Research Article ISSN 2639-9512

Stem Cell & Regenerative Medicine

Treating Parkinson Disease with Autologous Telomerase-Positive Stem Cells, Update 2021

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Received: 08 February 2021; **Accepted:** 28 February 2021

Citation: Young HE, Speight MO. Treating Parkinson Disease with Autologous Telomerase-Positive Stem Cells, Update 2021. Stem Cells Regen Med. 2021; 5(1): 1-13.

ABSTRACT

Parkinson disease (PD) is the second most common progressive neurodegenerative disorder that affects older adults. PD is characterized by a low level of dopamine being expressed in the striatum and a deterioration of dopaminergic neurons and associated neural networks in the substantia nigra of the midbrain. Current medical, surgical, and rehabilitative treatments for PD have long-term side effects and do not halt the progression of the disease. Stem cell therapies generating dopaminergic neurons from fetal brain tissue, human embryonic stem cells, human induced pluripotent stem cells, mesenchymal stem cells, human neural stem cells, direct reprogramming of somatic cells and direct reprogramming of stem cells by either gene editing, and/or gene transfer have elicited keen interest as to eventual therapeutics for Parkinson disease. Unfortunately, thus far, these experimental therapies have proved to be of limited therapeutic value in clinical trials. Using a neurotoxin-induced animal model of PD, transplantation of a naïve telomerase positive pluripotent stem cell clone demonstrated reconstitution of dopaminergic neurons and associated neural networks when stereotactically injected into neurotoxin-lesioned substantia nigra pars compactum of the ventral midbrain. Two IRB-approved clinical trials in small cohort studies (n=8 & n=4), with a combined sample size of n=12, demonstrated that intranasal infusion of autologous telomerase positive totipotent cells followed by intravenous infusion of telomerase positive pluripotent stem cells and mesodermal stem cells had a positive influence on patient symptomology with Parkinson's Disease. No adverse effects were reported by any participant or their respective caregiver for the entire combined small cohort study (n=12). Taken together as a 2021 update of this on-going clinical study, 33% (n=4) showed moderate to no benefit of telomerase positive stem cell treatment by demonstrating a continued decline in symptoms after treatment; 33% (n=4) remained in stasis after the first month after treatment; and 33% (n=4) resolved their symptoms. The results suggest that autologous telomerase positive stem cells, TSCs, PSCs, and MesoSCs, are safe and efficacious (66%) to reduce the symptoms in participants with Parkinson's disease.

Keywords
Parkinson's Disease, Stem Cells, Autologous, Adult, Telomerase Positive, Totipotent Stem Cells, Pluripotent Stem Cells, Mesodermal Stem Cells, Hoehn-Yahr Scoring.

Introduction
Parkinson disease (PD) is the second most common progressive neurodegenerative disorder, after Alzheimer's disease, affecting older adults. It is a complex, multisystem disorder encompassing both neurologic and systemic nonmotor indications. PD is a mixture of both slowly and rapidly progressing forms. It is characterized by a low level of dopamine being expressed in the striatum and a deterioration of dopaminergic neurons and associated neural networks in the substantia nigra pars compactum of the ventral midbrain. While greater than, 90% of the PD cases are idiopathic (having no known origin) and without a clear etiology, mutations in many genes have been linked to rare familial forms of the disease. In all, over 300 genes have been implicated in the pathophysiology of Parkinson's disease. Idiopathic Parkinson's disease is associated with risk factors, such as genetic (e.g., PARK16, BST1, SNCA, LRRK2, GBA, MAPT), family history, age, pesticide exposure, environmental chemicals, consumption of dairy products, history of melanoma, traumatic brain injury, and synthetic heroin use. Reduction of risk factors have been reported in association with smoking, caffeine consumption, physical activities, higher serum urate concentrations, use of ibuprofen and other common medications. However, PD's ultimate cause(s) is/are unknown, although there may be an immune component through an inflammatory or autoimmune response. Several autoantibodies directed against antigens associated with Parkinson disease have been identified in participants with PD. Currently; the only prevention technique for Parkinson's disease appears to be physical activity [1-11].

The clinical diagnosis of Parkinson's disease is based on history and physical examination. History can include prodromal features, such as rapid eye movement, sleep behavior disorder, hyposmia, constipation; characteristic movement difficulties, such as resting tremor, rigidity, postural instability, disordered balance, slowness, disordered gait, falls; psychological or cognitive problems, such as cognitive decline, depression, anxiety, and hallucinations. Physical examination demonstrates motor symptoms during physical examination, such as resting tremor, rigidity, bradykinesia, stooping posture; and non-motor symptoms, such as depression and anxiety (neurobehavioral disorders); cognitive impairment (dementia); orthostasis and hyperhidrosis (autonomic dysfunction); and sensory impairments [1,3,5,7,12,13].

As Parkinson disease symptoms worsen over time, there is diminished neurotransmitter levels, oxidative stress, mitochondrial dysfunction, and perturbed protein homeostasis. This results in death of dopaminergic neurons in substantia nigra pars compactum of the ventral midbrain and development of Lewy Bodies, which are brain deposits containing a substantial amount of alpha-synuclein [1,3,13].

Current therapies for Parkinson's Disease include improving physical function, levodopa, cholinesterase inhibitors, methylphenidate, deep-brain stimulation, and exercise [5]. Drugs that enhance intracerebral dopamine concentrations or stimulate dopamine receptors remain the mainstay for treatment of the disease. Current conventional therapies available for Parkinson's disease only treat the symptoms, rather than the underlying cause of the disease. Treatments include medications, such a levodopa with and without other medications, and other approaches, such as exercise, physical therapy, occupational therapy, and speech therapy [1,3,12]. Although levodopa is the most effective medication available for treating motor symptoms of PD, other drugs, such as monoamine oxidase Type-B inhibitors (selegiline, rasagiline), amantadine, anticholinergics, beta-blockers, or dopamine agonists may be used first to prevent levodopa-related motor complications. Motor fluctuations can be managed by modifying levodopa regimen or by adding monoamine oxidase Type-B inhibitors, catechol-O-methyltransferase inhibitors, or dopamine agonists. Impulse control disorders are managed by either reducing or eliminating dopaminergic medication, such as dopamine agonists [2,14].

The use of medications, surgery including deep brain stimulation, and rehabilitation have been established as current therapies for PD. Strong therapeutic benefit has been shown using these therapies, but none of them has proven effective at stopping the progression of PD. Approaches, such as deep brain stimulation and treatment with enteral suspension of levodopa-carbidopa, can help individuals with medication-resistant tremors, worsening symptoms, and dyskinesias [1,7,12]. Unfortunately, current medical, surgical, and rehabilitative treatments for PD have long-term side effects and do not halt the progression of the disease [18]. Nutritional modalities have offered some hope for slowing the progression of Parkinson' disease or lessening side effects of medications. The overall benefits, while positive, appear limited [19,20]. Fortunately, cell therapy may prove beneficial for restoration of neuronal structure and function [15-17].

Dopaminergic neurons, derived from fetal brain tissue, human embryonic stem cells, human induced pluripotent stem cells, mesenchymal stem cells, medicinal signaling cells, human neural stem cells, direct reprogramming of somatic cells or direct reprogramming stem cells by either gene editing, and/or gene transfer, have elicited keen interest as to the eventual treatment of Parkinson disease. However, thus far, these experimental therapies under development have proved in clinical trials to be of limited therapeutic value [1,4,13,15,17,18,21-26].

Use of fetal brain tissue as a standardized treatment regimen is fraught with problems in terms of low availability and high variability, as well as moral and ethical issues with respect to tissue harvesting [22,25,27]. An alternative to the use of fetal brain tissue is to use human embryonic stem cells [27]. Similar problems arise from moral and ethical issues with respect to tissue harvest, immuno-rejection, and grafting procedures when using human embryonic stem cells. In addition, implantation of human

embryonic stem cells in the naïve-undifferentiated state can cause teratoma formation [15]. Current major technical and logistical challenges include source of human embryonic stem cells, establishment of human embryonic stem cell lines, GMP (good manufacturing process) compliance to the differentiation protocol and reagents used, characterization of the cell products in terms of identity, safety, and efficacy to restore functional dopaminergic neurons to the substantia nigra [28,29].

An alternative to the use of fetal brain tissue is to use induced pluripotent stem cells (iPSCs) [24]. IPSCs are generated from differentiated somatic cells by transfecting the Yamanaka factors, Oct4, Sox2, c-Myc, and Klf4, into the somatic cells using retroviral vectors. The endogenous cellular machinery and transcriptional feedback mechanisms are preserved in these newly generated pluripotent stem cells. However, similar problems arise with respect to source of differentiated somatic cells to harvest, immuno-rejection, and grafting procedures when using iPSCs. Unfortunately, iPSCs can also engender teratoma formation when transplanted into an individual in the naïve-undifferentiated state. In contrast, iPSC lines can be established with specific risk factors to ascertain their differing response to treatment. iPSC lines can then be genetically corrected; pre-differentiated into neuronal/ neural crest ectodermal lineage cells, e.g., pyramidal cells, Purkinje cells, dopaminergic neurons, motor neurons, interneurons, radial glial cells, astrocytes, oligodendrocytes, Schwann cells, ganglion cells, melanocytes, adrenal medulla, etc.; sorted for the appropriate neurons; and the neurons subsequently transplanted back into patients in hopes of re-establishing function. With development of the latest genomic modification strategies, dopaminergic neuron differentiation technologies, and directed cell transplantation studies, PD research is poised to generate state-of-the-art Parkinson disease-modifying therapies. Like ESCs, major technical and logistical challenges include establishment of iPSC lines, GMP compliance to the differentiation protocol and reagents used, immuno-rejection, characterization of the cell products in terms of identity, safety, and efficacy to restore function of dopaminergic neurons to the substantia nigra compacta of the ventral midbrain [6,15,21,22,26-33]. There remain challenges to therapeutic implementation of ESCs and iPSCs. Based on karyotypic analysis of 1,163 ESC cultures and 552 iPSC cultures the average abnormal karyotype incidence is 12.5% in both settings [34]. In 2018, the first human trial for PD iPSC transplantation began in Japan [19,30].

Cell transplantation into the brain presents several immunological challenges. To avoid allogeneic graft rejection from either ESCs, ESC-lines or iPSC-lines, the adaptive immune system should be abolished. However, the innate immune response will still be present after transplanting cells into the brain. Modulation of the innate immune system can increase success rate in clinical trials by enhancing cell differentiation and cell survival [18]. Mesenchymal stem cells / medicinal signaling cells (MSCs) are an attractive alternative therapeutic candidate because of their high capacity for self-renewal, immunomodulatory activity with respect to allogeneic versus autologous transplants, high ethical acceptance, and no teratoma formation. MSCs can be obtained from different adult and fetal tissues, e.g., bone marrow, adipose tissue, placenta, umbilical cord, and dental tissues [23,24]. The neuroregenerative, anti-inflammatory, and immunomodulatory properties of mesenchymal stem cells are mainly mediated by secretion of an array of biomolecules encased within exosomes. Not only can cultured MSCs spontaneously produce neurotrophic factors, but they can also be genetically programmed to synthesize and secrete similar factors in vivo [24,25,34].

Exosomes are being studied to alleviate problems associated with neurodegenerative diseases, such as PD. The paracrine effects of mesenchymal stem cells, via exosomes, play a crucial role in modulating inflammation and immunosuppression during transplantation of stem cells. Extracellular vesicles containing microRNA (miRNA) mediate biological function through gene regulation [36].

Endogenous regeneration of dopaminergic neurons from neural stem cells remains highly controversial. Neural stem cells are located in the subventricular zone of the lateral ventricles and in the dentate gyrus of the hippocampus. Neural stem cells derived from Parkinson patients exhibit aberrant mitochondrial morphology and aberrant functionality, making them less than an ideal treatment for Parkinson disease [36,38]. While pluripotent stem cells can be induced from somatic cells using the Yamanaka factors, e.g., Oct4, Sox2, c-Myc, and Klf4, the ectopic expression of c-Myc causes tumorgenicity in subsequent offspring and the retroviruses themselves used to transfect the genes can cause insertional mutagenesis in the cells. The generation of iPSCs from neural stem cells with the minimal number of factors, may be more advantageous to clinical therapies. To that end, iPSCs were generated from neural stem cells by reprogramming with Oct4 and Sox2. Results showed that inducing pluripotency in neural stem cells could reduce the number of reprogramming factors necessary for reprogramming [39]. As an alternative to using reprogrammed neural stem cells, direct lineage reprogramming of glial cells to form neurons, or fibroblasts to form dopaminergic neurons, is advantageous. There are no ethical concerns, no risk of tumor formation, and no need for even transplantation using this technology [38,40].

While use of stem cell-derived dopaminergic neurons, derived from fetal brain tissue, human embryonic stem cells, human induced pluripotent stem cells, mesenchymal stem cells, human neural stem cells, direct reprogramming of somatic cells or direct reprogramming stem cells by either gene editing, and/or gene transfer are promising approaches to the eventual treatment of Parkinson disease, these experimental therapies under development, thus far, have proved of limited therapeutic value in clinical trials [1,4,13,15,17,18,21-27].

We offer an alternative to the above experimental stem cell technologies, i.e., the use of naturally occurring endogenous, adult-derived telomerase positive stem cells, e.g., totipotent stem cells (TSCs), pluripotent stem cells (PSCs), and mesodermal stem cells (MesoSCs), as a potential treatment option for Parkinson's

disease, for restoration of dopaminergic neurons, dopaminergic-neural networks, loss of symptoms, and gain of function. In culture, telomerase positive TSCs and PSCs were shown to differentiate under the influence of human recombinant proteins and exosomes isolated from selective adult differentiated tissues to form cells of the neural ectodermal lineage, e.g., neurons, interneurons, oligodendrocytes, astrocytes, ganglion cells, and radial glial cells [41-44]. In animal models of disease, implantation of naïve telomerase positive TSCs and PSCs were shown to be induced by local factors to form dopaminergic neurons, neural networks, pyramidal neurons, interneurons, and glial cells [45-47]. And in previous human clinical trials of Parkinson disease, age-related dry macular degeneration, and Alzheimer's disease, transplantation of autologous telomerase positive stem cells was shown to be both safe and efficacious, up to 75%, to restore function in the respective participants [46-49]. In contrast to the induced neurogenic activities of TSCs and PSCs, MesoSCs were shown to act in a supporting role to revascularize damaged tissues in the cerebral cortex [44], after myocardial infarction [50,51], and in chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis [52-54].

Materials and Methods

Autologous telomerase positive TSCs, PSCS, and MesoSCs were utilized in IRB-approved study protocols for Parkinson's disease. Inclusion criteria were any male or female, age 18 to 120, with diagnosed Parkinson's disease. Four additional males were assessed in this ongoing clinical trial. Participants were instructed to follow informed consent guidelines for telomerase positive stem cells for clinical therapy, e.g., avoidance of alcohol, tobacco products, vaping, recreational drugs, lidocaine, and chemotherapeutic drugs because they kill telomerase positive stem cells; limit use of caffeine because it prevents differentiation of the undifferentiated stem cells; and limit the use of corticosteroids because they prematurely differentiate TSCs and PSCs into cells of the mesodermal lineage [55]. The participants were instructed to ingest combinatorial nutraceuticals (CN) (DFRD, Macon, GA) daily for a minimum of 30 days prior to initial and subsequent harvests to increase proliferation of their telomerase positive stem cells, thus making themselves their own endogenous sterile bioreactors for their telomerase positive stem cells propagation. Participants were to stay well hydrated with aqueous fluids for ease of stem cell harvest. And to limit moderate to excessive exercising during a two-week window before harvest and after treatment to maximize directed stem cell repair responses. Eighteen hours before harvest, the participants were requested to ingest glacial caps (GC, DFRD) to induce reverse diapedesis of the telomerase positive stem cells into the blood stream for peak stem cell release and harvest [55].

Harvesting telomerase positive TSCs, PSCs, and MesoSCs occurred using venipuncture, withdrawing 300 to 400cc's of blood, based on the body weight of the participants. The TSCs, PSCs, and MesoSCs were separated from the RBCs, WBCs, and platelets using 'FDA-mandated minimal manipulative procedures', coupled with gravity/zeta potential and differential density gradient centrifugation using serum, sterile saline, and sterile distilled water gradients. The telomerase positive stem cells were segregated into individual populations and activated. The stem cell isolation protocol for two males that received a single treatment of autologous stem cells required ~72-hours to complete, whereas the other two males that received six treatments with autologous telomerase positive stem cells every other month for one year, required ~24-hours for processing the telomerase positive stem cells for each treatment.

Following isolation, segregation, and activation, the stem cell treatment regimen consisted of intranasal infusion of autologous TSCs, followed by infusion of pooled PSCs and MesoSCs by intravenous (IV) infusion, preferably into the median cubital vein. The autologous TSCs were concentrated into 0.5cc's of sterile saline and split into two aliquots of 0.25cc is each. Each participant was instructed to clean the mucus out of their nostrils with sterile 0.65% saline, after which participants were placed into the reverse Trendelenburg position. Each nostril received a 0.25cc aliquot of TSCs, placed dropwise onto the olfactory epithelium in the roof of the superior nasal meatus. The recipient remained in that position for five minutes and then placed in the upright position. Pooled PSCs and MesoSCs were diluted into 250cc's of heparin/saline and given by IV infusion [49].

Results

A modified Hoehn-Yahr (H-Y) scoring system was used to determine severity of Parkinson disease symptoms, both before and after treatments [9,14,56-58]. We expanded the H-Y scoring scale from 5 to 10 to better distinguish the severity of symptoms between the participants (Figure 1) [47]. All participants in this study had pre-treatment Hoehn-Yahr scores between 6 to 8.5. Two of the participants, even after six treatments, did not vary significantly from their pretreatment values, at 1-month, 7-months, and 14-months after treatment. In contrast, the other two participants with a single autologous telomerase positive stem cell treatment displayed no symptoms of Parkinson's disease within one month after treatment and beyond (Figure 2).

Discussion

The use of medications, e.g., levodopa, cholinesterase inhibitors, methylphenidate; surgery, including deep brain stimulation; and rehabilitation exercises have been established as current therapies for Parkinson Disease. Individuals with medication-resistant tremors, worsening symptoms, and dyskinesias can be helped with deep brain stimulation and treatment with enteral suspension of levodopa-carbidopa [1,7,12]. Strong therapeutic benefit has been shown using these approaches, but none of them have been proven effective at stopping the progression of PD [5,18]. Due to recent advances, stem cell therapy may prove beneficial for restoration of neuronal structure and function [15-17].

Stem cell-derived dopaminergic neurons, derived from fetal brain tissue, human embryonic stem cells, human induced pluripotent stem cells, mesenchymal stem cells, medicinal signaling cells,

Parkinson Disease

Original Hoehn-Yahr 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 = 1 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 1: Original Hoehn-Yahr scoring system expanded to a ten-point scale for histogram graphing, where zero = normal with no Parkinson's disease symptoms and 10 = confinement to bed. Reprinted with permission from Young HE, Hyer L, Black Jr AC, et al. Treating Parkinson Disease with adult stem cells. J Neurol Disord 2013; 2:1 [47].

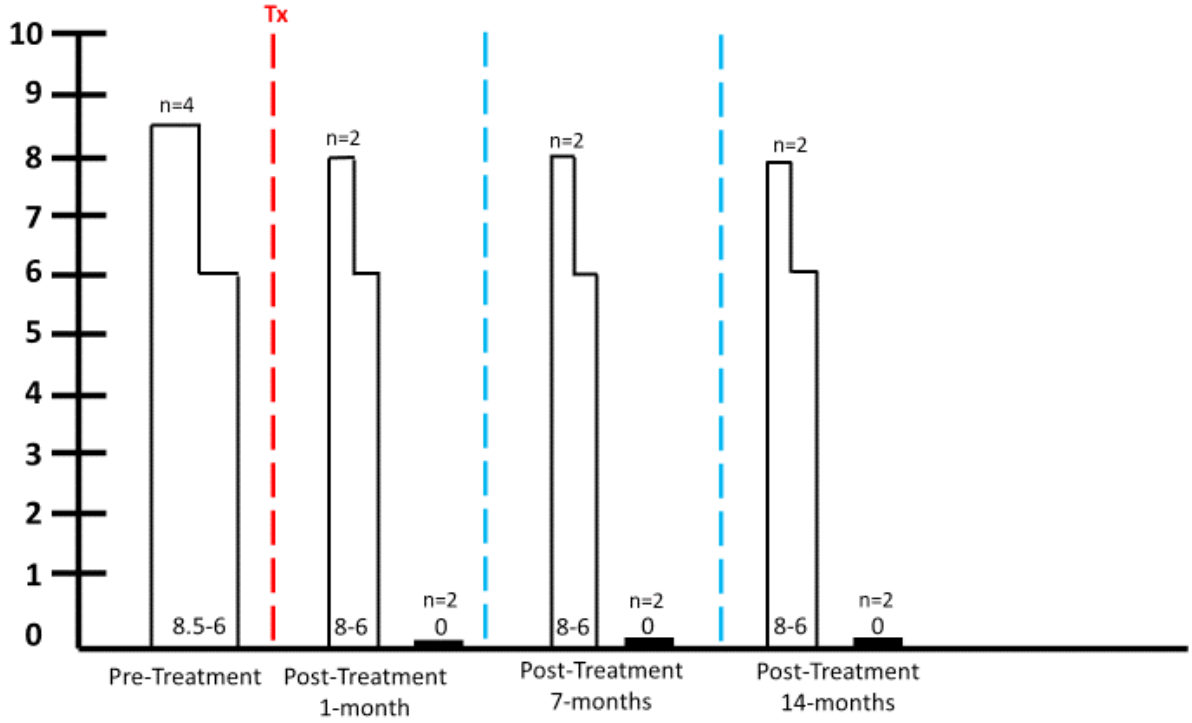

Figure 2: Hoehn-Yahr extended scoring system, for current study with a sample size of n=4. Four participants started study with H-Y score range of 8.5 to 6.0. One-month post treatment, two participants had H-Y score range of 8-6 and two participants had H-Y score of 0. Similar H-Y scores were noted for same participants at 7-month and 14-month during post-treatment follow-up assessments.

human neural stem cells, direct reprogramming of somatic cells or direct reprogramming of stem cells by either gene editing, and/or gene transfer are promising approaches to the eventual treatment of Parkinson disease. However, thus far, the current experimental therapies under development have proved of limited therapeutic value in clinical trials [1,4,13,15,17,18,21-26].

We offer an alternative to the above stem cell technologies, i.e., the use of naturally occurring endogenous, adult-derived telomerase positive stem cells, e.g., totipotent stem cells (TSCs), pluripotent stem cells (PSCs), and mesodermal stem cells (MesoSCs), as a potential treatment option for Parkinson's disease for restoration of dopaminergic neurons and gain of function.

The generation of a Parkinson Disease model in adult rats was created using a stereo tactically injected dopaminergic neurotoxin, 6-hydroxydopamine (6-OHDA), to study subsequent treatment with adult-derived telomerase positive stem cells [45]. In this model, animals were either stereotactically injected with saline or with the neurotoxin 6-OHDA. At two weeks post injection, representative animals were euthanized, the brains processed, and stained histochemically for tyrosine hydroxylase activity, to denote presence (saline) or absence (6-OHDA) of active dopaminergic neurons (Figure 3). The tissues were counterstained with methyl green to distinguish host neurons and glial cells. Control animals previously injected with saline showed no loss of tyrosine hydroxylase activity (Figure 3A). Experimental animals, previously injected with 6-OHDA, showed a zone absent of tyrosine hydroxylase activity at the injection site (Figure 3B). This was indicative of loss of both dopaminergic neurons and their associated neural networks. The remaining experimental animals (previously injected with 6-OHDA) were stereotactically injected with either saline (control) or a genomically-labeled clone of naïve pluripotent stem cells (Scl-40b). Scl-40b was generated from adult rat PSCs by repetitive serial dilution single cell clonogenic analysis, genomically-labeled with Lac-Z using lipofectin, and characterized [44]. Extensive characterization studies of Scl-40b were undertaken. Under the influence of human recombinant proteins and/or exosomes derived from selective differentiated cell types [59,60], Scl-40b would form all differentiated cell types of the body, except the gametes and the nucleus pulposus of the intervertebral disc. The phenotypic marker expressing induced cells indicated formation of cells of the ectodermal lineage, e.g., dopaminergic neurons, pyramidal neurons, interneurons, astrocytes, oligodendrocytes, radial glial cells, and derivatives of neural crest, e.g., ganglion cells, melanocytes, and adrenal medulla (Figure 4) [44].

Six weeks following their second stereotactic injection, the saline injected animals demonstrated glial cells along their needle tracks as well as a disintegration of the dopaminergic neural networks at the original 6-OHDA injection site (Figure 5). In contrast, experimental animals injected with the Scl-40b clone demonstrated a line of cells within the needle track that stained positive for tyrosine hydroxylase activity as well as their associated neural networks (Figure 6). The results suggested the potential that adult-derived pluripotent stem cells could be transplanted into the substantia nigra of Parkinson's patients and assist in the restoration of dopaminergic neurons and their respective neural networks.

As a follow-up to this animal study of Parkinson's disease, an IRB-approved human clinical trial was undertaken. However, a few parameters were changed based on less invasive procedures to introduce the telomerase positive stem cells to the substantia nigra of the midbrain in adult humans to effectively treat Parkinson's disease.

Instead of using a universal clone of allogeneic telomerase positive

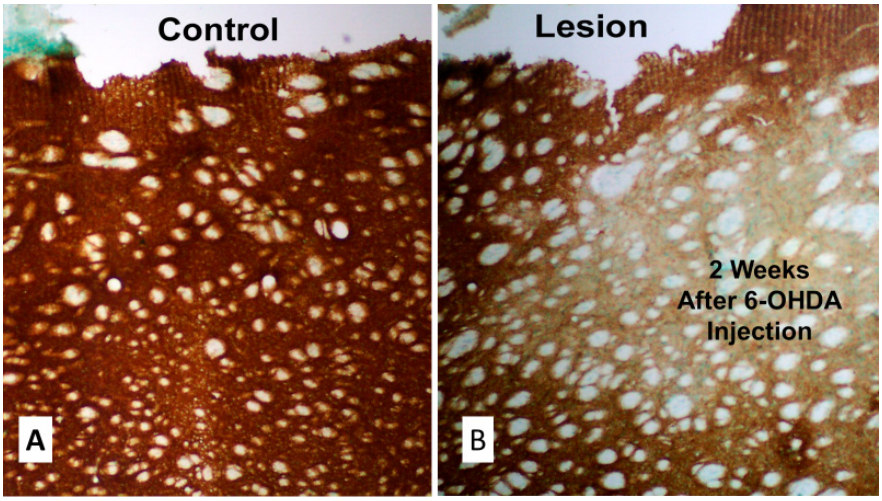

Figure 3: Rat model of Parkinson's disease created by stereotactically injecting dopaminergic neurotoxin, 6-hydroxydopamine (6-OHDA), into substantia nigra pars compacta of the adult rat ventral midbrain. A, Control section two weeks after injection of saline only. Note dark brown reaction product indicating tyrosine hydroxylase activity in area of midbrain, indicating presence of dopaminergic neurons and their associated neural networks. B, Experimental section two weeks after injection 6-OHDA, note loss of tyrosine hydroxylase staining at injection site, indicating loss of both dopaminergic neurons and their associated neural networks. Reprinted with permission from Young HE, Duplaa C, Katz R, et al. Adult-derived stem cells and their potential for tissue repair and molecular medicine. J Cell Molec Med 9:753-769, 2005 [45].

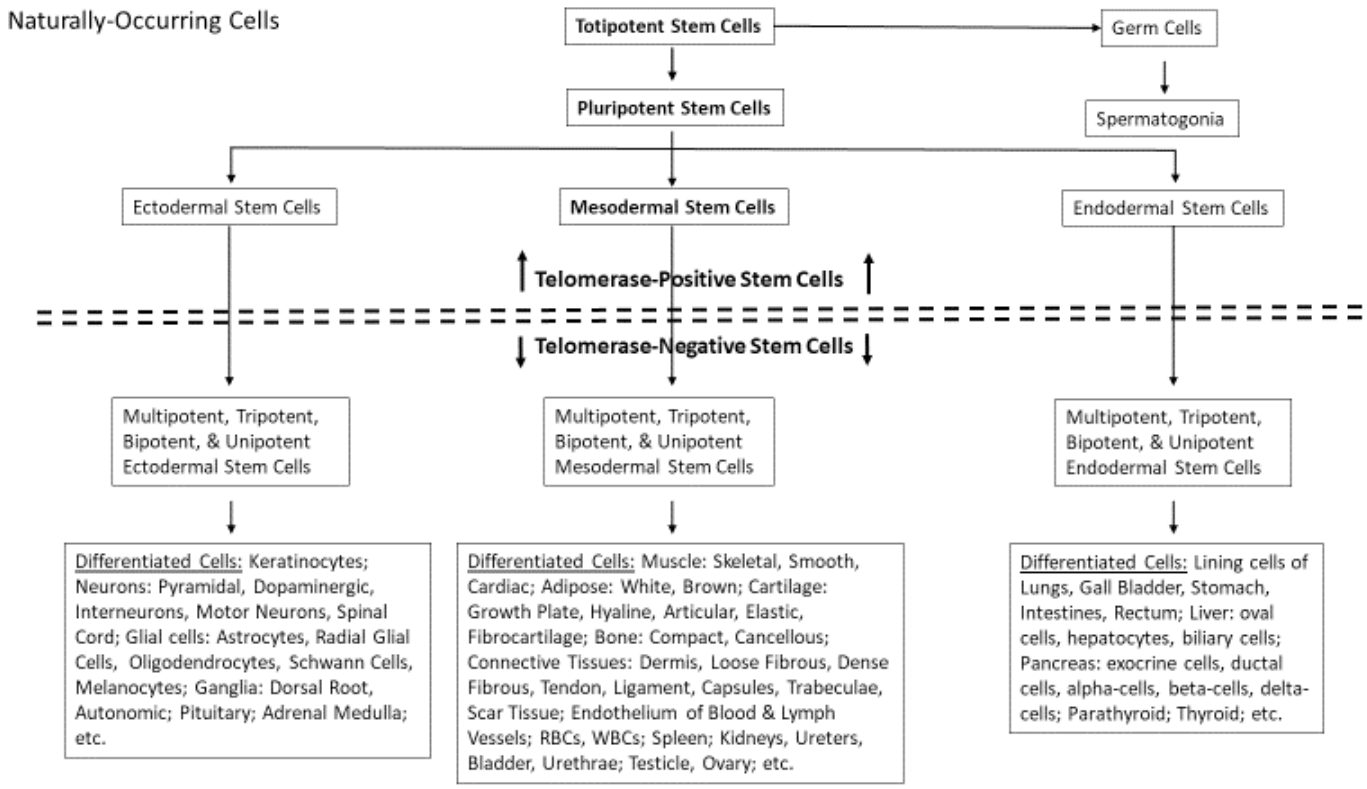

Figure 4: Diagram of respective downstream differentiation potentials of telomerase-positive stem cells with essentially unlimited proliferation potential (above dotted line) and telomerase-negative progenitor stem cells and differentiated cells, which conform to Hayflick's limit of 50-70 population doublings before senescence and cell death (below dotted line). Telomerase positive stem cells, telomerase negative progenitor stem cells, and differentiated cell are found within the body. Scl-40b, a clone of pluripotent stem cells (PSCs) derived by repetitive serial dilution single cell clonogenic analysis, demonstrated the potential to form all cells of the body from ectodermal, mesodermal, and endodermal germ layer lineages, including their respective progenitor stem cells and differentiated cell types, but would not form the gametes or the nucleus pulposus of intervertebral disc, and would not dedifferentiate into the more primitive undifferentiated totipotent stem cells. Reprinted with permission from Young HE, Speight MO. Characterization of endogenous telomerase-positive stem cells for regenerative medicine and a review. Stem Cell Regen Med 2020; 4(2):1-14 [44].

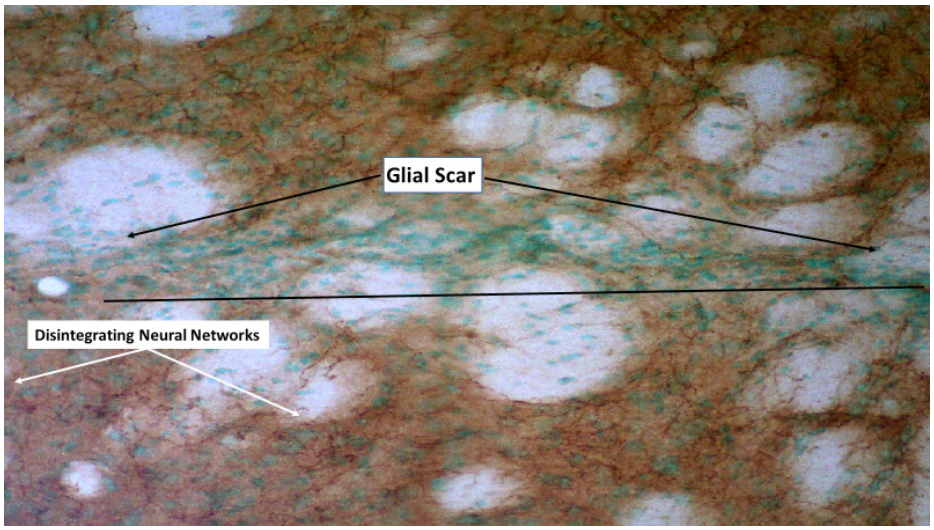

Figure 5: Rat model of Parkinson's disease injected stereotactically with neurotoxin 6-OHDA to create zone devoid of tyrosine hydroxylase activity (staining) indicative of lost dopaminergic neurons and disintegrating dopaminergic neural networks. Section depicts experimental animal (Figure 3B) six weeks after injection with saline. Sections stained histochemically for tyrosine hydroxylase activity and counterstained with methyl green to denote host cells (neurons and glial cells). Note line of green-stained glial cells in needle track, indicating a glial scar, along with disintegrating dopaminergic neural networks. Reprinted with permission from Young HE, Duplaa C, Katz R, et al. Adult-derived stem cells and their potential for tissue repair and molecular medicine. J Cell Molec Med 9:753-769, 2005 [44].

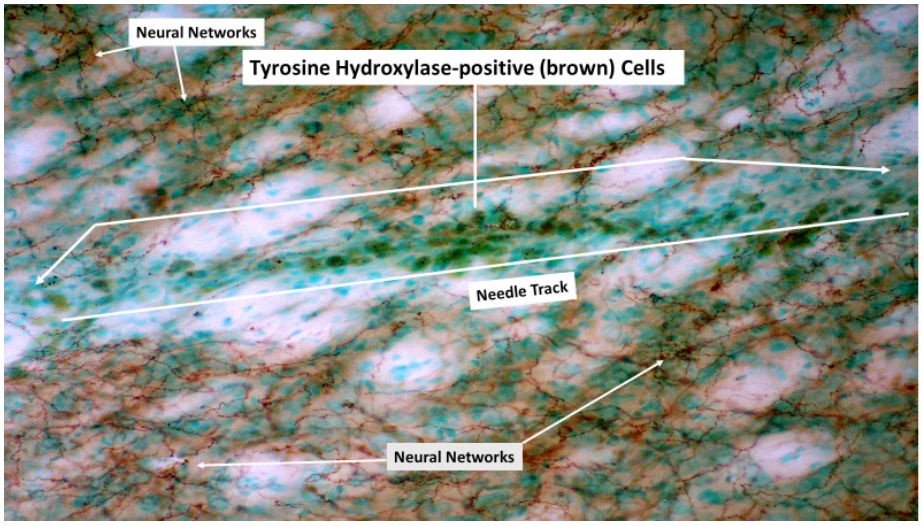

Figure 6: Rat model of Parkinson's disease injected stereotactically with neurotoxin 6-OHDA to create zone devoid of tyrosine hydroxylase activity (staining) indicative of lost dopaminergic neurons and disintegration of neural networks. Section depicts experimental animal (Fig. 3B) six weeks after injection with telomerase positive genomically-labeled pluripotent stem cell clone, Scl-40b. Sections stained histochemically (brown) for tyrosine hydroxylase activity and counterstained with methyl green to denote host cells (neurons and glial cells). Note tyrosine hydroxylase-positive cells within needle track of experimental animal and development of dopaminergic neural networks along all sides of the tyrosine hydroxylase-positive cells. Reprinted with permission from Young HE, Duplaa C, Katz R, et al. Adult-derived stem cells and their potential for tissue repair and molecular medicine. J Cell Molec Med 9:753-769, 2005 [44].

stem cells for these studies, we opted to use autologous telomerase positive stem cells from the participants themselves. This was done for several reasons. At this point in our research timeline, we did not know whether an allogeneic clone of telomerase positive stem cells from a donor would elicit a graft versus host disease (GvHD) response, where either the graft rejects the host or the host rejects the graft [61,62]. A GvHD response would necessitate the use of immunosuppressants with their own inherent morbidities. To ensure that a GvHD response did not occur, only autologous telomerase positive stem cells were utilized.

An intranasal approach was chosen for the telomerase positive stem cells rather than stereotactic surgery for a less invasive means for the stem cells to gain access to the brain. Previously, telomerase negative mesenchymal stem cells had been introduced to the brain in a clinical trial treatment for Parkinson's Disease via the intranasal route [49,63-65]. They were chosen for that role due to their perceived ability to form all cells of the mesodermal lineage as well as dedifferentiating into all cells of both the ectodermal and endodermal lineages [66-69].

However, based on the results from our characterization studies [44], we would disagree on the perceived abilities of mesenchymal stem cells, utilizing human recombinant proteins and exosomes derived from selective differentiated cell types [59,60] with clones of telomerase positive totipotent stem cells (TSCs), pluripotent stem cells (PSCs), ectodermal stem cells (EctoSCs), mesodermal stem cells (MesoSCs), endodermal stem cells (EndoSCs), and telomerase negative mesenchymal stem cells (MSCs), all derived by repetitive single cell clonogenic analysis, for a side-by-side comparison analysis of differentiation capabilities.

The results from those studies corresponded to the results from Pittenger et al. [70] which showed that the original stem cell population termed by Caplan as a 'mesenchymal stem cell (MSC)' [71] consisted of a telomerase-negative tripotent progenitor stem cell capable of only forming cartilage, fat, and bone. In our hands, only telomerase-positive clones of TSCs, PSCs, and EctoSCs could be induced to form neurons, astrocytes, oligodendrocytes, and radial glial cells; only clones of TSCs, PSCs, and MesoSCs could be induced to form all cell types in the mesodermal lineage; only clones of TSCs, PSCs and EndoSCs could form all cell types of the endodermal lineage; and only TSCs, PSCs, MesoSCs, and MSCs would form fat, cartilage, and bone (Figure 4). In addition, none of the PSC, EctoSC, MesoSC, EndoSC, or MSC clones would dedifferentiate to form cells of an alternative lineage [44,59,60].

Due to inherent size of mesenchymal stem cells (>12 microns), a hyperosmotic solution of mannitol was required to shrink the olfactory epithelium to create channels to allow migration of the MSCs between the olfactory cells to gain access to the brain [49,63-65]. Following mannitol treatment, the olfactory epithelium would swell to their normal shape. Unfortunately, for older-aged individuals, such as those with Parkinson's disease, two or more mannitol treatments of the olfactory epithelium created permanent channels between the olfactory cells, allowing a greater chance for bacterial meningitis [49].

To circumvent the potential size problem and use of mannitol with respect to intranasal delivery of the stem cells, we opted to use the smallest telomerase positive stem cells, i.e., the totipotent stem cells (TSCs). Previous characterization studies of TSCs demonstrated that these very small cells (0.1 to 2.0 microns in size) would form all cell types of the body, including gametes and the nucleus pulposus of the intervertebral disc. Extensive

characterization of three separate clones of TSCs with human recombinant proteins and exosomes derived from differentiated cell types noted that these stem cells would form downstream pluripotent stem cells, endodermal stem cells, mesodermal stem cells, and ectodermal stem cells, as well as all telomerase negative progenitor stem cells, and differentiated cell types of the neural ectodermal lineage, including dopaminergic neurons, pyramidal neurons, interneurons, astrocytes, oligodendrocytes, radial glial cells; ganglion cells, melanocytes, and other derivatives of neural crest (Figure 4) [44,59,60]. To keep the TSCs from being trapped in nasal mucus secretions, the participants washed the mucus from their nostrils with 0.65% saline prior to TSC application to the olfactory epithelium [47-49].

Multiple parameters were assayed during the clinical trial of telomerase positive stem cells for the treatment of Parkinson disease [46,47]. These parameters included repeated CIBIC ratings, Hoehn-Yahr (symptom) scores, repeated Schwab-England scale of daily living, repeated UODRS total scores, repeated FAQ scores, repeated Epworth sleepiness scale, repeated Beck depression inventory total scores, cognition: repeated trails-A, cognition: repeated trails-B, and Caregiver Burden: repeated Zarit Burden scale total scores.

Hoehn-Yahr scoring (Figures. 1and2) prior to stem cell treatment noted that the participants exhibited initial scores of 6.0 (bilateral disease – mild to moderate disability with impaired postural reflexes) to 8.5 (severely disabled – able to stand with assistance). At 1-month after treatment, there were two groups, e.g., 2.0 (unilateral involvement with minimal functional disability) to 4.0 (bilateral involvement without impairment of balance), n=4, and 1.0 (unilateral involvement with no functional disability), n=4. By seven-months after treatment, there were three groups of participants, e.g., n=2 had a H-Y score of 5 (bilateral disease - independent); n=4 had a H-Y score of 4.0 (bilateral involvement without impairment of balance) to 1.0 (unilateral involvement with no functional disability); and n=2 had a H-Y average score of 0.75 (unilateral involvement with no functional disability). By 14-months after treatment, there were the same three groups, with similar or slightly altered H-Y scores, one group displaying slightly worse symptoms and the other group displaying slightly better symptoms. In toto, the percentages for participants in the study were 25% (n=2) for participants regressing, 50% (n=4) for participants in stasis, and 25% (n=2) for participants getting better, for a combined efficacy of treatment of 75% (Figure 7) [46,47].

The current small cohort study (n=4) details the addition of four additional participants to the on-going IRB-approved Parkinson Disease treatment with autologous telomerase positive stem cell clinical trial. These additional four participants had an initial H-Y score of 6.0 to 8.5, like the previous cohort (n=8) [46,47]. Two participants displayed minimal to no improvement in Parkinson's symptoms, H-Y score of 6.0 to 8.0. While the other two participants completely lost all symptoms of Parkinson's disease throughout the time course of the study (Figure 2).

One possible difference affecting potential stem cell activity is

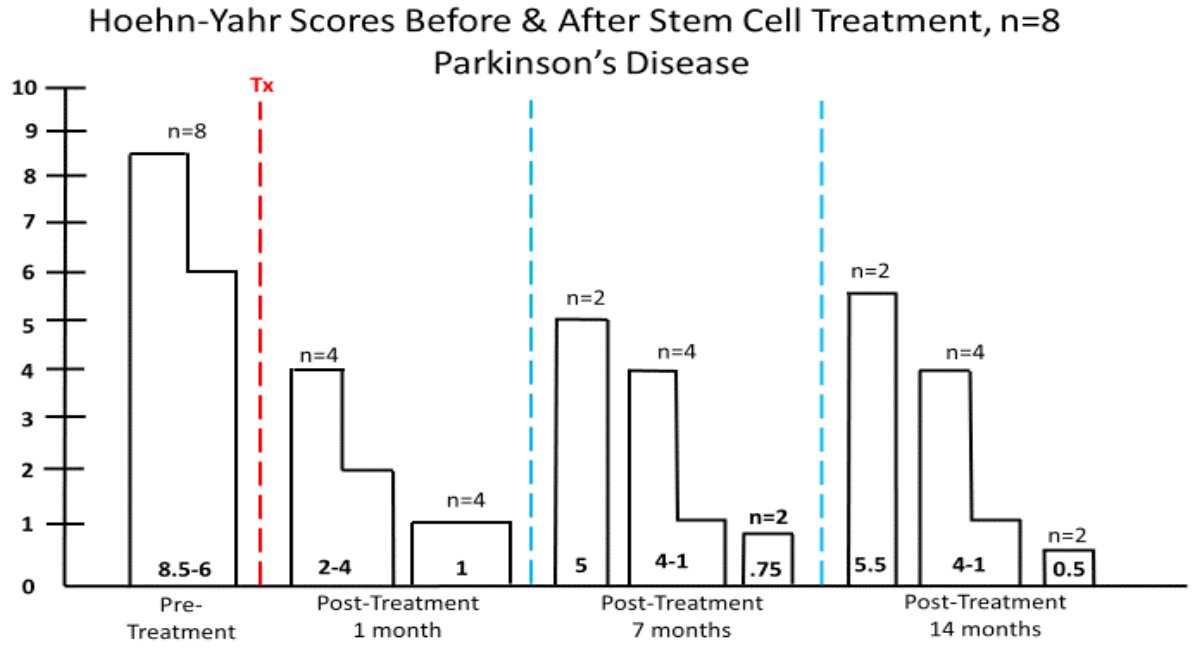

Figure 7: Hoen-Yahr Scoring expanded to 10-Point scale where confinement = 10 and normal = 0. IRB-approved small cohort clinical trial (n=8) through 14-month period. Pre-treatment range of H-Y scores between 8.5-6. At 1-month post-treatment, sorted into two groups: H-Y score of 4-2 (n=4) and H-Y score of 1.0. At 7th-month follow-up assessment sorted into three groups: H-Y score of 5.0 (n=2); stabilized H-Y score of 4-1 (n=4); H-Y score (n=2) of 0.75. At 14th-month follow-up assessment sorted into three groups with same participants in each group as in the 7th-month assessment: H-Y score of 5.5 (n=2); H-Y score of 4-1 (n=4); and H-Y score of 0.5 (n=2). Reprinted with permission from Young HE, Hyer L, Black Jr AC, et al. Treating Parkinson Disease with adult stem cells. J Neurol Disord 2013; 2:1 [47].

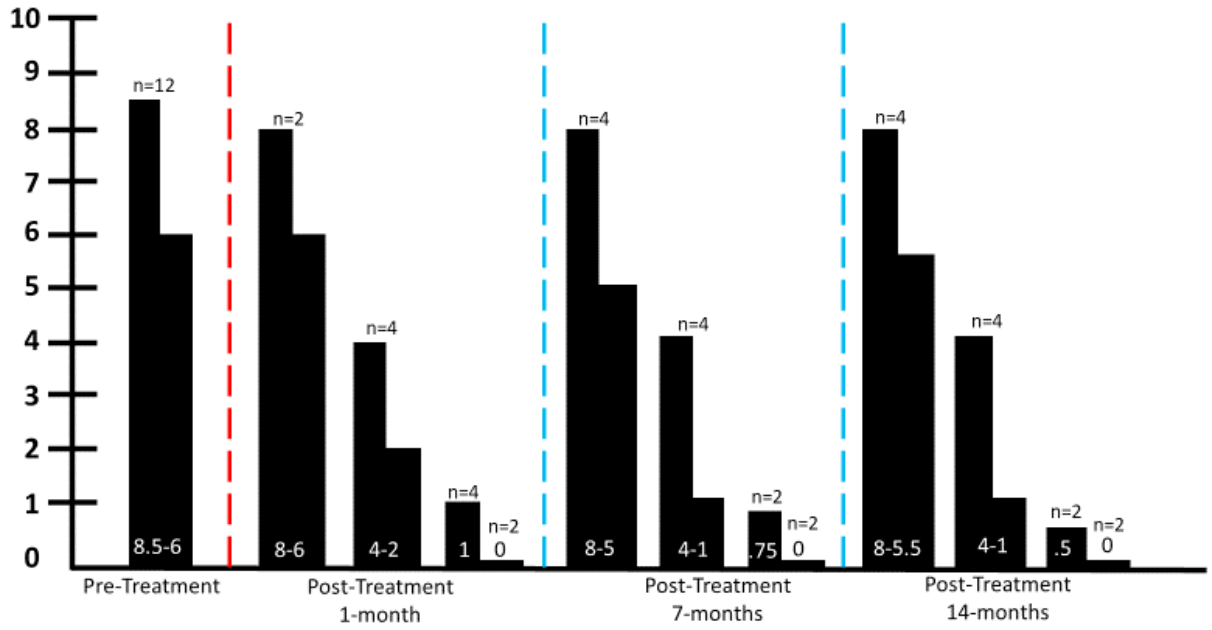

Figure 8: Combined data for small cohort clinical trial (n=12), encompassing 2013 Parkinson trial [43] and additional four participants (current trial). No adverse effects were noted from participants or their caregivers from either trial. 33% (n=4) showed moderate to no benefit of telomerase positive stem cell treatment at 1-month (H-Y: 8-6), and either no benefit or a slow increase in Hoehn-Yahr scores from 7-month (H-Y: 8-5) to 14-month (H-Y: 8-5.5) post-treatment assessments. 33% (n=4) decreased their Hoehn-Yahr scores by about half by 1-month after treatment (H-Y: 4-2), but then remained in stasis at 7-months (H-Y: 4-1) and 14-months (H-Y: 4-1) during post-treatment assessments. The remaining 33% (n=2 + n=2) were either completely void of Parkinsonian symptoms (H-Y: 0, n=2) or continued to decrease in Hoehn-Yahr score at each assessment period following treatment, e.g., 1-month (H-Y: 1.0, n=2), 7-months (H-Y: 0.75, n=2), and 14-months (H-Y: 0.5, n=2). Reprinted with permission from Young HE, Hyer L, Black Jr AC, et al. Treating Parkinson Disease with adult stem cells. J Neurol Disord 2013; 2:1 [47] and the current study.

compliance to informed consent guidelines. One caregiver from the group showing minimal improvement was overheard saying "this stem cell B*** S*** isn't going to work, so why should I bother with all these rules and regulations". Indeed, failure to follow informed consent guidelines also occurred with participants in the concurrent chronic obstructive pulmonary disease clinical trial [54] and in the age-related dry macular degeneration clinical trial [48], with similar outcomes, i.e., failure to follow informed consent guidelines resulted in failure of telomerase positive stem cell treatment(s) to improve quality of life. Another potential difference affecting stem cell activity between the two groups may be the period for isolation. As noted in the concurrent Alzheimer's clinical trial [45], the period for isolation of telomerase positive stem cells for the group showing minimal improvement was ~24-hours and ~73-hours for the group showing maximal improvement of symptoms. This time difference 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. Since separate studies show similar differences, these points need to be addressed in future studies of telomerase positive stem cell treatment of chronic diseases.

When combining the data from the 2013 study [46,47] and the current study, this is still a small cohort combined study (n=12). Thirty-three percent (n=4) of the participants demonstrated moderate to no improvement of their Parkinsonian symptoms with stem cell treatment(s). Thirty-three percent (n=4) of the participants, maintained stasis from one-month post treatment to the end of the study. And 33% (n=4) of the participants significantly improved their quality of life with the loss of most of their Parkinsonian symptoms, for a combined efficacy of 66%, as measured by the Hoehn-Yahr scoring system (Figure 8).

Conclusion

A Parkinsonian animal model was created by stereotactically injecting a dopamine neurotoxin into the substantia nigra pars compacta of the ventral midbrain of adult rats. This was done to test the capability of telomerase positive stem cells to regenerate dopaminergic cells. Scl-40b, a naïve PSC clone, having the capability to form cells of the neural ectodermal lineage, was stereotactically injected into the lesion site. The results showed the generation of new dopaminergic neurons and their associated neural networks. An IRB-approved small cohort (n=8) clinical trial was conducted for the treatment of Parkinson's Disease using the participant's own endogenous adult-derived autologous telomerase positive stem cells, i.e., TSCs given by intranasal delivery and PSCs and MesoSCs by given intravenous infusion. One to two months following administration of the stem cells, all participants noted a positive response. By the 7-month and 14-month post, treatment assessments the participants sorted into

three groups. The first group (25% of participants, n=2) began to decline, but at a much slower rate than before their stem cell treatment. The second group (50% of participants, n=4) remained in stasis following their response at one month. The third group (25% of participants, n=2) almost completely lost all their Parkinsonian symptoms as measure by the Hoehn-Yahr scale. We have since added four participants to this clinical trial for PD. Two participants showed no benefit of treatment, while two participants showed complete loss of symptoms. This discrepancy in findings might be attributed in failure to follow informed consent guidelines and/or the processing time needed to isolate, segregate, and activate the telomerase positive stem cells prior to treatment. No adverse effects were reported by any participant or their respective caregiver for the entire combined small cohort (n=12). Taken together as a 2021 update of this on-going clinical study, 33% (n=4) showed moderate to no benefit of telomerase positive stem cell treatment by continuing to show a decline; 33% (n=4) remained in stasis after the first month of treatment; and 33% (n=4) resolved their symptoms. The results suggest that autologous telomerase positive stem cells, TSCs, PSCs, and MesoSCs, are both safe and efficacious (66%) to reduce the symptoms of Parkinson's Disease in diagnosed individuals.

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