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Blunt Force Trauma-Induced Total Bilateral Visual Impairment of 13 Years Duration Treated with Autologous Telomerase-Positive Stem Cells

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ABSTRACT

Loss of visual acuity may result from genetics, cancer, metabolic disorders, trauma, or aging. Adult telomerase-positive totipotent stem cells have been identified in multiple species of animals, including humans. Characterization studies to identify differentiated cells utilized three clones of adult-derived totipotent stem cells (TSCs) and treated them with induction factors consisting of chemical mediators, human recombinant proteins and cell-specific exosomes. Results demonstrated that the TSC clones would form cells expressing phenotypic markers of the neural ectodermal lineage, e.g., ectodermal stem cells, ectodermal progenitor stem cells, neurons, ganglion cells, glial cells, and neural crest derivatives. Autologous TSCs were shown to partially restore function in clinical trial participants with Alzheimer's disease, Parkinson's disease, and Age-related Dry Macular Degeneration. It was hypothesized that following intranasal infusion, TSCs would migrate to areas associated with the visual pathway to repair and/or regenerate damaged and/or missing cells, thus restoring function. In this small cohort study (n=1), the presenting symptom for a 17-year-old female was total bilateral visual impairment (complete blindness) of 13-years duration, due to severe head trauma from an automobile accident at four years of age. Following her first TSC treatment, she could see indistinct black shapes on a background of a dark shade of gray. Following her second TSC treatment she could see background as a lighter shade of gray and a black square with slightly more distinct borders. No adverse effects were noted after either autologous TSC treatment. These results suggest that two treatments with TSCs were both safe and somewhat efficacious in helping to partially restore her 'night' vision.

Keywords

Blindness, Trauma-induced, Adult, Stem Cells, Totipotent Stem Cells, Telomerase Positive, Regenerative Medicine, Total Bilateral Visual Impairment, ESCs, iPSCs, MSCs.

Introduction

Worldwide as of 2015, 940 million people had some sort of vision loss; 240 million people were diagnosed as having low vision; and another ~40 million people were diagnosed with total bilateral visual impairment. The developing world contains most people over the age of 50 with low visual acuity. Visual impairments have considerable impact on worldwide gross national product (GNP), due to cost of treatment(s) and inability of the individuals to work. Visual impairment is defined as reduced vision not corrected with

either contact lenses or glasses. The World Health Organization (WHO) classifies visual impairment (Table 1, Figure 1), when vision is measured with the better eye [1-3].

Table 1: Visual Impairment Defined by the WHO using Best Eye with Best Possible Corrective Lenses.

Visual Acuity	Definition
20/20	Normal vision
20/30 to 20/60	Near normal vision to mild vision loss
20/70 to 20/160	Moderate low vision or moderate visual impairment
20/200 to 20/400	Severe low vision or severe vision impairment
20/500 to 20/1,000	Profound low vision or profound visual impairment
Greater than 20/1,000	Near total blindness or near-total visual impairment
No Light Perception (NLP)	Total blindness or total visual impairment

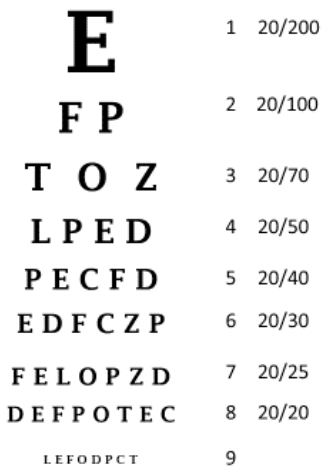

Figure 1: A Snellen eye chart used to measure visual acuity. Named for Dr. Herman Snellen, a Dutch Ophthalmologist who developed the chart in 1862 [115].

In the United States, partial sightedness indicates some sort of visual problem, and can be further subdivided based on the ability of the individual to see objects. Near-sightedness (myopia) is not being able to see distant objects clearly; whereas far-sightedness (hyperopia), is not being able to see close objects clearly. Low vision refers to individuals who are unable to read at a normal viewing distance even with visual correction. Legally blind indicates that an individual has visual acuity of less than 20/200 in their best eye with vision correction (either glasses or contact lenses). Totally blind is considered as total bilateral visual impairment or no light perception of any kind (Figure 2) [4].

The major causes of total bilateral visual impairment are genetics, cancer, metabolic disorders, neurodegenerative disorders, trauma, and aging. Neurodegenerative disorders include almost all retinal dystrophies and share the loss bilaterally of retinal pigmented epithelium, photoreceptor cells, and/or retinal ganglion cells [5]. Traumatic eye injuries, including blunt force trauma and penetrating eye injuries, are the leading cause of unilateral (monocular) blindness in the United States [6]. Although not as frequent an occurrence as unilateral blindness, blunt force head trauma and/or bilateral penetrating eye injuries can also cause disruptions at one or more sites along the visual pathway from retina to primary visual cortex, e.g., eye globe, optic disc, optic nerve (Fig. 3); optic chiasm (Fig. 4); optic tract, lateral geniculate body, optic radiations, and primary visual cortex, that would engender total bilateral visual impairment (Figure 5).

Traumatic optic neuropathy is characterized by sudden vision loss following facial trauma leading to variable visual field defects [7]. Ocular trauma, either by blunt force or penetrating injuries, is the leading cause of non-congenital unilateral blindness in children less than 20-years-of-age where visual prognosis is poorer in penetrating injuries than in mild blunt force trauma. Most ocular trauma injuries in children occur from sports, recreation, or automotive accidents [8-10]. Most automotive injuries arise from deployment of air bags, which can cause corneal abrasions, hyphemia (collection of blood within anterior chamber of the eye), vitreous hemorrhage, retinal hemorrhages, retinal tears, and retinal detachments [10-15]. In children up to age 14, serious automotive-associated ocular injuries could be prevented if children were not sitting in the front seat during an accident, with subsequent deployment of air bags [16].

Currently, there is no pharmacological or surgical cure for unilateral (monocular) or total bilateral (binocular) visual impairment. Current research for resolving neurodegenerative diseases and trauma is focusing on regenerative medicine, a multidisciplinary field that focuses on repairing, replacing, and/or regenerating damaged or missing tissues and organs in hopes of restoring their histoarchitecture and physiological function. Important players in the realm of regenerative medicine are stem cells, secreted paracrine exosomes, and gene therapy. Treatments combining the best of these important players support the concept that clinically meaningful regeneration to correct visual impairment may be achievable in the future [17].

Specific types of stem cells have been proposed to regenerate cells and their cell processes in the retina, which is the initiation site of the visual pathway. The cells in the retina are pigmented epithelial cells, photoreceptor cells, and retinal ganglion cells. Proposed stem cells utilized for their treatment include embryonic stem cells, induced pluripotent stem cells, embryonic retinal progenitor cells, mesenchymal stem cells (MSCs) and medicinal signaling cells (MSCs). The MSCs can be derived from either bone marrow, adipose tissue, umbilical cord Wharton's jelly, umbilical cord blood, dental pulp, or periapical mesenchymal cyst [5-29].

We would hypothesize an alternative to embryonic stem cells, induced pluripotent stem cells, embryonic retinal progenitor cells, mesenchymal stem cells, or medicinal signaling cells. This alternative being naturally occurring autologous telomerase positive stem cells. These autologous stem cells, e.g., endogenous adult (post-natal) telomerase positive totipotent stem cells (TSCs), pluripotent stem cells (PSCs), and mesodermal stem cells (MesoSCs), would be a potential treatment option for monocular or binocular total visual impairment by restoring function to cells

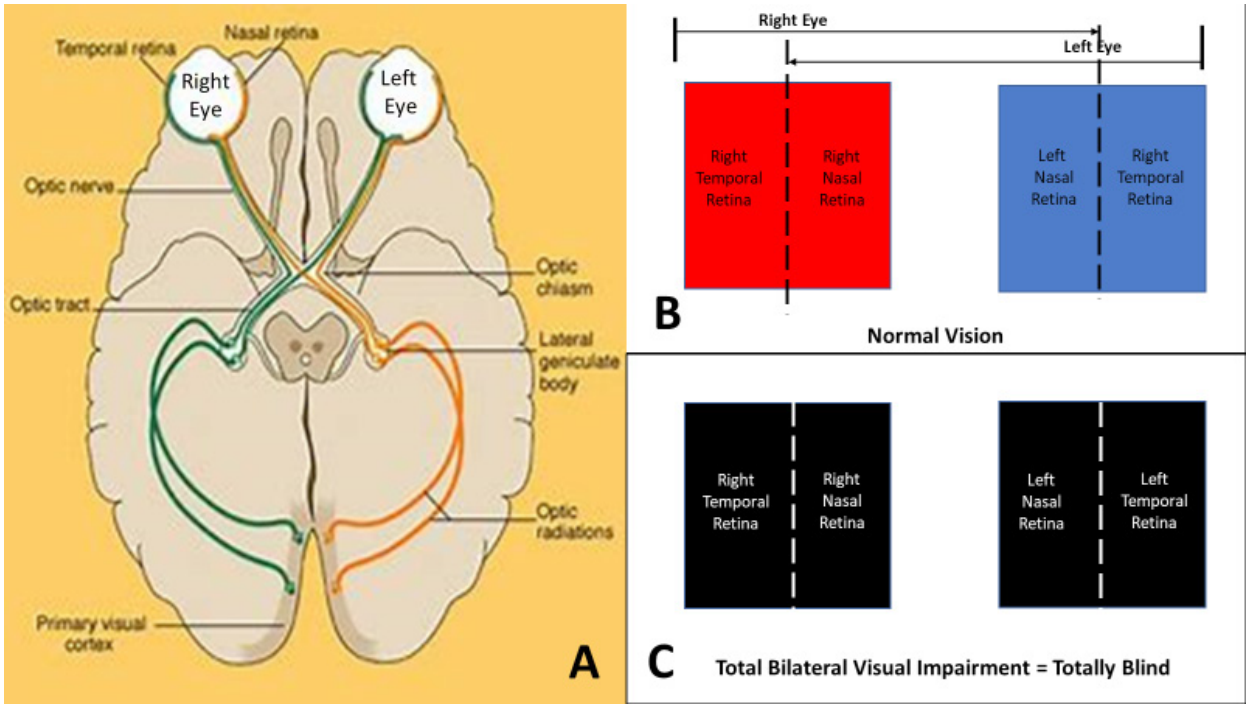

Figure 2: Visual pathways for right and left eyes. A: signal pathway from retinas (temporal and nasal) to optic nerve, to optic chiasm where signals from temporal retinas stay ipsilateral (same) side and signals from nasal retinas cross over to contralateral (opposite) side to continue as optic tracts. Optic tracts synapse with cell bodies in lateral geniculate nuclei and send optic radiations (fiber tracts) to primary visual cortex in occipital lobes of cerebral hemispheres. B: Right visual field = right temporal retina, right nasal retina, and left nasal retina. Left visual field = left temporal retina, left nasal retina, and right nasal retina. C: Total bilateral visual impairment (totally blind) = total vision impairment in right temporal retina, right nasal retina, left nasal retina, and left temporal retina, or bilaterally in their associated optic discs, optic nerves, optic tracts, optic radiations, and primary visual cortex within occipital lobe of the cerebral cortex. A: Reprinted with permission from Visual Pathway Lesions http://kellogg.umich.edu/theeyeshaveit/anatomy/visual_pathway_lesions.html.

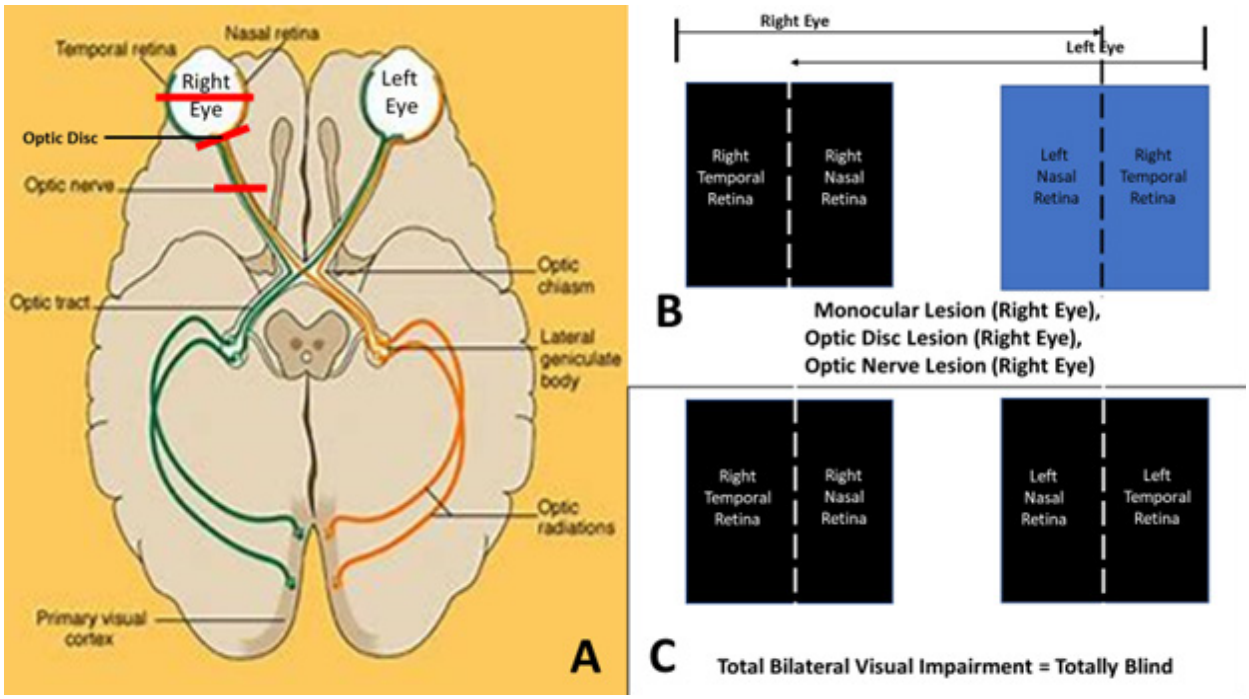

Figure 3: Diagram representation of three lesions that can cause a monocular vision loss of the right eye, affecting the right temporal and nasal retina, the optic disc, and/or the right optic nerve. Any of these three lesions will engender vision in the left eye only. To cause total bilateral visual impairment would necessitate bilateral lesions. A: Original illustration reprinted with permission from Visual Pathway Lesions http://kellogg.umich.edu/theeyeshaveit/anatomy/visual_pathway_lesions.html.

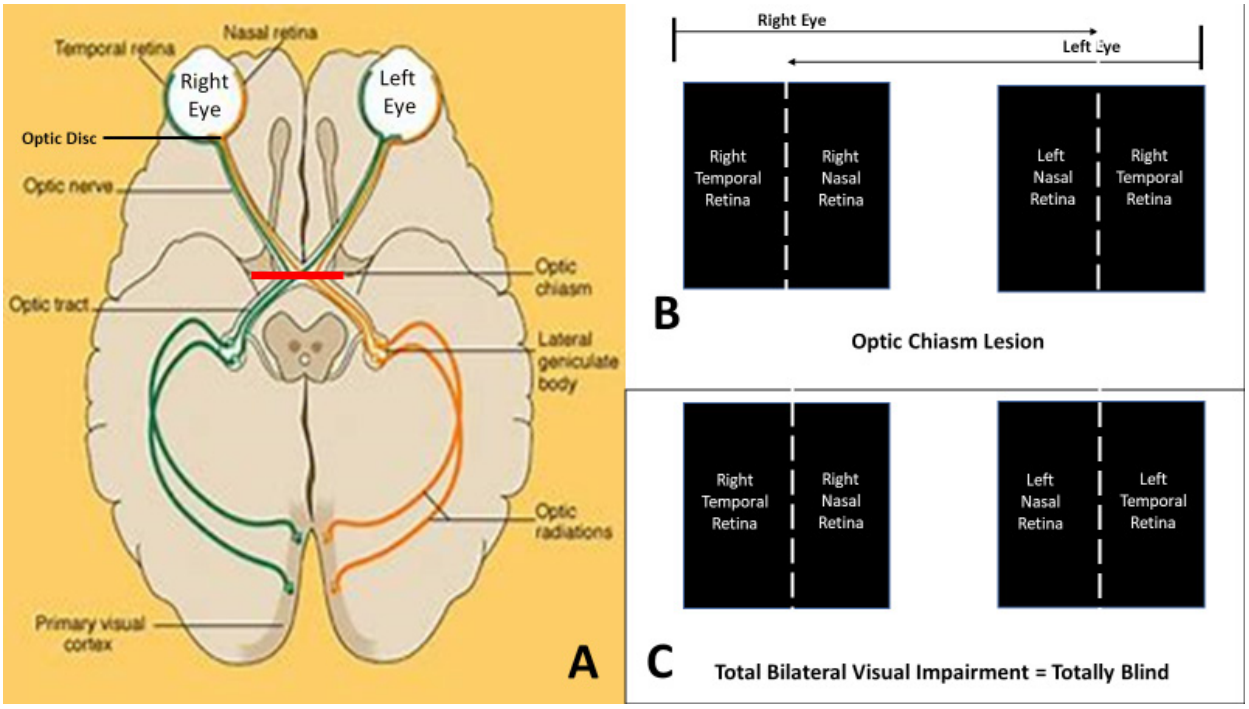

Figure 4: A lesion through the optic chiasm would cause total bilateral visual impairment, due to severance of both temporal and nasal retinas through their respective optic tracts. A: Original illustration reprinted with permission from Visual Pathway Lesions http://kellogg.umich.edu/theeyeshaveit/anatomy/visual_pathway_lesions.html.

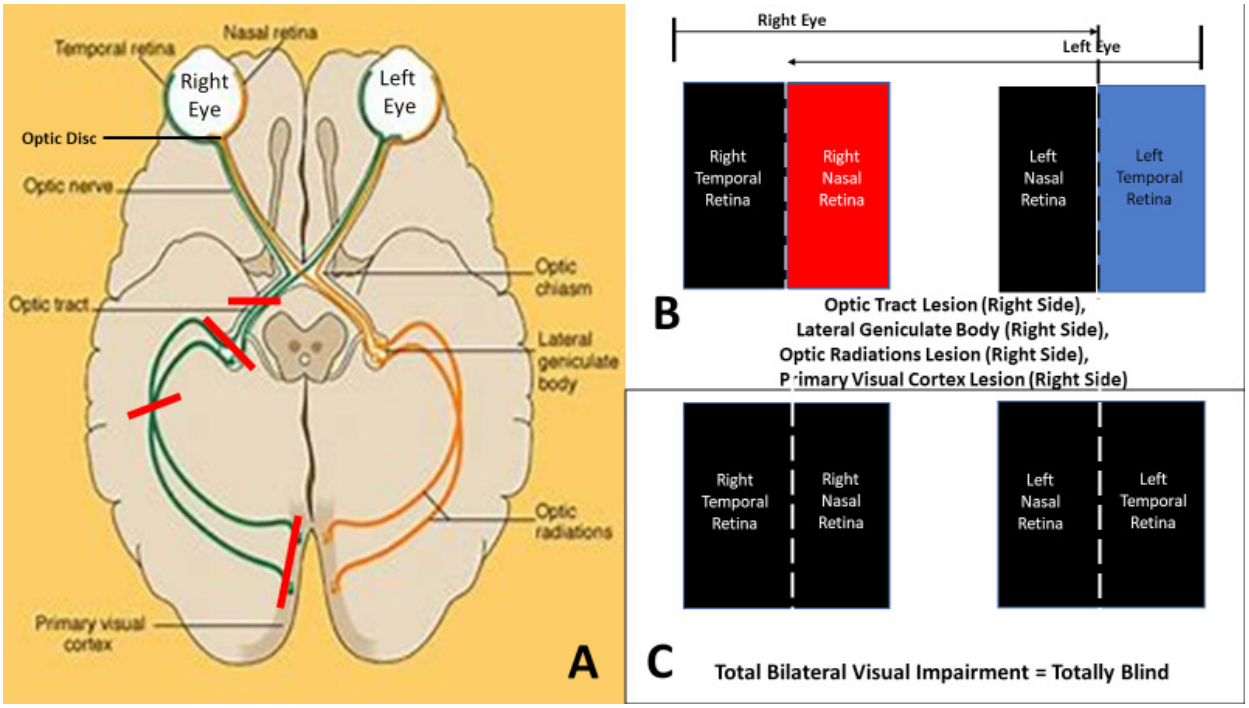

Figure 5: Diagram representation of four lesions (e.g., optic tract, lateral geniculate body, optic radiations, primary visual cortex within occipital lobes of cerebral cortex), any of which can cause visual impairment in the right temporal lobe and left nasal retina. This would result in tunnel vision in the right eye, whereby there is a central area of visual acuity and absence along periphery; and macular degeneration of the left eye, whereby there is vision loss in the center area and acuity along the periphery. To cause total bilateral visual impairment would necessitate either bilateral lesions in any of the lesion sites or two lesions, one on right side and one on left side, of the four lesion sites noted. A: Original illustration reprinted with permission from Visual Pathway Lesions http://kellogg.umich.edu/theeyeshaveit/anatomy/visual_pathway_lesions.html.

throughout the visual pathway, through regeneration of damaged cells from the retina to the primary visual cortex.

In culture [30-33], in animal models [34-37], and in human clinical studies [37-39], TSCs and PSCs were shown to differentiate into neuronal cell types, home to sites of tissue damage, replace damaged (neuronal) tissues, and restore function to the individual. MesoSCs have been shown to act in a supporting role to revascularize damaged tissues in cerebral cortex [34-37], in cardiovascular disorders [40,41] and in pulmonary disorders [42-44]. These safety and efficacy clinical studies suggested the potential for telomerase-positive stem cells to be used as treatment modalities for individuals with various types of visual impairment.

Bilateral visual impairment can be caused by interruption anywhere along the length of the visual pathway from the retina to the primary visual cortex, e.g., retina, photoreceptors, retinal ganglion cells, optic disc, optic nerve, optic chiasm, optic tract, lateral geniculate body, optic radiations, and primary visual acuity centers in the bilateral occipital lobes of the cerebral cortex (Figures 3-5). Since telomerase-positive adult stem cells have an inherent homing guidance system to migrate to areas of damaged tissues and to restore the function of those tissues by repair and/or regeneration [34,35,37,38-44], it was hypothesized that the telomerase positive stem cells would migrate along the visual pathway from the retina to the primary visual cortex and restore function to any damaged cells and their cell processes, thereby resolving visual impairment in the individual. In this small cohort study (n=1), a 17-year-old female, with total bilateral visual impairment since age 4 due to head trauma from an automobile accident, was treated with two successive rounds of fresh isolate autologous telomerase positive stem cells.

Materials and Methods

Autologous adult-derived telomerase-positive stem cells, e.g., totipotent stem cells (TSCs), pluripotent stem cells (PSCs), and mesodermal stem cells (MesoSCs) were tested in an IRB-approved study protocol for neurodegenerative disorders, e.g., Parkinson disease [35,37,38], Age-Related Dry Macular Degeneration [39], Alzheimer's disease [45], total bilateral visual impairment [this study], traumatic spinal cord injury [46], traumatic brain injury [47], chronic idiopathic demyelinating polyneuropathy [48], stroke [49], and Amyotrophic Lateral Sclerosis [50] in participants 18 to 120 years-of-age. Special permission was obtained to treat the 17-year-old female, since she was younger than the approved 18-120-year age group.

Stem cell participants were mandated to follow the informed consent guidelines for telomerase-positive stem cells for clinical therapy [51]. These guidelines consisted of a defined protocol to maximize the number of telomerase-positive stem cells for harvest and subsequent repair of the tissues. The informed consent guidelines 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 telomerase positive stem cells from differentiating. Avoid use of corticosteroids because they prematurely differentiate TSCs and PSCs into the mesodermal lineage. Ingestion of combinatorial nutraceuticals (CN) (DFRD, Macon, GA) daily for a minimum of 30 days prior to initial harvest and then throughout subsequent treatments to increase the number of telomerase-positive stem cells within the person for harvest. Stay well hydrated with aqueous-based fluids two weeks before stem cell harvest to allow ease of harvesting stem cell. Limit moderate to excessive exercising during a two-week window around fresh isolate stem cell harvest/treatment to maximize directed repair responses. And to ingest glacial caps (GC, DFRD) 18 hours before a stem cell harvest to mobilize telomerase positive stem cells into the blood stream [51].

The harvesting of the 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 (TSCs, PSCs, and MesoSCs) were separated from the blood elements (RBCs, WBCs, platelets) utilizing FDA-mandated 'minimal manipulative procedures' encompassing gravity/zeta potential and differential density gradient centrifugation with serum, saline, and distilled water density gradients [35,37-50]. Utilizing these procedures, the blood elements were separated from the telomerase positive stem cells. The stem cells were further segregated into individual populations of TSCs, PSCs, and MesoSCs, and activated. The TSCs were concentrated into two aliquots of 0.25cc's each. The subjects' nostrils were cleaned of mucus with 0.65% sterile saline. The subject was placed into the reversed Trendelenburg position (nostrils pointing upward) and one 0.25cc aliquot applied to each nostril in a dropwise fashion (Figure 6). The subject remained in the reversed Trendelenburg position for an additional five minutes after the second application to ensure that the TSCs had sufficient time to migrate to the olfactory bulbs lying just dorsal to the cribriform plate. The participant was then placed into an upright position. The activated PSCs and MesoSCs were pooled and diluted into 250cc's of sterile normal heparin/saline and infused into the median cubital vein by regular intravenous (IV) infusion. The participant was cautioned to rest the remainder of their stem cell treatment day. Following the above protocol, the participant received two autologous telomerase positive stem cell treatments [35,37-39,45-50].

Results

A white placard with red and blue boxes containing black squares was used to test visual impairment in the participant (Figure 7A). Before stem cell treatment began, she stated that "I can't see anything, everything is black" (see representation in Figure 7B). Two months following her first telomerase positive stem cell treatment, she stated that "I can see a fuzzy black spot on a dark gray fuzzy background" (see representation in Figure 7C). Two months following her second stem cell treatment, she stated that "I can see a more distinct fuzzy black square on a lighter gray fuzzy background" (see representation in Figure 7D).

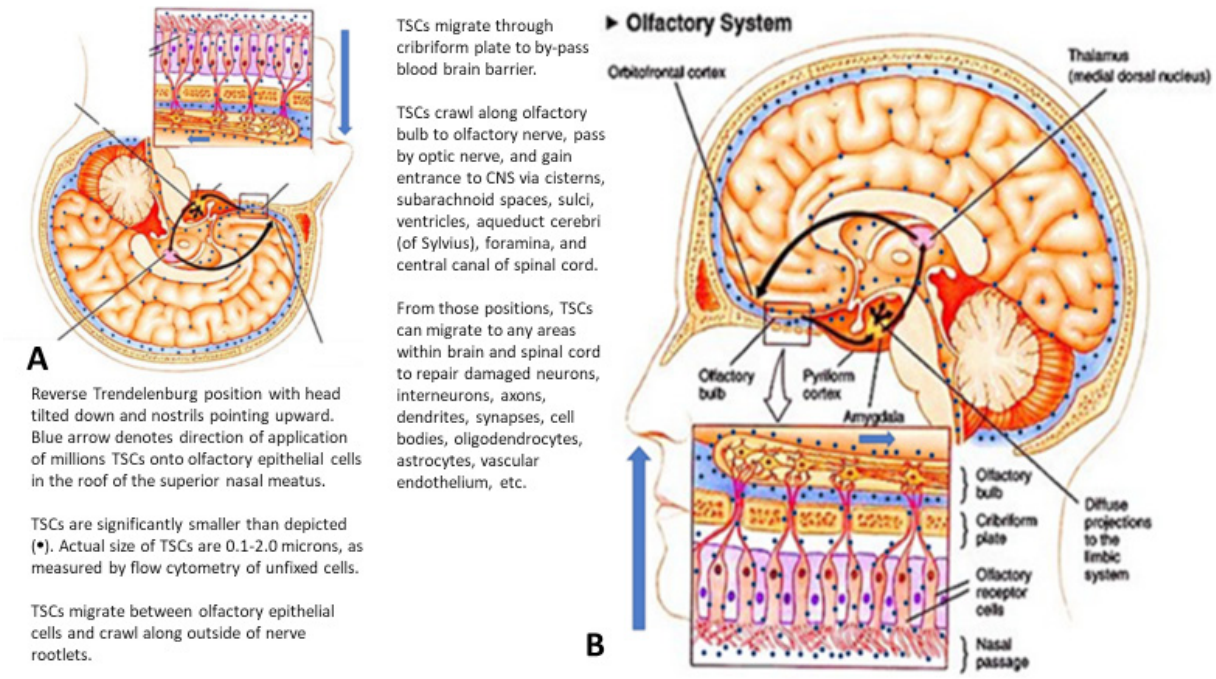

Figure 6: Diagram of TSCs by-passing blood-brain barrier at cribriform plate to gain entry to central nervous system, e.g., brain and spinal cord. The nasal mucus from each nostril is removed by washing with 0.65% sterile saline. The patient is placed into the reversed Trendelenburg position (nostril openings pointing towards ceiling) and millions of TSCs are deposited dropwise onto the olfactory epithelium in the roof of the superior nasal meatus. The TSCs migrate between the olfactory cells, migrate along the outside of the olfactory nerve rootlets, through the cribriform plate, to the olfactory bulbs. The TSCs then migrate from the olfactory bulbs to the olfactory nerves, then along the olfactory nerve, passing by the optic nerves, to gain access to outside of brain via cisterns and inside of brain and spinal cord via cisterns, subarachnoid spaces, sulci, lateral ventricles, third ventricles, aqueduct cerebri (of Sylvius), fourth ventricles, foramina, and central canal of spinal cord. Time frame for migration of TSCs from being deposited onto olfactory epithelium in roof of superior meatus in nose to appearance in the caudal equina of spinal cord averaged 45 minutes. Original illustration reprinted with permission from "Smell is a Symphony", March 19, 2012, Neuroscience News. March 24, 2012. Neuro News & Cosmos Clues, New Model of Olfactory System, https://protoplasmix.wordpress.com/2012/03/31/new-model-of-the-olfactory-system/?blogsub=confirming#subscribe-blog.

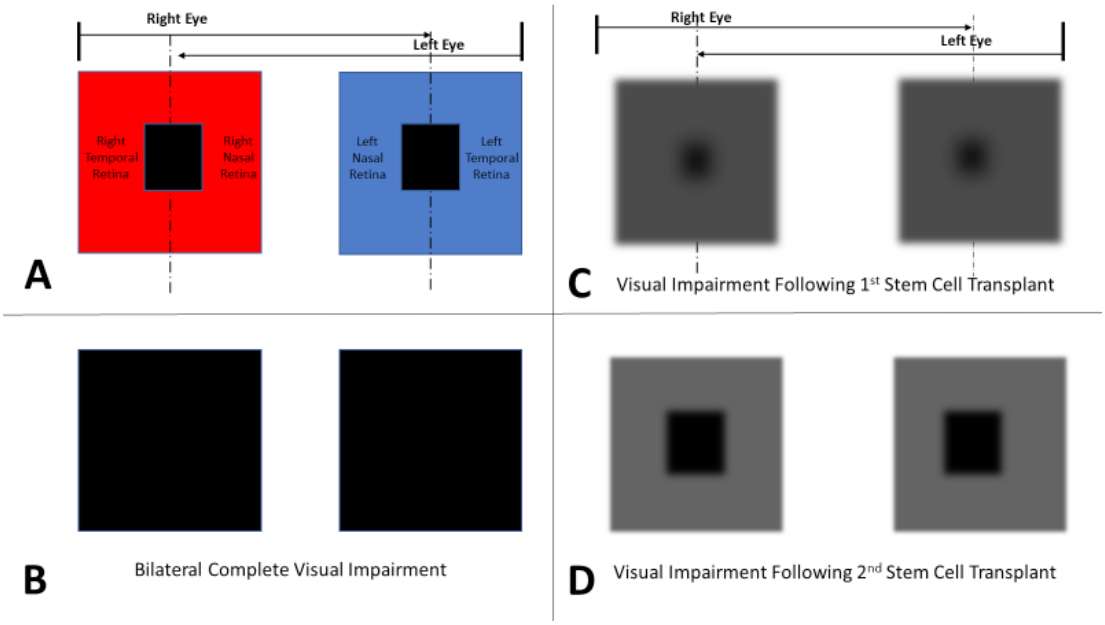

Figure 7: A: A white placard with red and blue boxes containing black squares was used to test visual impairment in the participant. **B:** Before stem cell treatment began, she stated that "I can't see anything, everything is black". **C:** Two months following her first telomerase positive stem cell treatment, she stated that "I can see a fuzzy black spot on a fuzzy dark gray background". **D:** Two months following her second stem cell treatment, she stated that "I can see a slightly less fuzzy black square on a slightly less fuzzy lighter gray background".

Discussion

National Eye Institute (NEI) of the National Institute of Health (NIH) developed an 'Audacious Goals Initiative' in 2013 for restoration of usable vision in humans through regeneration of retinal pigmented epithelium, photoreceptor cells, neurons, retinal ganglion cells, and neural connections from the eye and throughout the visual system and creating a cellular environment for neuro-regeneration by the year 2025 [52]. However, regeneration of lost cells may be insufficient due to damage of other neurons and non-neuronal cells, gliosis, and fibrosis, which may make regeneration of neuronal cells problematic. There are advantages for doing clinical trials in patients with acute versus chronic problems. In acute instances, supporting cellular and structural constituents are still likely to be present. In contrast, chronic conditions may make it easier to detect incremental improvement in visual function. AGI-funded NEI studies have developed three groups to study stem cell-based therapies: 1) noninvasive functional imaging systems, 2) novel neuronal factors in visual system, and 3) model systems to study regenerated neurons [53-57].

The major causes of total bilateral visual impairment are genetics, cancer, metabolic disorders, neurodegenerative disorders, trauma, and aging. Neurodegenerative disorders include almost all retinal dystrophies and share the loss of retinal pigmented epithelium, photoreceptor cells, and/or retinal ganglion cells (Figure 3) [5]. Traumatic eye injuries, including blunt force trauma and penetrating eye injuries, are the leading cause of monocular blindness in the United States. Although of rare occurrence, both blunt force head trauma and/or bilateral penetrating eye injuries can cause disruptions at one or more sites along the visual pathway from retina to primary visual cortex that would engender total bilateral visual impairment, e.g., retinal (1-3) (pigmented epithelium , photoreceptor cells, retinal ganglion cells), optic disc (4), optic nerve (5), optic chiasm (6), optic tract (7), lateral geniculate body (8), optic radiations (9), and primary visual cortices (10) of bilateral occipital lobes of cerebral cortex (Figure 8) [6].

Significant barriers remain in the ability to restore visual function following traumatic injury or disease-induced regeneration. To date, neither medications nor surgical interventions are sufficient to halt or reverse lost vision [60-64].

With the advent of regenerative medicine for chronic and incurable diseases, where other therapies have proved less than adequate, there is hope that information gleaned from these studies can be applied to treating participants with total visual impairment. Current sites in the visual pathway for regenerative medicine are the retina (pigmented epithelial cells, photoreceptors, and ganglion cells) and the optic nerve (retinal ganglion cell axons). Current players in regenerative medicine for these sites are stem cells, paracrine exosomes, and gene therapy [5-29].

Retina: Retinal Pigmented Epithelial Cells, Photoreceptors, & Retinal Ganglion Cells

Neurodegenerative disorders, including almost all retinal dystrophies share the specific loss of retinal pigmented epithelium, photoreceptor cells, and/or retinal ganglion cells. Retinal ganglion cell axons travel through the optic nerve and carry all visual signals to the lateral geniculate bodies where they synapse, and the visual signals continue through the optic radiations to the primary visual cortex. After injury, retinal ganglion cell axons usually fail to regrow and die, leading to irreversible vision loss [65].

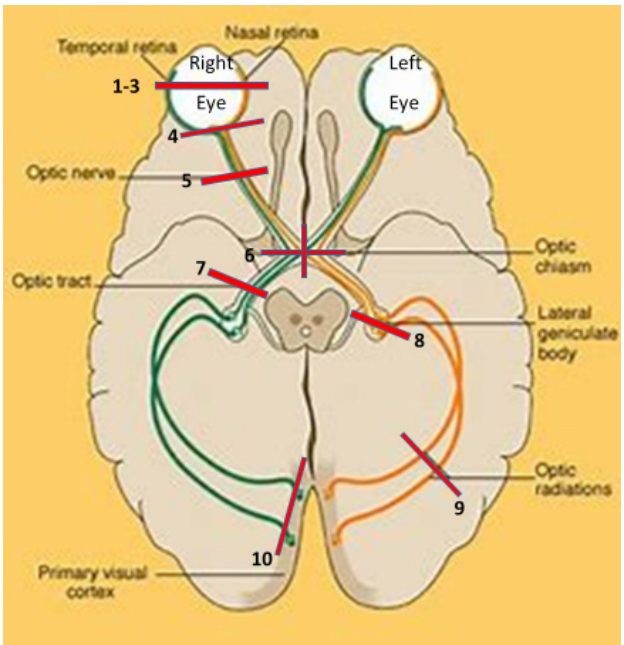

Figure 8: Diagrammatic representation of one or more potential bilateral lesions necessary to engender complete total visual impairment, e.g., retinal (1-3) (pigmented epithelium , photoreceptor cells, retinal ganglion cells), optic disc (4), optic nerve (5), optic chiasm (6), optic tract (7), lateral geniculate body (8), optic radiations (9), and primary visual cortices (10) of bilateral occipital lobes of cerebral cortex. Diagram reprinted with permission from Visual Pathway Lesions http://kellogg.umich.edu/theeyeshaveit/anatomy/visual_pathway_lesions.html.

Retinal degenerative diseases are the leading cause of irreversible blindness. Cell death of retinal pigmented epithelial cells and photoreceptors is main cause of vision loss in retinitis pigmentosa, Stargardt disease, Leber congenital amaurosis, and age-related macular degeneration, which are major causes of vision loss and blindness worldwide [65-68]. Based on preliminary Phase-1/-2 trials of retinal pigmented epithelium transplantation, no single approach is likely to succeed in overcoming photoreceptor loss in all cases [69].

Optic Nerves to Primary Visual Cortex

Damage to axonal processes/cells in the optic nerve, optic chiasm, optic tract, lateral geniculate body, optic radiations, and/or primary visual cortex can lead to cell death, with no avenue for replacement [65-69]. Traumatic optic nerve injuries are a leading cause of irreversible blindness worldwide and causes progressive visual impairment, which can be attributed to dysfunction and death of retinal ganglion cells. Significant barriers remain in the ability to restore visual function following traumatic injury or disease-induced regeneration. Current treatments for optic nerve injuries include high dose corticosteroids, optic nerve decompression, or high dose corticosteroids with optic nerve decompression. However, to date, neither medications nor surgical interventions are sufficient to halt or reverse lost vision [7,63,65,67].

Induced Pluripotent Stem Cells (iPSCs), Embryonic Stem Cells, Embryonic Retinal Progenitor Cells

Both stem cells and progenitor cells have been studied in acute cases of visual impairment as potential therapeutic interventions for degenerative eye diseases. Cell replacement therapies need these cells as well as their associated circuitry for repair to be successful. They have been studied with respect to replacing photoreceptor cells, retinal pigmented epithelial cells, lost neurons, or ganglion cells; restoring neural circuit; or as paracrine mediated exosome therapies to protect compromised retinal neurons from death or induce growth of new synapses. Sources of these cells include pluripotent stem cells, fetal cells, and postnatal tissues.

Induced pluripotent stem cells (iPSCs) can be programmed to specific retinal cell fates with high yields and acceptable purity for clinical trials. One potential approach is the generation of these cells using iPSCs to develop new sources of cells. iPSCs can generate retinal pigmented epithelial cells, photoreceptor cells, retinal ganglion cells for therapies [5,18,67]. Early-stage clinical trials of retinal pigmented epithelium and photoreceptors generated from human embryonic cells (ESCs) or iPSCs, retinal progenitor cells, as well as paracrine-containing exosomes derived from mesenchymal stromal cells (MSCs / medicinal signaling cells) from bone marrow, adipose tissue, dental pulp, or umbilical cord tissue, have shown preliminary safety and a potential for visual acuity benefits. Intravitreally injected autologous bone marrow mononucleated cells for hereditary retinal dystrophy demonstrated no evidence of toxicity with a potential visual acuity benefits but no structural or functional changes. Undifferentiated neural stem cells may act as both a reservoir for differentiating neurons as well as providing paracrine factors via exosomes [20-27].

Human embryonic stem cells have been proposed as a source of replacement cells in regenerative medicine, including visual-related lesions and diseases. Their spontaneous plasticity and unlimited capacity for self-renewal raises concerns relating to their safety, tumor formation, potential immuno-rejection, and risk of differentiating into unwanted cell types. Two prospective phase -I/-II studies were performed to assess safety and tolerability of pre-induced hESC-into retinal pigmented epithelium and transplanted beneath the retina. Results of these studies show evidence of medium-term and long-term safety, graft survival, and a potential for biological activity in pre-differentiated hESCs for visual diseases [67]. Embryonic retinal progenitor cells have been suggested as a possible replacement cell type for retinal ganglion cells [19].

MSCs & Extracellular Vesicles (Exosomes)

Currently, most studies concerning regenerative medicine focus on tissue repair, regeneration, and return of function. MSCs are among the most researched topics and represent one of the current hot topics in regenerative medicine. MSCs can be isolated from bone marrow, adipose tissue, umbilical cord Wharton's Jelly, umbilical cord blood, placental tissues, and dental pulp [28-32,68-87]. MSCs are telomerase negative, and thus have a defined 70 population doubling biological clock that begins at birth. Due to storage limitations and because cell senescence and death occurs during in vitro expansion past their biological clock, their clinical application is challenging [36,88].

Within the scientific literature, the acronym MSC has two different meanings. On one hand, the acronym "MSC" is defined as a 'mesenchymal stem cell' expressing cell surface cluster of differentiation (CD) markers CD105, CD117, CD123, and CD166 and, as defined by their function, is a telomerase negative tripotent progenitor stem cell with the capacity to form fat, cartilage, and bone [74,75]. On the other hand, the acronym "MSC" is defined as a 'medicinal signaling cell' expressing cell surface markers CD73, CD90, and CD105 and, as defined by its function, is a telomerase negative stem cell that secretes extracellular vesicles in the form of exosomes that contain proteins, genomic material, and lipids for intercellular communication in normal and pathological processes [73,76-87]. Unfortunately, the terms mesenchymal stem cells (MSCs) and medicinal signaling cells (MSCs) are used interchangeably within the scientific literature. This interchangeability between mesenchymal stem cells and medicinal signaling cells can be confusing for those not well versed in stem cell literature since MSCs have decidedly different CD marker profiles (CD105, CD117, CD123, and CD166 versus CD73, CD90, and CD105) and different functions (form fat, cartilage, and bone versus secreting exosomes for intercellular communication).

MSCs (medicinal signaling cells) secrete extracellular vesicles (exosomes), containing factors with unique anti-inflammatory, anti-apoptotic, anti-microbial, anti-oxidative, and reparative properties. This is accomplished through a paracrine signaling pathway. The secreted exosomes not only have the same effects as MSCs (medicinal signaling cells), but they also have the advantages

of targeted delivery, low immunogenicity, and high repairability. Exosomes are 30-150 nm extracellular vesicles secreted by most cell types, both in vitro and in vivo. They exert their function by transporting proteins, nucleic acids, and lipids between cells and organs, and to act as intercellular communicators in normal and pathological biological processes. Currently, extracellular vesicles from MSCs (medicinal signaling cells) are being used as therapeutic agents by themselves without their parent MSCs (medicinal signaling cells), since they lack the capacities to self-replicate, ectopic differentiation, tumor formation, genetic instability, and cellular rejection of the immune system MSC (medicinal signaling cell)-derived extracellular vesicles (e.g., EVs, exosomes). The EVs (exosomes) act as mediators by exchanging proteins and genetic information (microRNAs, mRNAs, long non-coding RNAs, and phospholipids) between MSCs (medicinal signaling cells) and target cells. EVs (exosomes) appear to be a better choice than MSCs (medicinal signaling cells) for intravitreal injection because of their small size. They can pass through biological barriers more easily and their contents can be genetically modified to serve as drug delivery vehicles [28,73,76-87].

Gene Therapy

Therapeutic options for retinal degenerative diseases remain limited. Almost 30 years have passed since the first causative gene for retinitis pigmentosa was discovered. Since then, 250 genes have been identified and therapies utilizing those genes are being pursued [88]. Thus, gene therapy and stem cell-based approaches offer potential therapeutics for visual degenerative diseases [66].

Data from Clinical Trials

Many stem cells, e.g., ESCs, iPSCs, MSCs, embryonic retinal ganglion cells, etc., currently in clinical trials for ocular therapies do not have preclinical safety or efficacy data. Although some early phase trials suggest acceptable safety profiles [20]. Other reports involving autologous, non-ocular MSCs have been linked to severe blinding complications. We would hypothesize that in those instances, intravitreal injection of MSCs (tripotent mesenchymal stem cells with potential to form fat, cartilage, and bone) occurred instead of injection of MSCs (medicinal signaling cells secreting exosomes) for intercellular signaling, due to their perceived interchangeability of function. These types of adverse events have raised concerns about safety of retinal stem cell transplantations and whether patients are protected from harm [20,89,90]. In clinical trials of MSCs (medicinal signaling cells), or their exosomes, with various degenerative diseases, including those of ocular origin, it was noted that MSCs (medicinal signaling cells) engendered no adverse events and were safe to transplant. However, their efficacy for reversing the effects of the diseases were less than stellar to non-existent [89-100].

Stem cell and/or progenitor cell interventions have potential to address blinding retinal diseases, affecting millions of people worldwide. However, currently, no FDA-approved stem cell therapies for retinal disease exist. Therefore, there is a need for continued clinical trials to test various types of stem cells [20,21].

Telomerase Positive Stem Cells

Most of the clinical trials use ESCs, iPSCs, embryonic retinal ganglion cells, or medicinal signaling cells and their associated extracellular vesicles/exosomes to treat acute monocular visual impairment. In contrast, we chose a different option for treating bilateral visual impairment of 13-years duration in a 17-year-old female. That option being using her own autologous (post-natal) telomerase positive stem cells. The endogenous telomerase positive stem cells, e.g., TSCs, PSCs, and MesoSCs, were chosen for several reasons. These reasons included their inherent biological clock, unlimited proliferation potential due to presence of the telomerase enzyme, ability to form multiple cell types, inherent homing mechanism to migrate to sites of tissue damage, responding to locally released environmental cues (e.g., exosomes) to form only those cells needed to replace damaged or missing cells, and their ability to restore function to damaged tissues.

Biological Clock & Proliferation Potential

All human cells at birth (e.g., progenitor cells and differentiated cells), or when they start to differentiate into progenitor cells and/or directly into differentiated cells (e.g., ESCs, iPSCs, TSCs, PSCs, and MesoSCs), have 70 telomeres at the ends of each of their chromosomes. With each cell division, a telomere is lost at the ends of each chromosome. When the ends of each chromosome contain zero telomeres, the cells are programmed to die. This is considered their biological clock [33,101].

The difference between telomerase positive ESCs, iPSCs, TSCs, PSCs, and MesoSCs versus telomerase negative progenitor stem cells (e.g., mesenchymal stem cells, medicinal signaling cells, embryonic retinal ganglion cells, etc.) and telomerase negative differentiated cell types (e.g., retinal pigmented epithelium, photoreceptor cells, retinal ganglion cells, lateral geniculate bodies, etc.) is the presence of the enzyme telomerase. In their respective undifferentiated states, the telomerase enzyme adds a telomere to the ends of each of their chromosomes after cell division, maintaining the telomere number at 70. This gives the ESCs, iPSCs, TSCs, PSCs, and MesoSCs essentially the capacity for unlimited proliferation potential if they remain in the undifferentiated state [36,102,103].

Ability to Form Multiple Cell Types

ESCs (derived from blastomeres of the morula) (Figure 9) and TSCs (isolated from adult connective tissues) (Figure 10) have the capability to form all cells of the body, including the gametes. ESCs (derived from blastomeres of the inner cell mass), iPSCs (derived by retroviral insertion of the Yamanaka factors, e.g., Oct4, Sox2, c-Myc, and Klf4, into adult differentiated cells), and PSCs (isolated from adult connective tissues) have the capability to form all cells of the body, except the gametes [36,104-108] (Figures 9 and 10).

The difference between ESCS/iPSCs versus TSCs/PSCs is the programming in which multiple cell types can be formed. ESCs and iPSCs are programmed to spontaneously differentiate in the absence of inductive factors. In the uterus, naïve ESCs

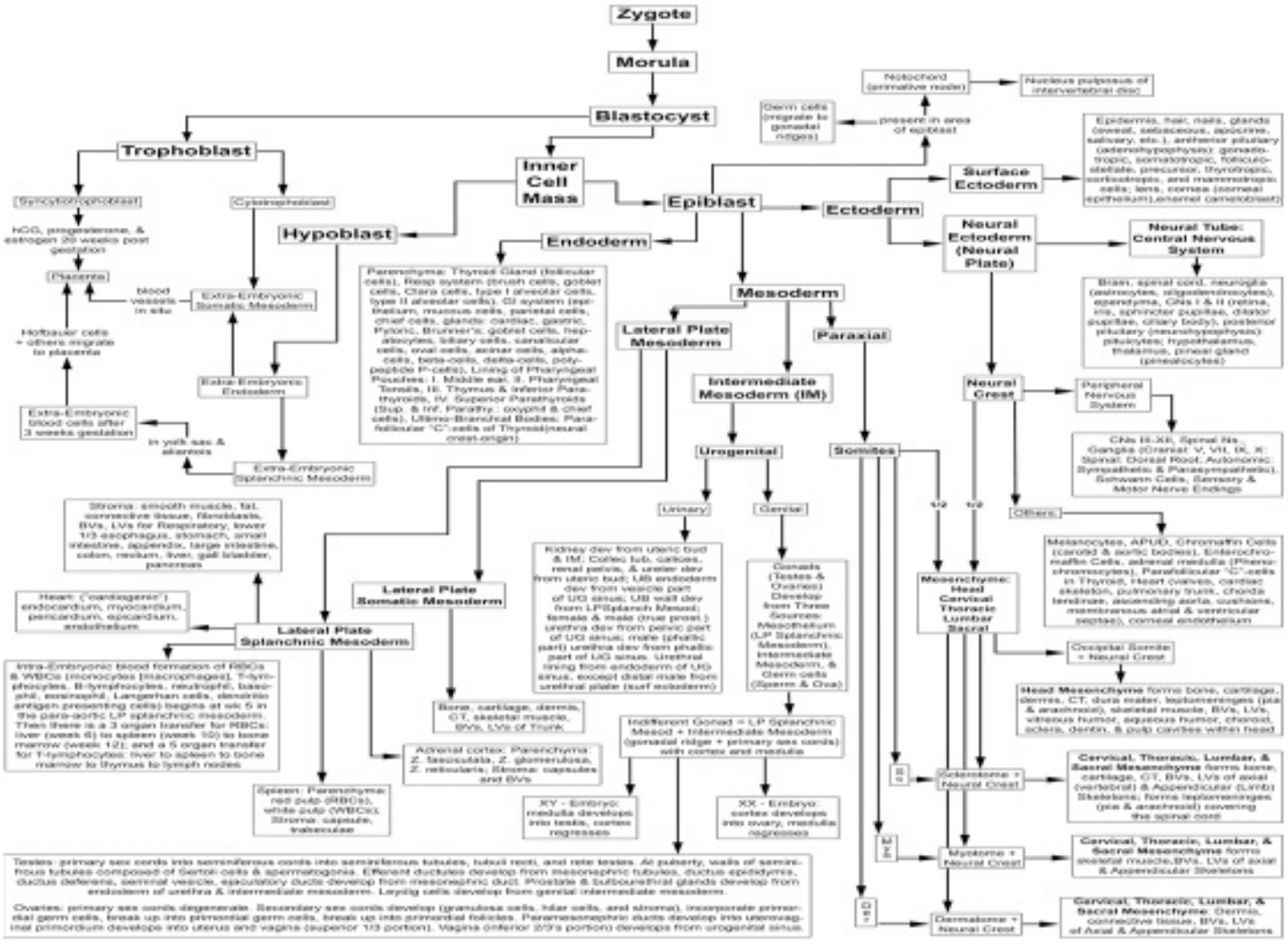

Figure 9: Lineage Map of unidirectional cell differentiation pathway for cells derived from the embryonic zygote. Reprinted with permission from Young HE, Black Jr, AC. Adult Stem Cells. Anat Rec Part A 2004; 267A:75-102 [104].

will spontaneously differentiate to form an intact individual. If implanted in the undifferentiated naïve state into another tissue site, both ESCs and iPSCs will form multiple cell types in a jumbled mass, termed a teratoma. To prevent spontaneous differentiation in culture that forms structures termed embryoid bodies, an anti-differentiation factor, such as leukemia inhibitory factor (LIF), is used which allows proliferation of the cells without differentiation. To prevent teratoma formation if transplanted in vivo, the ESCs and iPSCs are pre-differentiated into the cell type(s) of choice before transplantation. This negates the ability of either ESCS or iPSCs to spontaneously differentiate. At this point they assume the characteristics of differentiated cells; they lose their plasticity; and they lose their unlimited proliferation potential [31,33,102].

In contrast, TSCs and PSCs are programmed to remain in a quiescent hibernating state in the tissue unless acted upon by inductive factors. If implanted in the undifferentiated naïve state without access to inductive factors, TSCs and PSCs will just sit there and do absolutely nothing. Inductive factors are needed to induce the TSCs and PSCs (and MesoSCs) into specific cell types. This can be accomplished in culture using chemical mediators, human recombinant proteins, or exosomes derived from differentiated cells [36,39]. And this can be accomplished in vivo if telomerase positive stem cells have access to local environmental cues (exosomes) from the damaged and undamaged surrounding tissues (Figures 10,11,14,16,17,19,21) [30-45,102,104,107,108].

Homing to Sites of Tissue Damage & Responding to Local Cues

Telomerase positive TSCs, PSCs, and MesoSCs are programmed with a homing mechanism to migrate to areas of tissue damage. This was seen in our animal models for Parkinson disease, myocardial infarction, and pulmonary disease, where the stem cells were implanted at distant sites and migrated to reach damaged areas to repair the damage. The result of TSC-repair, PSC-repair, and MesoSC-repair of tissue damage was also seen in the Parkinson Disease, Myocardial Infarction, and Pulmonary disease clinical studies.

In the Parkinson disease animal model, a clone of PSCs was genomically-labeled with Lac-Z and designated as Scl-40β.

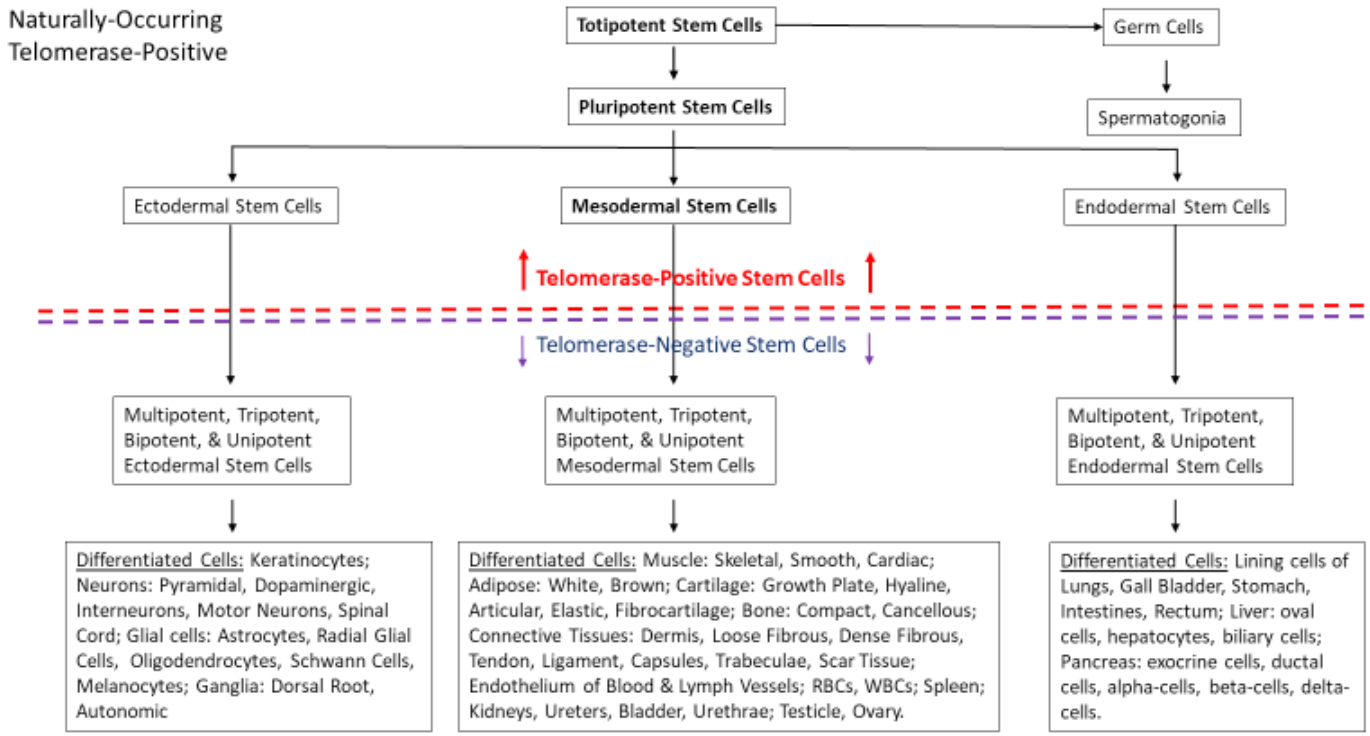

Figure 10: Differentiation potential of telomerase positive stem cells as assessed by induction with chemical agents, human recombinant proteins, and cell-specific exosomes. Phenotypic expression markers for cell types were identified immunocytochemically by enzyme-linked immuno-culture assay (ELICA) and molecularly by expressed genes. Reprinted with permission from Young HE, Speight MO. Characterization of endogenous telomerase-positive stem cells for regenerative medicine, a review. Stem Cell Regen Med 2020; 4(2):1-14.] 103-106 [33].

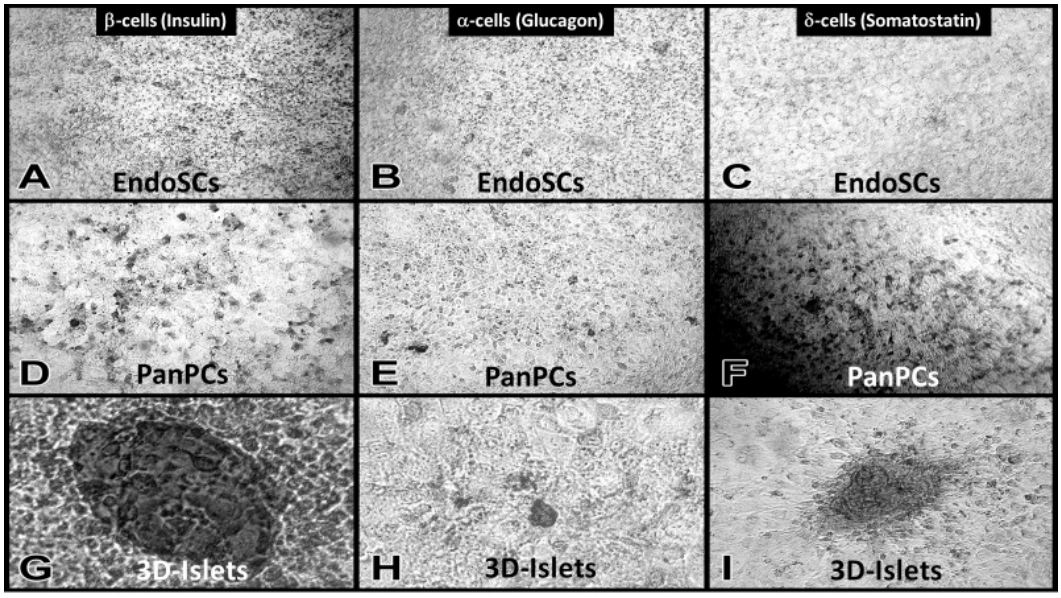

Figure 11: The hypothesis being tested was whether a telomerase positive pluripotent stem cell clone would respond to exosomes derived from cultured cells and a cocktail of recombinant proteins to form three-dimensional pancreatic islets. Naïve undifferentiated telomerase positive pluripotent stem cell clone was incubated with exosomes derived from cultured endodermal stem cells and the PSCs differentiated into endodermal stem cells (EndoSCs) (A, B, C). Next the EndoSCs were incubated with exosomes derived from pancreatic progenitor cells. And the EndoSCs differentiated into pancreatic progenitor cells (PanPCs) (D, E, F). Finally, the PanPCs were incubated with a cocktail of recombinant proteins [116] to generate three-dimensional structures (G, H, I). Based on immunocytochemical staining with antibodies to insulin (A, D, G), glucagon (B, E, H), and somatostatin (C, F, I), the above step wise induction scenario of PSCs to EndoSCs to PanPCs to 3D-Islets, formed islets that produced insulin, glucagon, and somatostatin. Reprinted with permission from Young HE, Black AC Jr. Differentiation potential of adult stem cells. In: Contemporary Endocrinology: Stem Cells in Endocrinology, L.B. Lester, ed., The Humana Press Inc., Totowa, NJ. Chap. 4, p. 67-92, 2005 [107].

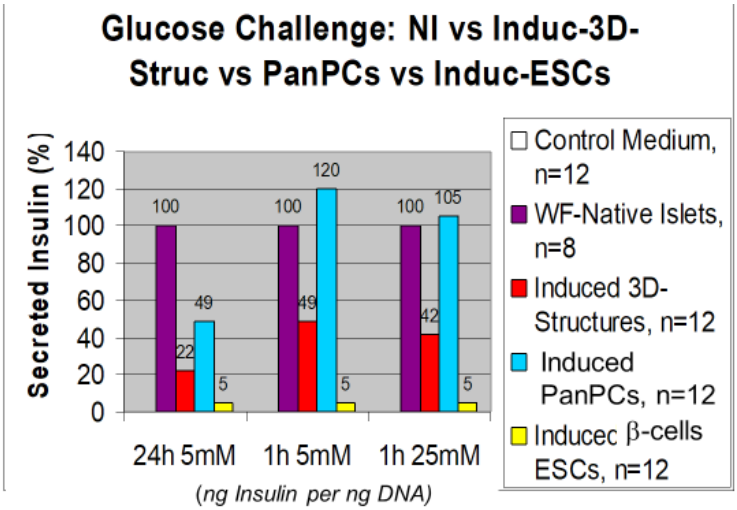

Figure 12: The hypothesis being tested was whether induced three-dimensional islet-like structures (3D-Islets) and induced pancreatic progenitor cells (PanPCs) would synthesize and secrete a comparable amount of insulin as induced insulin-secreting β-cells from embryonic stem cells (β-cell ESCs) versus native pancreatic islets isolated from Wistar-Furth rats (WF-Native Islets). In contrast to other reports for insulin synthesis and secretion where testing parameters were performed on different populations of cells, we used the same five cultures tested in series for a glucose challenge test, e.g., control medium only (contained bovine insulin), WF-native islets, 3D-Islets, PanPCs, and β-cell ESCs. The cultures were incubated for 24 hours with 5mM glucose, followed by 1 hour with 5mM glucose, followed by 1 hour with 25mM glucose. Insulin secretion was measured using a specific insulin-radioimmunoassay for rat insulin. We tested the rat-insulin RIA kit utilized against concentration gradients of bovine insulin, human insulin, porcine insulin, and rat insulin. And the rat-insulin RIA kit only measured rat insulin. As shown, with all values standardized to 100% insulin secretion for WF-Islets, 3D-Islets secreted approximately 50% insulin compared to native islets, whereas PanPCs synthesized and secreted approximately an equal amount of insulin. This was compared to induced β-cells from ESCs that synthesized and secreted approximately 5% of insulin compared to native islets [117]. Reprinted with permission from Young HE, Black AC Jr. Differentiation potential of adult stem cells. In: Contemporary Endocrinology: Stem Cells in Endocrinology, L.B. Lester, ed., The Humana Press Inc., Totowa, NJ. 2005; Chap. 4, p. 67-92 [107].

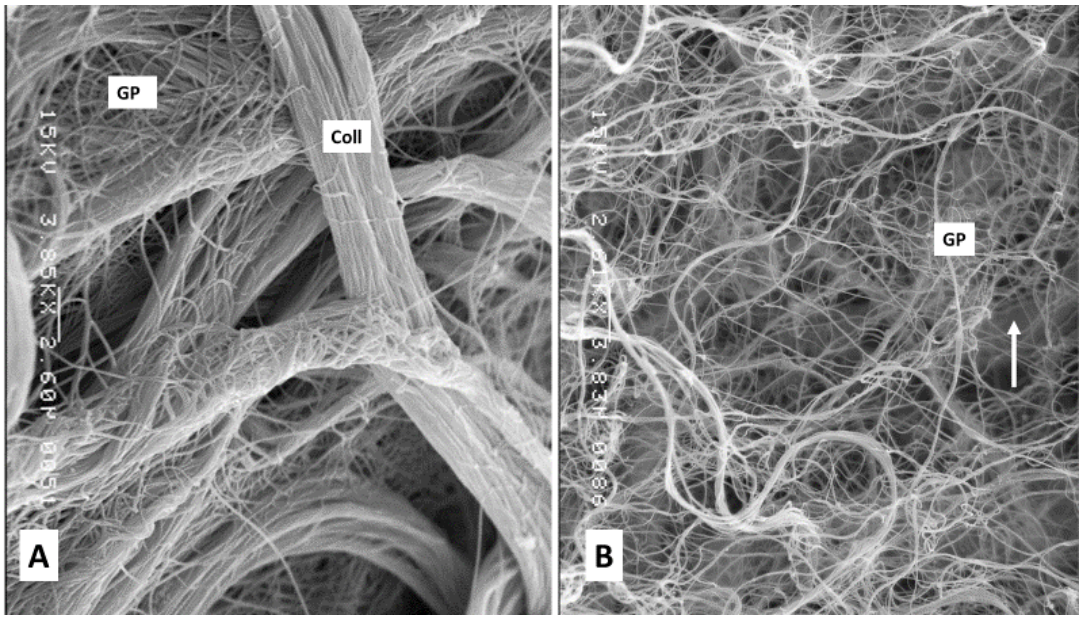

Figure 13: The hypothesis being tested was whether one could create a self-renewing pancreatic organoid capable to responding to a glucose challenge for type-I diabetes that would be protected from immune system destruction. The pancreatic composite was composed of a decellularized porcine pancreatic extracellular matrix embedded with allogeneic donor islets, surrounded by recipient's telomerase positive pluripotent stem cells, which was then surrounded by recipient's telomerase positive totipotent stem cells. Scanning electron micrographs of decellularized native porcine pancreatic extracellular matrices, using two different decellularization solutions, A & B, engendering matrix-A and matrix-B. **A:** Type-I collagen (Coll) bundles are distinct and overlain with strands of glycoproteins (GP). **B:** Type-I collagen bundles (arrow) are barely visible beneath multiple stands of glycoproteins (GP). Reprinted with permission from Young HE, Limnios JI, Lochner F, et al. Pancreatic islet composites secrete insulin in response to a glucose challenge. J Stem Cell Res 1(1) 001: 1-12, 2017 [108].

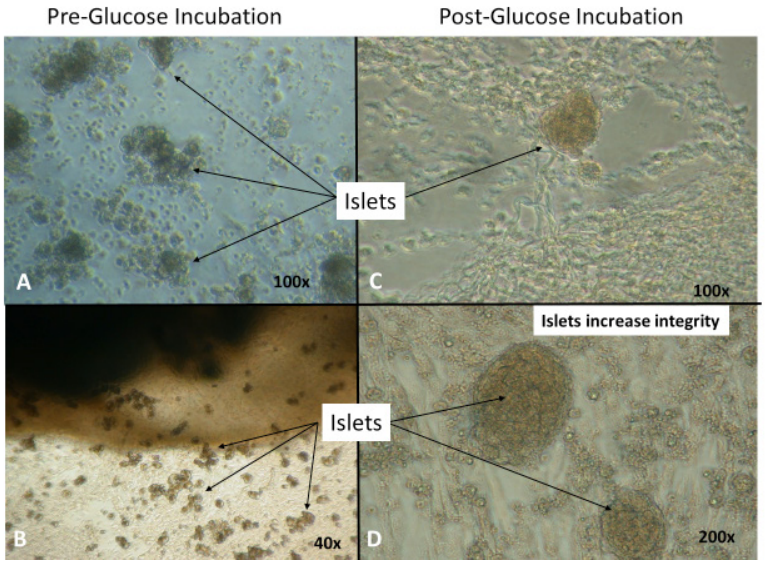

Figure 14: The pancreatic composite was composed of a decellularized porcine pancreatic extracellular matrix embedded with allogeneic donor islets, surrounded by recipient's telomerase positive pluripotent stem cells, which was then surrounded by recipient's telomerase positive totipotent stem cells. Our working theory was that the donor islets would induce (via islet paracrine exosomes) the recipient pluripotent stem cells to produce more islet cells. And to replace the pluripotent stem cells lost due to induction, the totipotent stem cells would form new pluripotent stem cells (via pluripotent paracrine exosomes). And with loss of totipotent stem cells to induction, there would be proliferation of totipotent stem cells (via totipotent autocrine exosomes) to maintain their numbers. And this proved to be the case. Isolated rat pancreatic islets were grown on decellularized native porcine pancreatic matrices (matrix-A = Figures A & C; and matrix-B = Figures B & D) utilizing the standard TSC-based culture medium. A & B are rat islets 48 hours after plating. C & D are rat islets 72 – 96 hours after plating. Note that islets in C & D are increasing their three-dimensional integrity, as if native islet cells were inducing surrounding TSCs and PSCs to convert to islet cells. These decellularized pancreatic matrices seeded with TSCs, PSCs, and native islets demonstrated an increase in the integrity of the islet structures throughout the 30 days in culture, before termination of the experiment. Reprinted with permission from Young HE, Limnios JI, Lochner F, et al. Pancreatic islet composites secrete insulin in response to a glucose challenge. J Stem Cell Res 1(1) 001: 1-12, 2017 [108].

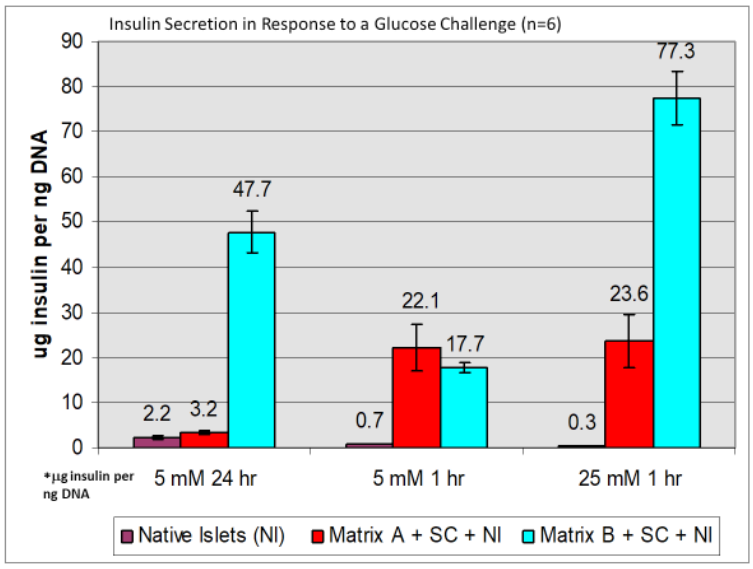

Figure 15: The hypothesis being tested was the ability of the pancreatic composites generated with matrix-A versus matrix-B (Figure 13) to synthesize and secrete insulin compared to native islets when tested with a glucose challenge. We used the same scenario as the previous experiment (Figure 12) in that the same cultures were used for all three glucose challenges, e.g., 5 mM glucose for 24 hours, followed by 5 mM glucose for 1 hour, followed by 25 mM glucose for 1 hr. Insulin secretion was measured using a specific insulin-radioimmunoassay for rat insulin. We tested the rat-insulin RIA kit against concentration gradients of bovine insulin, human insulin, porcine insulin, and rat insulin. And the rat-insulin was the only insulin detectable with the rat insulin RIA kit. In the previous experiment (Figure 12) the amount of insulin synthesized and secreted was nanogram quantities of insulin per nanogram DNA. In the current experiment, the amount of insulin secreted was 1,000-fold greater at microgram quantities of insulin per nanogram DNA. DNA. Reprinted with permission from Young HE, Limnios JI, Lochner F, et al. Pancreatic islet composites secrete insulin in response to a glucose challenge. J Stem Cell Res 1(1) 001:1-12, 2017.

Adult rats were stereotactically injected with a neurotoxin into the substantia nigra pars compactum of the ventral midbrain to kill dopaminergic neurons and to disintegrate their associated neural networks. Scl-40β was then stereotactically injected into the lesion site. Besides generating new dopaminergic neurons and associated neural networks (Figure 16). Scl-40β also migrated back into the cerebral cortex along the needle tract and regenerated all cell types that were damaged, e.g., pyramidal neurons, interneurons, glial cells, and capillary endothelial cells (Figure 17) [34,35,37].

In the Parkinson clinical studies (n=12), while 83% of the participants experienced some reversal of symptoms by two months after treatment, by 14-months after treatment 33% demonstrated stasis of their symptoms and 33% demonstrated a reversal of their symptoms to normal or nearly normal levels (Figure 18) [37,38].

In the myocardial infarction-induced animal model, Scl-40β was injected into the tail vein to reach the heart by systemic blood flow. Once in the heart, Scl-40β repaired damage to the vasculature, myocardium, and cardiac connective tissue skeleton (Fig. 19) [31,40]. In the cardiovascular clinical studies (n=3) where autologous TSCs, PSCs, and MesoSCs were delivered by intravenous infusion through the median cubital vein, there was an increase in cardiac function in all trial participants (Figure 20) [40,41].

In the pulmonary fibrosis animal model, Scl-40β was injected into the vasculature and was visualized regenerating bronchioles, alveolar ducts, and alveolar sacs (Figure 21) [42]. In the clinical studies for idiopathic pulmonary fibrosis (IPF) (n=2) (Figure 22) [43] and chronic obstructive pulmonary disease (COPD) (Figure 23) (n=51) [44], 94% of participants (50/53) showed an increase in pulmonary function, some for as long as 8-10+ years after treatment. Both the animal models and the clinical trials suggested that the telomerase positive stem cells used a homing mechanism to migrate to the damaged tissues and repaired the damage, restoring function to the tissues.

Current Clinical Trial

History, physical exam, and laboratory tests of the participant in this small cohort (n=1) study noted a normal, healthy, 17-year-old female. She became totally bilaterally visually impaired at age 4 due to head trauma following an automobile accident. Thirteen years duration of total visual impairment suggested a chronic rather than acute problem in this individual and suggested the potential for death of all cells along the visual pathway. From birth to age 4 was sufficient time from for her to learn and associate the names of colors, e.g., black, white, shades of gray, red, blue, yellow, green, etc., with the visual representation of colors, e.g., red dress, blue sneakers, yellow sun, green grass, and black/shades of gray with respect to what she could see in the dark (night vision), etc.

Based on the information provided, it was unclear what portion of her visual pathway from retina to primary visual cortex was affected (Figures 2-5, 8), e.g., pigmented epithelial cells, photoreceptors, retinal ganglion cells and their axonal processes, optic disc, optic nerve, optic chiasm, optic tract, lateral geniculate bodies, optic radiations, and primary visual cortex in the bilateral occipital lobes of the cerebral cortex. Therefore, stem cells with a known ability to home to sites of tissue damage were chosen, i.e., the telomerase positive stem cells.

An intranasal infusion site for stem cell transplant was chosen to minimize repeated trauma to the participant if multiple stem cell treatments were required to restore function. Since the intranasal site was selected to gain entry to the visual pathway, TSCs were chosen because of their extremely small size (0.1 to 2 microns); their ability to form any cell type of the body (including those of the ectodermal lineage, e.g., choroid, retinal pigmented epithelium, photoreceptor cells, ganglion cells, cell bodies, cell processes: axons and dendrites, synapses, pyramidal neurons, interneurons, glial cells, etc.) (Fig. 10); and their ability to traverse the spaces between the olfactory epithelial cells without use of mannitol (Figure 6), that would potentially form permanent channels for potential bacterial meningitis [33,35-39,102,109-112].

Crawling along the outside of the nerve rootlets to the olfactory bulbs (Figure 6), would allow the TSCs entry across the blood-brain barrier at the cribriform plate. Then crawling along the olfactory nerves would allow access to sites along the visual pathway from the eye (Figure 8) necessary for repair to restore eyesight. Also, since her total visual impairment was trauma-induced rather than genetically induced, her own autologous TSCs could be utilized for treatment, thus negating any potential graft versus host disease response that may be incurred using allogeneic (donor) stem cells [113,114].

The “colors” she saw after her two autologous telomerase positive totipotent stem cell treatments were shades of gray and black (Figure 7). This suggested that photoreceptor rod cells were potentially being restored and that bilateral damage to her retinas was at least one potential area causing her bilateral total visual impairment. In addition, since her total visual impairment lasted for 13-years, this suggested that other cells, including the ganglion cells, their axons, synapses with cell bodies, and their axons, were regenerated as well to allow perception of at least a partial return of eyesight (night vision) in the primary visual cortex.

While two telomerase positive stem cell treatments were not sufficient to completely restore eyesight in an individual with chronic total bilateral visual impairment of 13-years duration (Figure 7), at least it was a start on that process. We would hypothesize that given sufficient numbers of autologous telomerase positive stem cell treatments that it would be possible to completely restore her eyesight. Unfortunately, that did not occur due to her returning to her special School for the Blind during the ensuing fall, winter, and spring semesters.

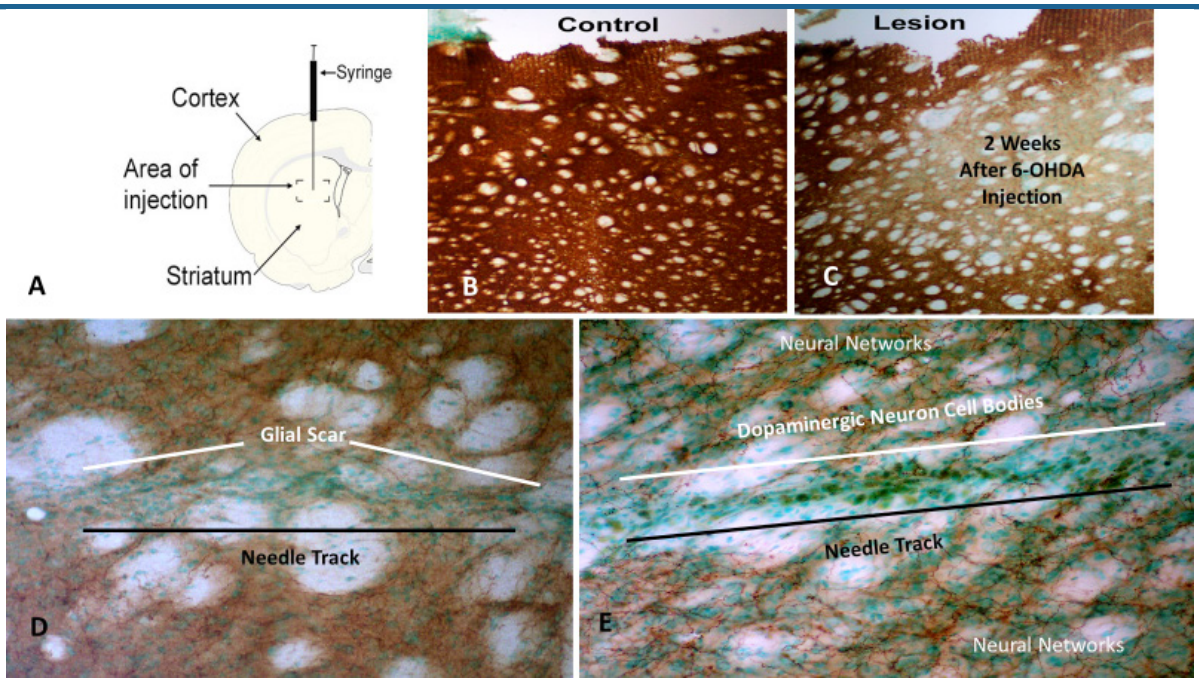

Figure 16: Parkinson Disease model in adult rats. A: Adult rats were stereotactically injected with either saline (B) or a neurotoxin, 6-hydroxydopamine (6-OHDA), into the substantia nigra pars compactum of the ventral midbrain to kill dopaminergic neurons and to disintegrate their associated neural networks (C). Either saline or a Lac-Z-genomically-labeled clone of pluripotent stem cells (Scl-40β) was then stereotactically injected into the lesion site. The animals were kept for additional six weeks, euthanized, their brains removed and processed for immunocytochemical staining for dopaminergic neurons via the enzyme tyrosine hydroxylase or Beta-galactosidase to distinguish Scl-40β naïve or differentiated cells. The tissue sections were counterstained with methyl green to distinguish host glial cells from Scl-40β. D: Lesioned area injected with saline only. Note a line of green cells, depicting a glial scar within the needle tract. Very few visible neural networks were present. E: Lesioned area injected with Scl-40β. Note a line of dopaminergic neurons were located within the needle tract as well as extensive neural networks on either side of the line of dopaminergic neurons. 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 2005; 9:753-769 [34].

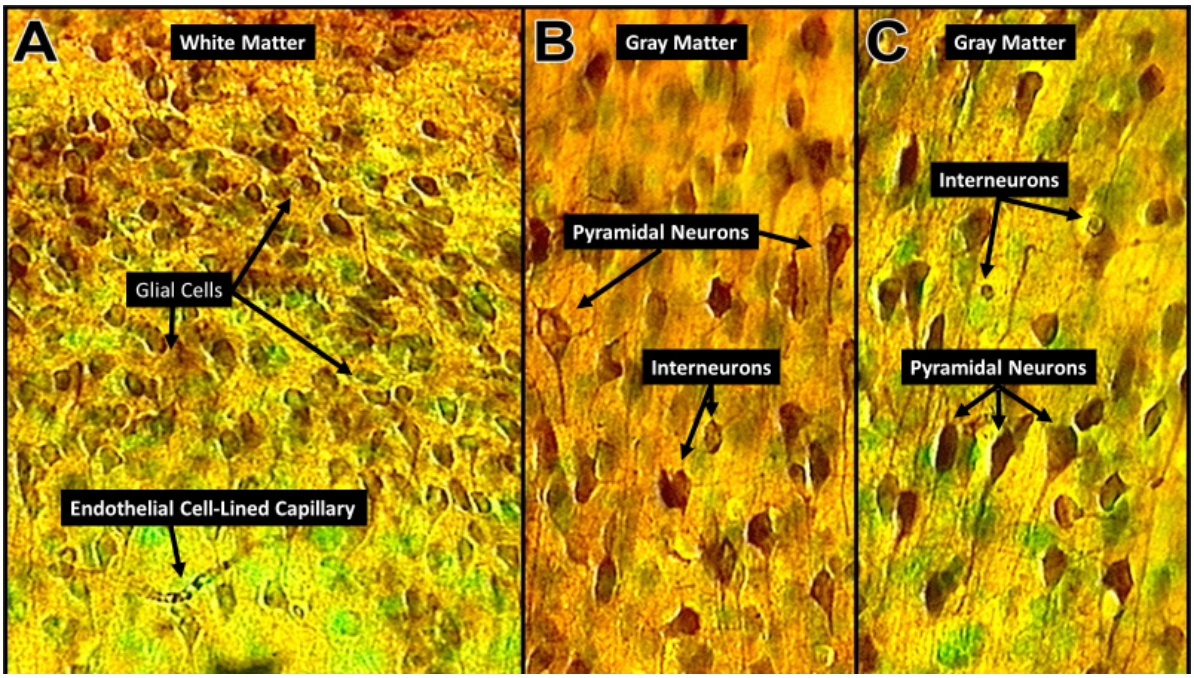

Figure 17: Scl-40β also migrated back into the cerebral cortex along the needle tract and regenerated all cell types that were damaged, A: White matter - glial cells and capillaries; B: Gray matter - interneurons and pyramidal neurons; and C: Gray matter - interneurons and pyramidal neurons. Reprinted 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 2005; 9:753-769 [34].

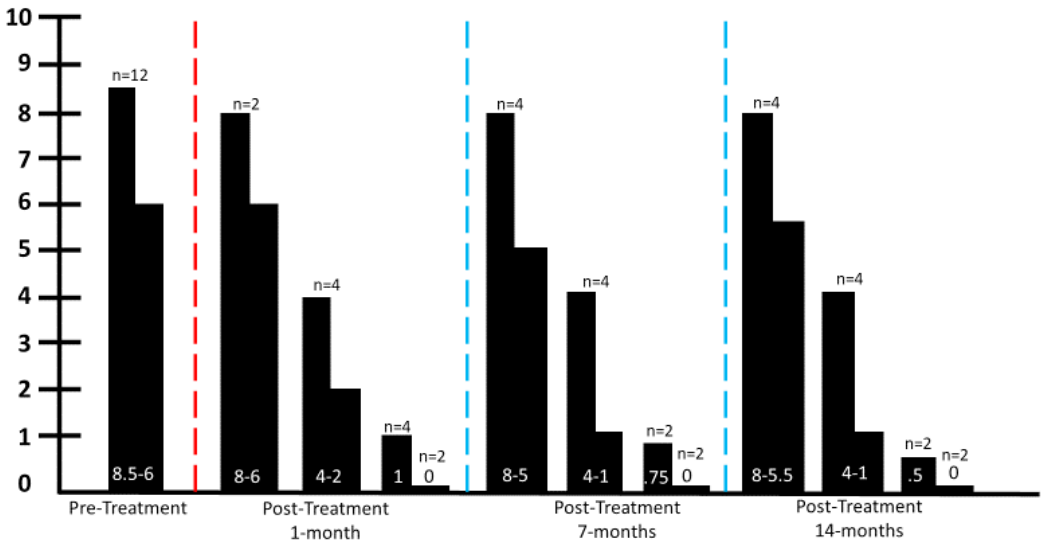

Figure 18: Combined Hoehn-Yahr scoring data for small cohort clinical trial (n=12), encompassing 2013 Parkinson trial [37] and additional four participants [39]. No adverse effects were noted from participants or their care givers from either trial. 33% (n=4) showed moderate to no benefit of telomerase 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 [37] and reprinted with permission from Young HE, Speight MO. Treating Parkinson Disease with Autologous Telomerase-Positive Stem Cells, Update 2021. Stem Cells Regen Med. 2021; [38].

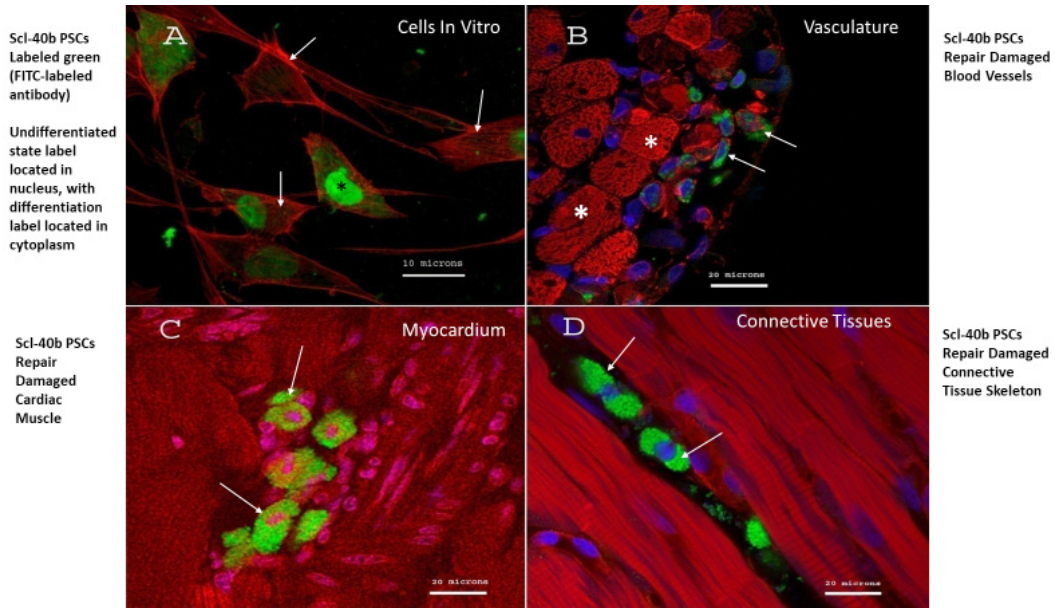

Figure 19: Laser confocal microscopy of Lac-Z transfected pluripotent stem cell clone, Scl-40β. **A:** Laser-scanning confocal micrograph of Scl-40β in culture on type-I collagen-coated tissue culture plastic. The f-actin in the cytoskeleton has been stained using rhodamine phalloidin (arrowhead). The β-galactosidase has been immunohistochemically labeled green (asterisk) using a fluoresceine isothiocyanate (FITC) fluorophore, denoting undifferentiated cells (where genomic label resides within nucleus). **B:** β-galactosidase-positive cells (arrowhead) localized in vascular endothelium of normal heart one week after infusion of cells into heart. β-galactosidase label in the cytoplasm denotes differentiated cell. End views of myofibril bundles stained with rhodamine phalloidin can be seen (asterisk). Cell nuclei (blue) are stained with topro-3 (a DNA intercalating dye). **C:** Differentiated β-galactosidase-positive cells (arrowhead) located within ischemic heart muscle one week after infusion of stem cells. **D:** Differentiated β-galactosidase-positive cells are located within the connective tissue skeleton of the heart replacing damaged cardiac connective tissues. Cell nuclei are stained with topro-3 (blue-stained nuclei). Reprinted with permission from Young HE, Duplaa C, Romero-Ramos M, et al. Adult reserve stem cells and their potential for tissue engineering. Cell Biochem Biophys, 2004; 40(1):1-80 [31].

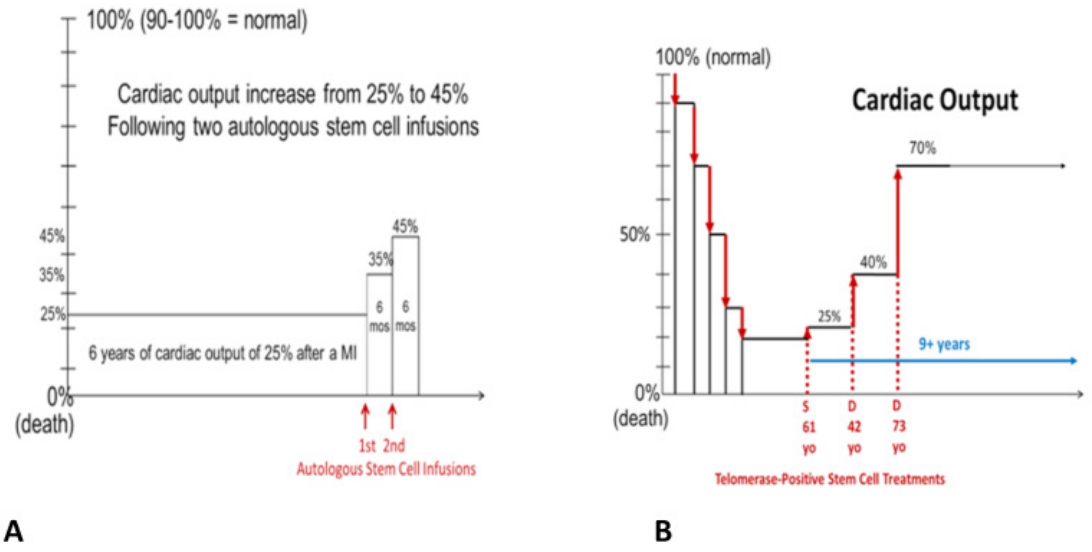

Figure 20: A: Human volunteer with cardiac output of 25% of six year's duration following myocardial infarction. Ingested combinatorial nutraceuticals 30 days before first stem cell harvest and throughout treatments. Stem cells were harvest by simple venipuncture, separated from blood cells, segregated into TSCs, PSCs, and MesoSCs and activated. TSCs were given by slow systemic infusion and PSCs and MesoSCs were given by regular systemic infusion. Treatment consisted of two successive autologous stem cell transplants six months apart from each other. Six months following first autologous stem cell transplant cardiac output rose from 25% to 35%. Six months following 2nd autologous stem cell transplant cardiac output rose from 35% to 45%. Reprinted with permission from Young HE, Limnios IJ, Lochner F, et al. Adult healing cells and cardiovascular disease: From bench top to bedside. J Stem Cell Res 2017; 1(3) 002:1-8 [40].

B: Systemic Lupus Erythematosus (SLE) participant treated with S, Self (autologous) and D, Donor (Allogeneic) telomerase positive stem cells. SLE participant's cardiac output dropped precipitously, 90% to 30%, during ingestion of hydroxychloroquine to slow progression of SLE. At time of first stem cell transplant, cardiac output was below 25%. First stem cell transplant (autologous) raised cardiac output to 25%. Second stem cell transplant from allogeneic 42-year-old A+ male raised cardiac output to approximately 40%. Third stem cell transplant from allogeneic 73-year-old O-negative male raise cardiac output to approximately 70%. A total of 29 adult-derived autologous and/or allogeneic telomerase-positive stem cell transplants thus far have maintained his cardiac output at approximately 70% for over nine years and counting. Reprinted with permission from Young HE, Speight MO. Cardiovascular disease treated with telomerase-positive stem cells. Stem Cells Regen Med. 2020; 4(2):1-8 [41].

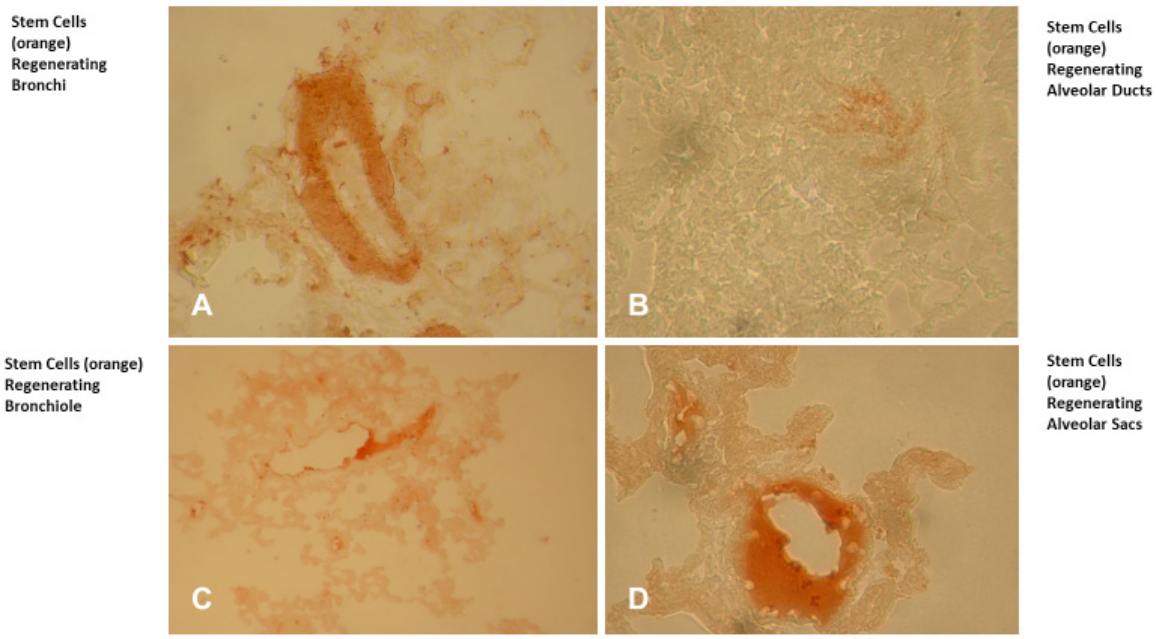

Figure 21: Adult rats treated with busulfan to induce pulmonary fibrosis. Scl-40β infused into vasculature. A: regenerating bronchioles. B: Regenerating alveolar ducts. C: Regenerating bronchiole. D: Regenerating alveolar sac. Reprinted with permission from Young HE, Black GF, Coleman JA, et al. Pulmonary diseases and adult healing cells: from bench top to bedside. J Stem Cell Res 2017; 1(2) 003:1-9 [42].

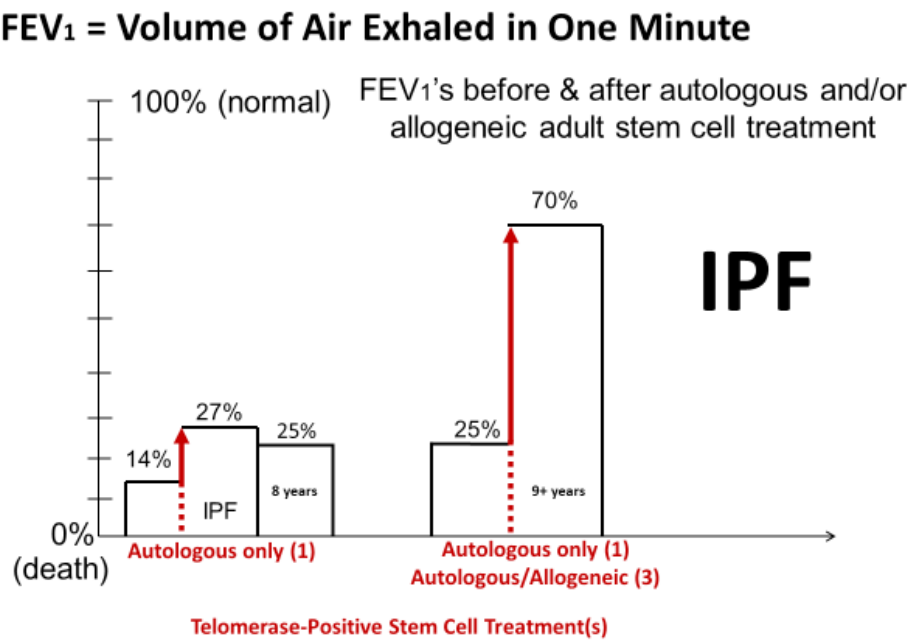

Figure 22: Endogenous telomerase-positive stem cell treatment of two individuals with idiopathic pulmonary fibrosis (IPF), with baseline FEV_1 values of less than 30% (Gold-4). The female, age 80 with a baseline FEV_1 of 14%, was transplanted with a single treatment of autologous telomerase positive stem cells (TSCs and PSCs by nebulization and MesoSCs by intravenous infusion). Within one month after treatment her FEV_1 rose to 27% [5], and then stabilized at 25% for eight years. The male, age 61 with a baseline FEV_1 of 25% was transplanted with a single autologous and three autologous/allogeneic (auto/allo) telomerase-positive stem cell treatments throughout a seven-year time frame. The autologous/allogeneic treatments consisted of pooled auto/allo-TSCs and auto/allo-PSCs by nebulization and autologous MesoSCs by intravenous infusion. His FEV_1 has stabilized at approximately 70% for the past nine years (and counting). Reprinted with permission from Young HE, Speight MO. Telomerase-positive stem cells as a potential treatment for idiopathic pulmonary fibrosis. Stem Cells Regen Med. 2020; 4(2):1-11 [43].

Conclusion

Loss of visual acuity may result from genetics, cancer, metabolic disorders, trauma, or aging. As of 2015, approximately 1.2 billion people suffer from some sort of visual impairment. The World Health Organization defines visual impairment based on vision in the best eye equipped with vision correction. There are several subcategories of visual impairment. Partial sightedness indicates some sort of visual problem. Near-sightedness (myopia) is not being able to see distant objects clearly. Far-sightedness (hyperopia) is not being able to see close objects clearly. Low vision refers to individuals who are unable to read at a normal viewing distance even with visual correction. Legally blind indicates that an individual has visual acuity of less than 20/200 in their best eye with vision correction (either glasses or contact lenses). Totally blind is considered as total bilateral visual impairment or no light perception of any kind. Since there is no known pharmacological or surgical cure for unilateral (monocular) or total bilateral (binocular) visual impairment, current research is focusing on regenerative medicine utilizing stem cells, secreted paracrine exosomes, and gene therapy. Proposed stem cells being studied and utilized for treatment include embryonic stem cells, induced pluripotent stem cells, embryonic retinal progenitor cells, mesenchymal stem cells (MSCs) and medicinal signaling cells (MSCs). In clinical trials published to date, most of these stem cells were safe to implant if they were pre-determined to a particular cell fate. However, their ability to reverse visual impairment was mostly non-existent. In contrast, based on previous in vitro, animal models, and human clinical trials, we proposed the use of telomerase positive stem cells as a treatment modality for visual impairment, e.g., endogenous adult-derived autologous totipotent stem cells, pluripotent stem cells, and mesodermal stem cells.

A 17-year-old female presented with total bilateral visual impairment of 13-years duration. The participant's visual impairment was the result of severe head trauma from an automobile accident at four years of age. She was given two telomerase positive stem cell treatments. Each treatment consisted of freshly isolated autologous totipotent stem cells infused intranasally and freshly isolated autologous pluripotent stem cells and mesodermal stem cells introduced into her bloodstream by intravenous infusion. Before her stem cell treatments began, she stated that she could not see anything, that everything was black. Following the first treatment the participant's vision was fuzzy, but she could distinguish black from a dark gray. Following the second treatment the participant's vision was less 'fuzzy' (slightly more distinct with sharper edges) and she could distinguish black from medium to light gray. No adverse events were reported during her stem cell treatments. The participant's results from two telomerase positive stem cell transplants suggested that autologous telomerase positive stem cells were safe for administration and slightly efficacious, with a partial restoration of ('night') vision.

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