types, e.g., downstream telomerase positive stem cells (GLSCs, EctoSCs, MesoSCs, EndoSCs) and differentiated cell types from ectoderm, mesoderm, and endoderm, but would not form either spermatogonia or the nucleus pulposus of the intervertebral disc [109].

Germ layer stem cells (GLSCs) are > 8 to 10 microns in size. They demonstrate complete absence of Trypan blue staining. They express moderate levels of SSEA-4, CD10, and CD90 on their cell surface. An optimal freezing and storage temperature for GLSCs is -80°C. GLSCs require a type-I collagen substratum for cell attachment and growth. Their default state in culture is quiescence without the presence of an anti-differentiation factor, such as leukemia inhibitory factor (LIF). They are unresponsive to a progression agent, such as insulin. They will respond to a proliferation inducing agent, with a cell doubling rate of 12-14 hours and form multiple confluent layers of cells. They will respond to inductive factors, either as human recombinant proteins or tissue-specific exosomes. A clone of GLSCs demonstrated capabilities of forming 62 separate and distinct cell types, e.g., downstream telomerase positive stem cells (EctoSCs, MesoSCs and EndoSCs) and differentiated cell types from ectoderm, mesoderm, and endoderm. They could not be induced to form either spermatogonia or the nucleus pulposus of the intervertebral disc [109].

Ectodermal stem cells (EctoSCs) are > 10 to 12 microns in size. They demonstrate complete absence of Trypan blue staining. They express CD56 and CD90 on their cell surface. An optimal freezing and storage temperature for EctoSCs is -70°C. EctoSCs require a type-I collagen substratum for cell attachment and growth. Their default state in culture is quiescence without the presence of an anti-differentiation factor, such as leukemia inhibitory factor (LIF). They are unresponsive to a progression agent, such as insulin. They will respond to a proliferation inducing agent, with a

cell doubling rate of 18-24 hours and form a single layer of cells at confluence, at which point they enter a quiescent state of activity. They will respond to ectodermal-specific inductive factors, either as human recombinant proteins or tissue-specific exosomes. However, they will not respond to inductive agents outside their ectodermal lineage. A clone of EctoSCs demonstrated capabilities of forming keratinocytes, pyramidal neurons, dopaminergic neurons, interneurons, motor neurons, sensory neurons, astrocytes, radial glial cells, oligodendrocytes, Schwann cells, melanocytes, dorsal root ganglion cells, autonomic ganglion cells, pituitary, adrenal medulla. They could not be induced to form any cells of the mesodermal or endodermal lineages [109].

Mesodermal stem cells (MesoSCs) are > 10 to 12 microns in size. They demonstrate complete absence of Trypan blue staining. They express CD13 and CD90 on their cell surface. An optimal freezing and storage temperature for MesoSCs is -70°C. MesoSCs require a type-I collagen substratum for cell attachment and growth. Their default state in culture is quiescence without the presence of an anti-differentiation factor, such as leukemia inhibitory factor (LIF). They are unresponsive to a progression agent, such as insulin. They will respond to a proliferation inducing agent, with a cell doubling rate of 18-24 hours and form a single layer of cells at confluence, at which point they enter a quiescent state of activity. They have been propagated past 690 population doublings while maintaining their karyotype and differentiation potential. They will respond to mesodermal-specific inductive factors, either as human recombinant proteins or tissue-specific exosomes. However, they will not respond to inductive agents outside their mesodermal lineage. A clone of MesoSCs demonstrated capabilities of forming skeletal muscle, cardiac muscle, smooth muscle, unilocular white fat, multilocular brown fat, hyaline cartilage, articular cartilage growth plate cartilage, elastic cartilage, fibrocartilage, compact cortical bone, spongy cancellous bone, dermis, loose fibrous connective tissue, dense fibrous connective tissue, tendon, ligament, organ capsules, trabeculae, scar tissue, endothelia of blood vessels and lymphatic vessels, RBCs, WBCs, spleen, kidney, ureter, urinary bladder, etc. They could not be induced to form any cells of the ectodermal or endodermal lineages. [106-109,111,123,124,127,130,133].

Endodermal stem cells (EndoSCs) are > 10 to 12 microns in size. They demonstrate complete absence of Trypan blue staining. They express CD90 on their cell surface. An optimal freezing and storage temperature for EndoSCs is -70°C. EndoSCs require a type-I collagen substratum for cell attachment and growth. Their default state in culture is quiescence without the presence of an anti-differentiation factor, such as leukemia inhibitory factor (LIF). They are unresponsive to a progression agent, such as insulin. They will respond to a proliferation inducing agent, with a cell doubling rate of 18-24 hours and form a single layer of cells at confluence, at which point they enter a quiescent state of activity. They will respond to endodermal-specific inductive factors, either as human recombinant proteins or tissue-specific exosomes. However, they will not respond to inductive agents outside their endodermal lineage. A clone of EndoSCs demonstrated capabilities of forming lining cells of the lungs, gall bladder, stomach, intestines, rectum; hepatocytes, oval cells and biliary cells of the liver; pancreatic exocrine cells, ductal cells, alpha-cells, beta-cells, and delta-cells; parathyroid, thyroid, etc. They could not be induced to form any cells of the ectodermal or mesodermal lineages. [109].

Telomerase Negative Progenitor Stem Cells

During the characterization studies [109] a mesenchymal stem cell clone, as a representative of a telomerase negative progenitor stem cell, was also cloned from a single cell using repetitive single cell clonogenic analysis. The mesenchymal stem cell (MSC) clone demonstrated the following characteristics. The cells were 15-20 microns in size in the unfixed state as measured by flow cytometry. They demonstrated complete absence of Trypan blue staining. An optimal freezing and storage temperature for MSCs was -196°C (liquid nitrogen). MSCs do not require a substratum for attachment because they synthesize and secrete the own type-I collagen substratum for cell attachment and growth. Their default state in culture is quiescence without the presence of an anti-differentiation factor, such as leukemia inhibitory factor (LIF). They are responsive to a progression agent, such as insulin. They will respond to a proliferation inducing agent, with a cell doubling rate of 24-48 hours and display contact inhibition at confluence. They will respond to only MSC-specific inductive factors for fat, cartilage, or bone. They are unresponsive to all other inductive factors, either as chemical compounds, human recombinant proteins or tissue-specific exosomes, tested in the characterization studies. A clone of MSCs demonstrated capabilities of forming three differentiated cell types, e.g., unilocular white fat, hyaline cartilage, and compact cortical bone. They could not be induced to form any other differentiated cell types [109].

Telomerase Negative Progenitor Stem Cell versus Telomerase Positive Stem Cells for Neurogenic Treatments

It appears that the differentiative capabilities of telomerase-negative mesenchymal progenitor stem cells have been confused with telomerase-positive totipotent stem cells and pluripotent stem cells, by investigators claiming that "mesenchymal stem cells (MSCs) can differentiate into not only cells of mesodermal lineages, but also transdifferentiate into endodermal and ectodermal derived elements, including neurons and glial cells" [75,82]. This was found not to be the case by us and others. In the characterization studies [109], utilizing conditioned medium with repetitive single cell clonogenic analysis-derived clones of telomerase-positive totipotent stem cells, pluripotent stem cells, mesodermal stem cells, and a clone of telomerase-negative mesenchymal stem cells, were generated by repetitive single cell serial dilution clonogenic analysis. The results from those studies corresponded to the results from Pittenger et al [63] which showed that the original stem cell population termed by Caplan as a 'mesenchymal stem cell' [62] consisted of a telomerase-negative tripotent progenitor stem cell capable of only forming cartilage, fat, and bone. In our hands, only telomerase-positive totipotent stem cells, pluripotent stem cells, and ectodermal stem cells could be induced to form cells of the neuronal lineage, e.g., neurons, astrocytes, oligodendrocytes, and radial glial cells [106-113,124,127-130].

Mesenchymal stem cells have been utilized in clinical trials for the treatment of Parkinson disease (PD) [131,132]. Due to the inherent size of the MSCs and utilizing an intranasal approach for the intracerebroventricular route of delivery of MSCs [83], there was a necessity of using a hyperosmotic solution of mannitol to shrink the olfactory epithelium to a sufficient extent to allow migration of the MSCs between the cells to gain access to the brain. Following treatment, the olfactory epithelium would swell to their normal shape and size and the normal histoarchitecture of the olfactory epithelium was restored. However, for older-aged individuals (i.e., post puberty), such as those with Alzheimer's disease, two or more treatments with the hyperosmolar mannitol solution created permanent channels forming direct conduits to the meninges. This enabled the increased propensity for bacterial meningitis in these individuals [83,131,132].

A Lac-Z genomically labeled clone of pluripotent stem cells, Scl-40b [128] was stereotactically injected through the cerebral cortex into the substania nigra of the midbrain in adult rats that had been previously injected with a dopaminergic neurotoxin (6-hydroxydopamine) killing dopaminergic neurons, and thus creating a Parkinson disease model [110-112]. Besides regenerating dopaminergic neurons in the substantia nigra and their associated neural networks, the genomically-labeled pluripotent stem cell clone also regenerated cells that were damaged in the cerebral cortex, e.g., pyramidal neurons, interneurons, glial cells, and endothelial-lined capillaries (Figures 2,3) [110,111].

An IRB-approved clinical trial was undertaken to assess the activity of telomerase positive stem cells for the treatment of Parkinson disease. Ten people started clinical trial, eight completed trial through the 14-month time-period. Before trial began, starting range of Hoehn-Yahr (H-Y) scores was between 8.5-6.0. At 1-month post-treatment, 4 showed H-Y range of 4.0-2.0, while 4 showed H-Y score of 1.0. At 7th month follow-up, 2 began to regress with H-Y score of 5.0; 4 stabilized at 4.0-1.0; and 2 improved to 0.75. At 14th month follow-up, same 2 individuals continue to regress with H-Y score of 5.5, but their scores were lower than when they started the trial. Same four that were stable at 7th month follow-up were stable at 14th month follow-up with H-Y scores of 4.0-1.0. The two individuals that showed improvement in their symptoms at 7th month follow-up continue to improve at the 14th month follow-up, with H-Y score of 0.5 (Figures 4,5) [111,112].

In the current small cohort (n=4) trial, four diagnosed Alzheimer's patients were treated with autologous and/or allogeneic telomerase positive stem cells (Table 1). There were two females, ages 72 and 92, and two males, ages 58 and 75. Treatment consisted of 18 hours before blood harvest to ingest glacial caps to mobilize telomerase positive stem cells into the blood stream. Then at harvest removing 400-ml of blood/stem cells, separating telomerase positive stem cells from blood cells by gravity/zeta potential and/or differential density gradient centrifugation with serum, saline, and sterile water. The telomerase positive stem cells were segregated into three populations, e.g., totipotent stem cells, pluripotent stem cells, and mesodermal stem cells and activated. The totipotent stem cells were given by intranasal infusion after the nostrils were washed with 0.65% sterile saline to remove the mucus. The patient was rotated to the reverse Trendelenburg position and the TSCs applied dropwise to the olfactory mucosa in the superior meatus of the nose. For participants receiving autologous stem cell transplants, both pluripotent stem cells and mesodermal stem cells were combined and infused through the median cubital vein. The 92-year-old female participant (age range of 92 to 94-years-of-age during her seven-telomerase positive stem cell treatments) received one autologous and six allogeneic stem cell transplants. Only the allogeneic pluripotent stem cells from her donors were infused through the median cubital vein.

Mini-Mental Status Examination, MMSE, was used to measure the severity and progression of cognitive impairment and to

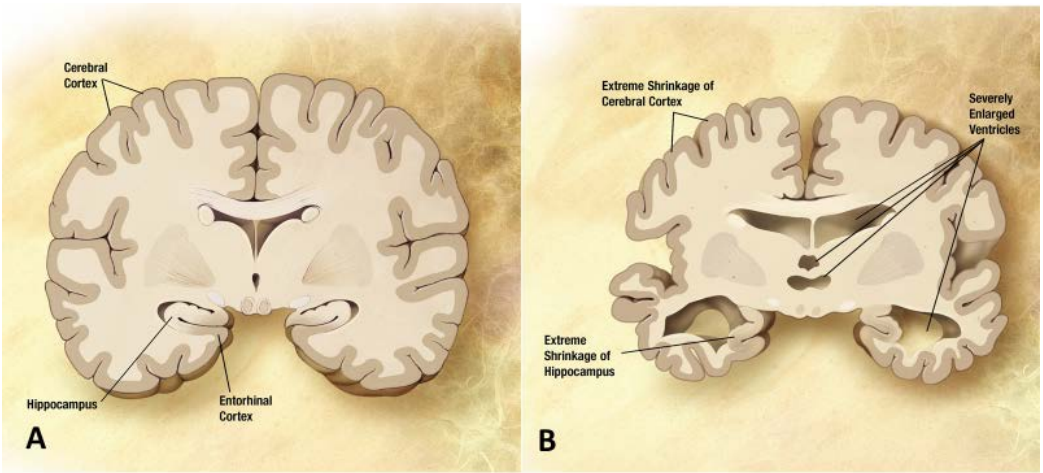

Figure 1: Note differences, comparing cross section of normal brain (Fig. 1A) to Alzheimer's brain (Figure 1B), with to respect to shrinkage of cerebral cortex, shrinkage of hippocampus, and enlargement of ventricles [11]. Reprinted with permission from derivative work: Garrondo (talk), 2008, Preclinicalslice_High.Jpg and Severslice_High.Jpg: Adear: "Alzheimer's Disease Education and Referral Center, a service of the National Institute of Aging [46].

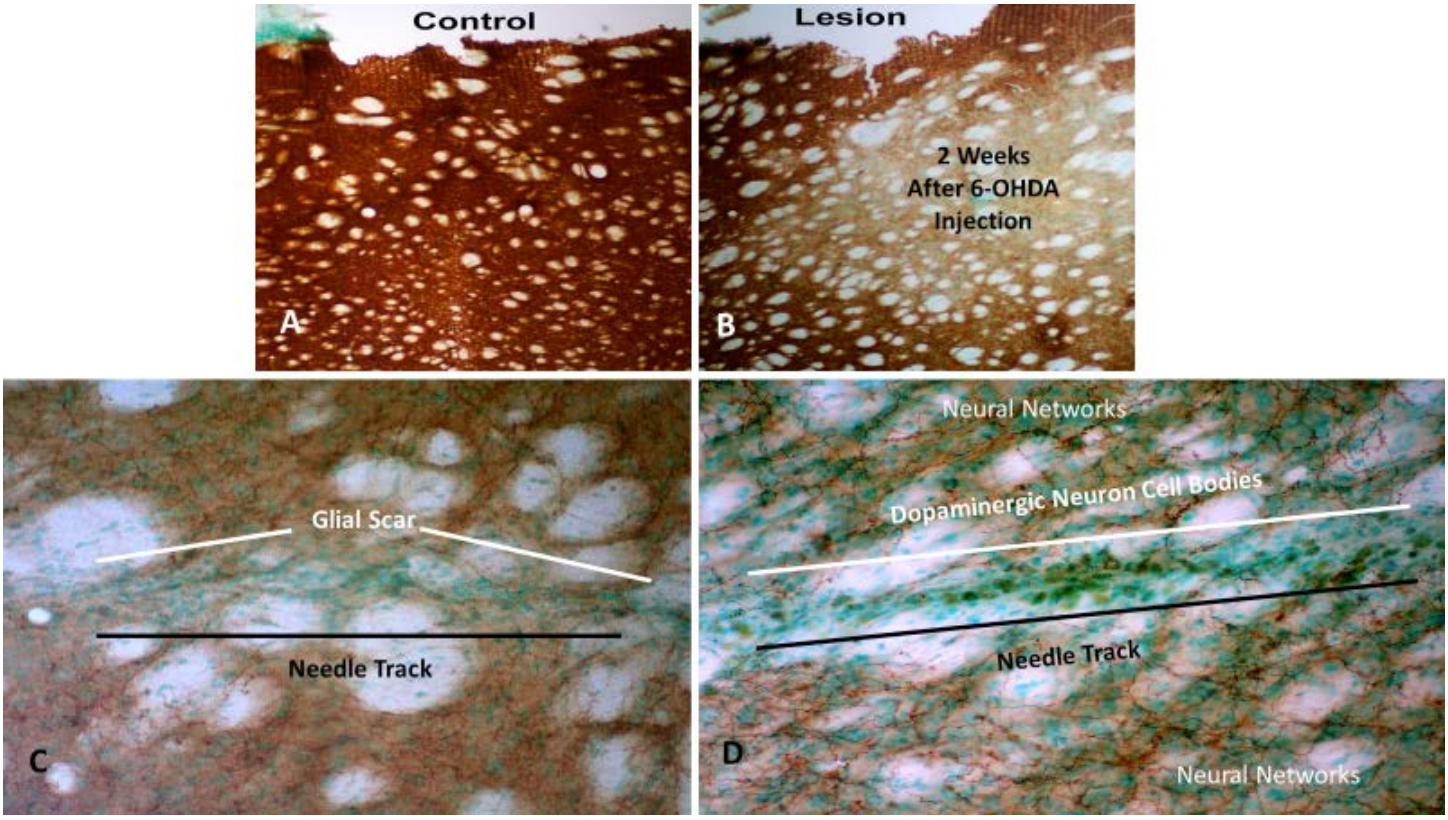

Figure 2: Animal model of Parkinson's disease created by stereotactic injection of neurotoxin 6-hydroxydopamine (6-OHDA) into substantia nigra of midbrain of adult rats. Wait two weeks to induce lesion, then stereotactically inject with either saline only or saline with genomically-labeled pluripotent stem cell clone, Scl-40b. Six weeks following stereotactic injections of saline only or saline with pluripotent stem cells, all animals were euthanized, brains removed, incubated with ELICA fixative, processed from cryosectioning, and incubated with antibody to tyrosine hydroxylase, key enzyme in pathway for production of neurotransmitter dopamine. Tissue was counterstained with methyl green [109,110]. A. Unoperated controls. Note tyrosine hydroxylase activity throughout all cells in region, difficult to distinguish neural networks from neuron cell bodies. B. Representative experimental animals two weeks after stereotactic injection of 6-OHDA. Note absence of tyrosine hydroxylase activity in central lesioned area. C. Experimental animals stereotactically injected with saline only into lesioned area within substantia nigra. Note glial scar (green-stained) within needle track, loss of dopaminergic neurons, and a decrease in definitive neural networks. D. Experimental animals stereotactically injected with saline containing genomically-labeled pluripotent stem cell clone Scl-40b into lesioned area within substantia nigra. Note neuronal cell bodies (presumptive dopaminergic neurons) positive for tyrosine hydroxylase activity within needle track and more definitive neural networks within lesioned area. Reprinted with permission from Young HE, Hyer L, Black AC Jr, et al, 2013 [111].

track changes over time [20-23]. It tests for complex commands (drawing a clock face), attention (spelling "world" backwards), calculation (serial "sevens"), orientation to place, orientation to time, repeating name prompts, recall, naming an object (pencil or watch), and repeating a phrase. The MMSE uses a 30-point scale to measure cognitive impairment, i.e., $0 \leq 9$, severe impairment; 10 – 18, moderate impairment; 19 – 23, mild impairment; and 24 – 30, normal cognition. MMSE was used to measure improvement and/or deterioration of cognitive impairment in participants before, during, and after treatment with telomerase positive adult stem cells.

Prior to stem cell treatment, the two females (ages 72 and 92) displayed difficulty in walking without assistance. Their initial MMSE scores were 0/30. They could not draw a clockface; they could not calculate serial "sevens"; they did not know where they were (stem cell clinic) or why they were there (stem cell treatment), they did not recognize who they were with (72-year-old: husband, stepson, or stepson's wife; 92-year-old: twin daughters); they did not know the year, the month, or the day (name or date); they did not know what had happened during the previous day, the previous week, previous month, previous year, or the sitting President of the United States.

The telomerase positive stem cells for both females were isolated using gravity/zeta potential and differential density gradient separation with serum, saline, and distilled water. The ex vivo activated TSCs were given by intranasal infusion and the ex vivo activated MesoSCs (autologous only) and/or PSCs (autologous and allogeneic) were given by intravenous infusion, requiring about 72 hours, from ingestion of glacial caps to intranasal and IV infusion of stem cells.

The 72-year-old female received a single autologous stem cell transplant. The 92-year-old female received one autologous and six allogeneic stem cell treatments. The day after treatment and for

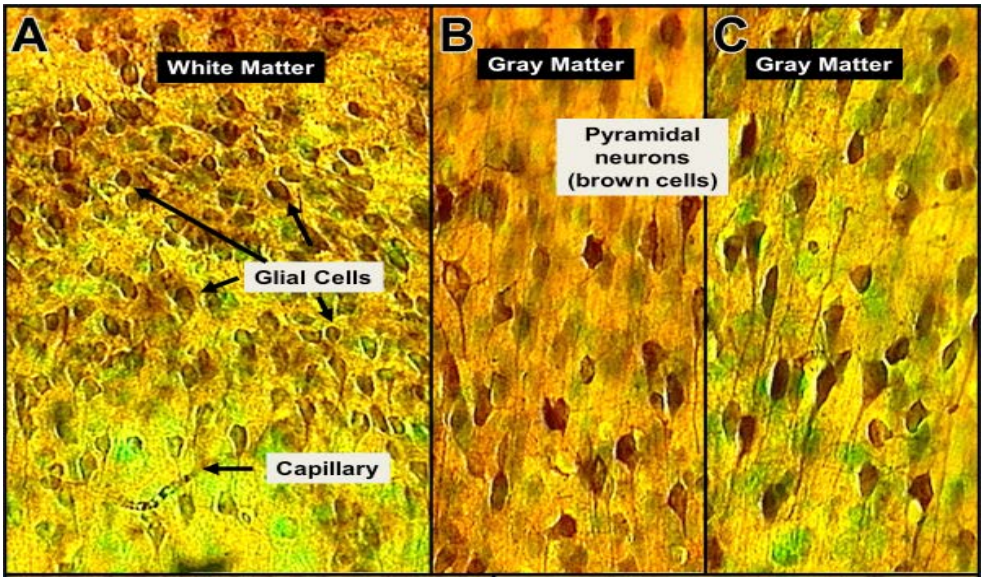

Figure 3: Animal model of Parkinson's disease created by stereotactic injection through cerebral cortex of neurotoxin 6-hydroxydopamine (6-OHDA) into substantia nigra of midbrain of adult rats. At termination of experiment, all animals were euthanized, brains removed, incubated with ELICA fixative, processed from cryosectioning, and cerebral cortices incubated with antibody to beta-galactosidase to distinguish differentiated Scl-40b cells versus resident cells. Tissue was counterstained with methyl green to identify resident cells. Note presence of genomically-labeled cells (brown) Scl-40b clone of pluripotent stem cells injected into substantia nigra of midbrain but migrated back along needle track to damaged area within cerebral cortex. A: White matter containing brown-labeled glial cells and endothelial-labeled capillary with green-labeled resident cells. B & C: Gray matter containing pyramidal neurons and interneurons. Reprinted with permission from Young HE, Hyer L, Black AC Jr, et al, 2013 [111].

Parkinson Disease

Original Hoehn-Yahr Stages

Stages:

1 - Only unilateral involvement, usually with minimal or no functional disability
2 - Bilateral or midline involvement without impairment of balance
3 - Bilateral disease: mild to moderate disability with impaired postural reflexes; physically independent
4 - Severely disabling disease; still able to walk or stand unassisted
5 - Confinement to bed or wheelchair unless aided

Expanded to 10-point scale for histogram so NORMAL = 0 and Confinement = 10

Scoring

1 = Unilateral involvement with no functional disability
2 = Unilateral involvement with minimal functional disability
3 = Midline involvement without impairment of balance
4 = Bilateral involvement without impairment of balance
5 = Bilateral disease – independent
6 = Bilateral disease – mild to moderate disability with impaired postural reflexes
7 = Severely Disabled – able to walk unassisted
8 = Severely Disabled – able to stand unassisted
9 = Confinement to wheelchair with aid
10 = Confinement to bed

Figure 4: Hoehn-Yahr scoring. Reprinted with permission from Young HE, Black Jr AC, Hyer L, et al, 2013 [112].

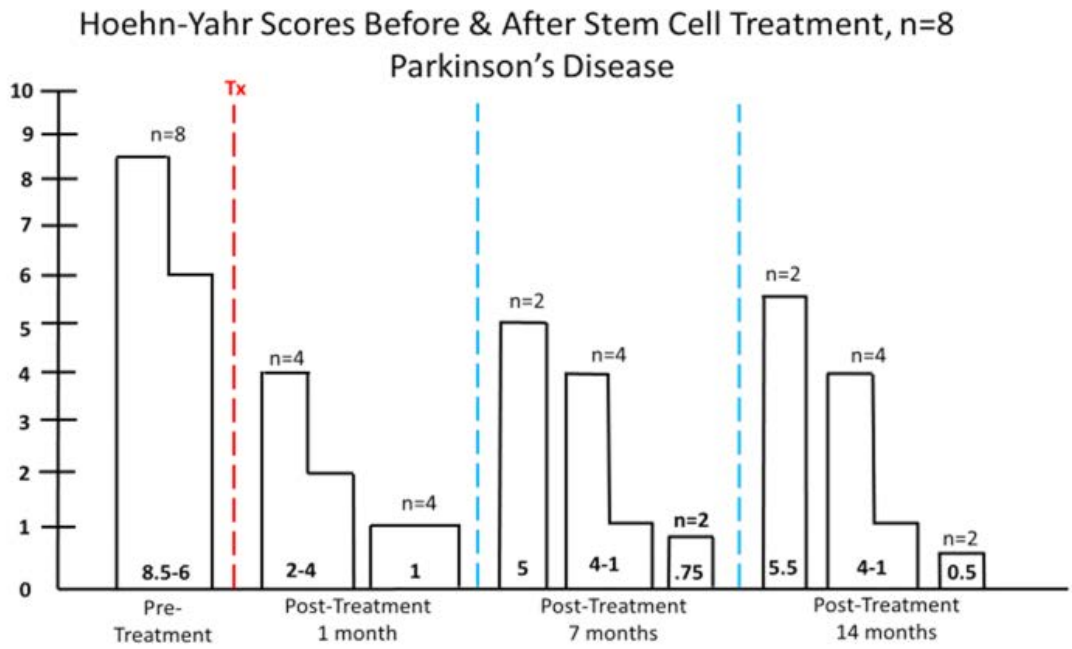

Figure 5: Hoen-Yahr Scoring expanded to 10 Point scale, with reversed where confinement = 10 and normal = 0. Ten* started trial and eight completed trial through the 14-month time-period. Before trial began, starting range of H-Y scores was between 8.5-6.0. At 1-month post-treatment, 4 showed H-Y range of 4.0-2.0, while 4 showed H-Y score of 1.0. At 7th month follow-up, 2 began to regress with H-Y score of 5.0; 4 stabilized at 4.0-1.0; and 2 improved to 0.75. At 14th month follow-up, same 2 individuals continue to regress with H-Y score of 5.5, but their scores were lower than when they started the trial. Same four that were stable at 7th month follow-up were stable at 14th month follow-up with H-Y scores of 4.0-1.0. The two individuals that showed improvement in their symptoms at 7th month follow-up continue to improve at the 14th month follow-up, with H-Y score of 0.5. Reprinted with permission from Young HE, Black Jr AC, Hyer L, et al, 2013 [112].

the next 4 months after treatment, both females could walk (and jog) without assistance. They could draw a clockface. They could calculate serial "sevens" (72-year-old) and serial "ones", "threes", "fives", "sevens", "nines", "11's", and "13's" (92-year-old). They knew they were visiting a stem cell clinic and they knew they were there for either assessment and/or continued treatments. They recognized who they were with (72-year-old: husband, stepson, or stepson's wife; and 92-year-old: twin daughters, stem cell physician, and stem cell isolator). They knew the date, day of the week, month, and year. They knew current events for the previous day, the previous week, month, and year. They knew the sitting President of the United States. Reversal of Alzheimer's symptoms lasted for four months in the 72-year-old receiving a single stem cell treatment and two years and four months for the 92-year-old that received one autologous and six allogeneic stem cell treatments. Unfortunately, once stem cell treatments ceased, approximately four months after their last respective treatment, the two females reverted to displaying similar Alzheimer's symptoms they had had before their telomerase positive stem cell treatments began.

While we initially thought that the AD symptom-free response to telomerase positive stem cell treatment in the two females was a placebo effect of knowing one is going to receive activated telomerase positive stem cells that did not appear to be the case with these two female participants. The placebo effect, e.g., the "Superman Syndrome", is the apparent feeling after infusion of exogenously activated telomerase-positive stem cells of being able to 'leap tall buildings at a single bound', 'out-run a speeding bullet', 'a fog being lifted', mental acuity, acute visual clarity, increased stamina, etc. These events had been reported on numerous occasions from multiple participants in our concurrent clinical studies (e.g., both recipients and donors receiving their activated telomerase-positive stem cells) being treated for neurogenic [111-113], cardiovascular [114,115], pulmonary [116-118], orthopedic [119], and autoimmune issues [120,121].

The "Superman Syndrome" can last up to two weeks post-transplant in most people, depending on the individual, their disease(s) being treated, and their respective comorbidities. Before their respective treatment, the two female Alzheimer's participants, with virtually no balance and initial MMSE scores of 0/30 had a clue that they were going to be receiving stem cells of any kind. This suggested against the "Superman Syndrome", in someone knowing they would be receiving stem cells. And for four months following their last treatment(s) they regained their balance and restored cognitive function with MMSE scores of 30/30. This suggesting that the "Superman Syndrome" that routinely lasts for two weeks, was not the instigator of the results seen in these two individuals. The results with these two female participants suggested that the restoration of balance and restoration of cognition could not be attributed to just a placebo event, but an actual phenomenon relating to the treatment with autologous and/or allogeneic telomerase positive stem cells.

The two males did not fare as well as their female counterparts. The procedure from ingestion of glacial caps to completing of telomerase positive stem cell transfusions took ~23 hours. The shortened time frame for the males was due to logistical problems with respect to time off work for their respective caregivers, travel time to and from clinic, and scheduling conflicts with clinic personnel.

The 58-year-old male, with an initial MMSE score of 0/30, received six autologous stem cell treatments, demonstrating a gain of three MMSE points total, 3/30, after his fourth stem cell treatment, but losing those MMSE points following his 5th and 6th treatments, 0/30. He did, however, show an increase in heart function by the end of his six stem cell treatments (Table 1). The 75-year-old male, with an initial MMSE score of 25/30, received three autologous stem cell treatments but only gained four MMSE points total for a MMSE score of 29/30, three after his first treatment and an additional point after his second treatment (Table 1).

The 58-year-old did not know he was to receive any stem cell treatments to alleviate his symptoms. On the other hand, the 75-year-old male had requested telomerase positive stem cell treatment for his diagnosed AD. However, the increase in MMSE scores for both individuals lasted up to two months following their respective stem cell treatments, after the third and fourth treatments for the 58-year-old (3/30) and during the three treatments for the 75-year-old (29/30). This suggested that their results were also not due to a two-week Superman Syndrome placebo effect.

Based on the limited numbers of participants, it is unknown why there was such a discrepancy in the increase in MMSE scores between males (avg 3.5) and females (avg 30). The 75-year-old male had a MMSE score of 25/30 prior to his first stem cell treatment. At most, the 75-year-old could have garnered just five additional MMSE points. The 58-year-old had a MMSE score of 0/30 prior to his first stem cell treatment. Theoretically, he could have garnered 30 MMSE points during his treatments, but only gained three and lost those during his fifth and sixth treatments. The cause of him losing the gained MMSE score is unknown. The theoretical 'best' MMSE average for the two males could have been 17.5, and not the 3.5 that was seen in this study.

We are attempting to determine what activity(ies) might have caused that discrepancy, 3.5 versus 17.5. Both female and male participants and their respective caregivers maintained Informed Consent Guidelines, that could, if not followed, disrupt the activities of the telomerase positive stem cells. Failure to follow Informed Consent Guidelines occurred in concurrent clinical trials studying autologous telomerase positive stem cells in patients with COPD [118] and age-related (dry) macular degeneration [113]. The results from these other studies demonstrated that failure to follow guidelines resulted in failure of the telomerase positive stem cells to restore damaged tissues to a functional state.

One possible difference affecting stem cell activity between the genders appeared to be the time frame in which the telomerase positive stem cells were processed. The time frame for isolation of telomerase positive stem cells from the females (or their donors for the 92-year-old) was approximately 73 hours, e.g., 18 hours prior to harvest ingestion of glacial caps; general health assessment and MMSE, ~1 hour; blood harvest, ~1 hour; processing blood, ~3 hours; gravity/zeta potential, ~24 hours; differential density gradient centrifugation with serum, saline, and water, ~4-6 hours; storage overnight; stem cell treatment, ~2 hours. The time frame for isolation of telomerase positive stem cells from the males was approximately 23 hours, e.g., 18 hours prior to harvest ingestion of glacial caps; general health assessment and MMSE, ~1 hour; blood harvest, differential density gradient centrifugation with serum, saline, and water, ~3 hours; stem cell treatment, ~1 hour. As stated in the reference notes, the shortened time frame of 23 hours for the males versus 73 hours for the females was due to logistical problems with respect to time off work for caregivers, travel time to and from clinic, and scheduling conflicts with clinic personnel. This potential discrepancy will be rectified in future studies.

Regardless of the discrepancy in average MMSE scores between male and female participants, no adverse events were reported for any participant (Table 1). Due to the limited number of participants treated, sample size of n=4, total efficacy for this study approximated 50%.

Conclusion

The cause of Alzheimer's disease (AD) is poorly understood. Genetics appear to play a major role, but other factors intervene as well. The presence of tau, aggregation of beta-amyloid protein, activated microglia, and massive losses of neurons and their synaptic processes have been associated with the disease. AD is an insidious and progressive loss of balance and cognitive memories from present time to distant past that occurs in reverse chronological order. There are no known pharmacological treatments clinically proven to decrease the risk for Alzheimer's disease, although some may temporarily forestall the eventual outcome of the disease. Death eventually occurs, usually 3-9 years after initial diagnosis. Currently, much of the promise for treating chronic and incurable diseases is centered on stem cell biology. Unfortunately, use of this technology for AD is still in its infancy. Embryonic stem cells and induced pluripotent stem cells cause tumor formation when implanted in a naïve undifferentiated state. However, when pre-induced to form neurons prior to transplantation there are still problems with immunosuppressive regimens to prevent graft versus host disease response in stem cells from allogeneic donors. In addition, pre-induction of autologous iPSCs into neurons has caused multiple neuropathies, thus making them of limited value as neuronal replacements. A Phase-2A clinical trial of telomerase negative mesenchymal stem cells reported no severe or long-term side effects with their administration. However, no significant clinical efficacy was noted. Neural stem cells have been investigated in rodent models of AD. While preliminary results show promise for decreasing symptoms associated with AD, there was limited engraftment success and limited survivability due to transplantation of the neural stem cells into a hostile environment. Based on our previous animal models and clinical studies, we

hypothesized that telomerase-positive stem cells would repair neuronal tissues leading to a reversal in cognitive decline. In a small cohort study (n=4) this report demonstrates that autologous and/or allogeneic telomerase-positive stem cells could be safely administered and resulted in both regaining balance and an increase in cognitive function, approximating 50% efficacy, for as long as the participants were being treated.

Future clinical studies for this disease will involve the use of a combinatorial nutraceutical mixture designed to mimic the effects of stimulating telomerase positive stem cells to proliferate in situ, mobilization of these stem cells into the blood stream, homing of these stem cells to damaged tissue sites, their on-site activation, and subsequent repair and/or regeneration of damaged or missing tissues to help restore balance and cognition for the treatment of Alzheimer's disease.

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