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Fresh isolate adult telomerase positive stem cells: An addition to Embryonic Stem Cells (ESCs), Induced Pluripotent Stem Cells (iPSCs), and/or Mesenchymal Stem Cells (MSCs) for regenerative medicine

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Abstract

Currently, embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and mesenchymal stem cells (MSCs) are the holy grail for regenerative medicine. Unfortunately, in clinical trials their efficacies have been less than ideal. While telomerase positive ESCs or iPSCs will form every cell type in the body, if implanted in their naïve state, they form teratomas. MSCs are a tripotent telomerase negative progenitor cell that will form fat, cartilage, and bone. MSCs' efficacy for treating conditions other than fat, cartilage, and bone average 1-5%. We offer healing adult telomerase positive stem cells (aTPSCs) as an addition stem cell to regenerative medicine. The aTPSCs are few in number and reside in connective tissues throughout the body in a quiescent state. Upon stimulation, the aTPSCs divide symmetrically to large numbers due to the presence of the telomerase enzyme. Coupled with their ability to differentiate into any progenitor or differentiated cell in the body from their naïve state under the direction of locally released exosome cues, make them an ideal candidate for regenerative medicine. When tested in clinical studies, both autologous and allogeneic aTPSCs demonstrated a 100% safety record and a cumulative efficacy of 86.4% for reversing signs and symptoms in 20 chronic diseases or traumatic injuries. The results suggested that aTPSCs retain all the positive aspects of ESCs, iPSCs, and MSCs, while exhibiting none of their negative aspects. Therefore, I propose that aTPSCs be an added category of stem cells for use in regenerative medicine to increase efficacy of proposed treatments.

Keywords: Telomerase; Adult Stem Cells; Chronic Diseases; Traumatic Injuries; ESCs; IPSCs

1. Introduction

The human body is composed of trillions and trillions of cells. These cells can be divided into three categories based on their function: telomerase-negative differentiated (functional) cells, telomerase-negative progenitor (maintenance) "stem" cells, and telomerase-positive (healing) stem cells [1].

Adult telomerase-negative differentiated cells (aTNDCs), comprise 50% of all cells of the body. They are the functional cells of the body and are represented by more than 220+ distinct cell types. The differentiated cells can be subdivided into parenchyma and stroma. Examples of parenchyma are neurons that transmit signals from the brain and spinal cord to the periphery, beta cells of the pancreas that secrete insulin, and cardiac muscle cells that pump blood throughout the body, etc. The stroma consists of the connective tissue structural framework of the body. Examples of stroma include dermis of the skin, organ capsules, trabeculae, and connective tissue coverings of nerve fibers, skeletal muscle fibers, pancreas, and heart, etc. Differentiated cells are missing the telomerase enzyme after birth, and thus have a defined biological clock of 70 population doublings from birth before pre-programmed senescence and cell death occur [1-8].

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Adult telomerase-negative progenitor cells (aTNPCs), comprise 40% of all cells of the body. The progenitor cells help to maintain the body in a functional state throughout the life span of the individual. As differentiated cells wear out, they are replaced by their cell associated progenitor cells. There are multiple subcategories of aTNPCs, based on the number of differentiated cell types they form [1-8]. There are multipotent progenitor cells, such as hematopoietic stem cells, that will form all cell types within the hematopoietic lineage but will not form any cell type outside that lineage [9-11]. There are tripotent progenitor cells, such as MSCs, e.g., mesenchymal stem cells, marrow stromal cells, multipotential stromal cells, mesenchymal stromal cells, that will form unilocular white adipose (fat) tissue, hyaline cartilage, and endochondral bone, but no other cell type [4,12-14]. There are bipotent progenitor cells, such as adipo-fibroblasts, that will form unilocular white adipose (fat) tissue and fibrocytes, but no other cell type [3,4]. And there are unipotent progenitor cells, such as osteoblasts that will form the osteocytes of bone, but no other cell type outside that lineage [1-4].

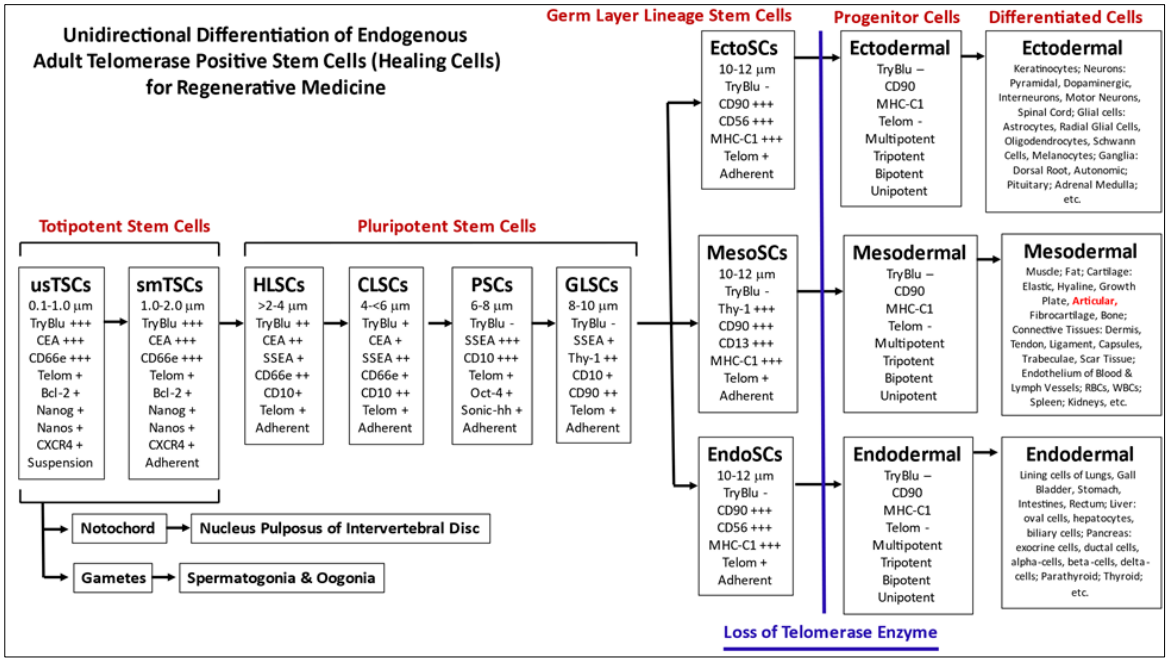

Figure 1 usTSCs, ultra-small totipotent adult telomerase-positive stem cells; TryBlu, 0.4% Trypan Blue staining; CEA, carcinoembryonic antigen; CEA-CAM-1, carcinoembryonic antigen-cell adhesion molecule-1; CD66e, carcinoembryonic antigen subtype 66e; Telom +, presence of telomerase enzyme; expressed genes: Bcl-2, Nanog, Nanos, CXCR4; Suspension, grows unattached in suspension cultures; sTSCs, small totipotent telomerase positive stem cells; Adherent, grows in cultures adherent to a type-1 collagen substrate; HLSCs halo-like pluripotent adult telomerase-positive stem cells; SSEA-4, stage-specific embryonic antigen-4; CD10, cluster of differentiation marker for neutral endopeptidase; CLSCs, corona-like pluripotent adult telomerase-positive stem cells; PSCs, pluripotent adult telomerase-positive stem cells; Expressed genes: Oct-4, Sonic-hh, Sonic hedgehog; GLSCs, pluripotent germ layer lineage telomerase-positive stem cells; CD90/Thy-1, cluster of differentiation marker for a heavily N-glycosylated glycophosphatidylinositol; CD56, cluster of differentiation marker for a neural cell adhesion molecule; MHC-1, major histocompatibility complex-1, located on all somatic cells of the body; EctoSCs, ectodermal telomerase-positive stem cells; Telom –, absence of the telomerase enzyme; MesoSCs, mesodermal telomerase-positive stem cells; CD13, cluster of differentiation marker for aminopeptidase; EndoSCs, endodermal telomerase-positive stem cells; progenitor cells (telomerase-negative); differentiated cells (telomerase negative)

As the name implies, telomerase negative progenitor cells are missing the telomerase enzyme at birth. Thus, they have a defined biological clock of 70 population doublings from birth. Similar to the aTNDCs, the telomerase negative progenitor cells will senesce and die at the termination of their lifespan [5-8]. Additionally, the aTNPCs decrease in number with increasing age of the individual [14].

Adult telomerase-positive stem cells (aTPSCs) comprise 10% of the cells of the body and are the TRUE stem (healing) cells of the body [1]. They are pre-programmed to heal/replace progenitor cells and differentiated cells damaged due to trauma and disease [2,4]. They are located within the connective tissue stroma throughout the body. The aTPSCs exist as a continuum of cell types, from most primitive to more differentiated. The aTPSCs have been arbitrarily assigned into categories and nomenclature abased on their unique sizes, patterns of Trypan Blue staining, cell surface markers, and

differentiation potentials. In order, from least differentiated telomerase positive stem cells to most differentiated, e.g., ultra-small totipotent stem cells (usTSCs, 0.05%), small totipotent stem cells (sTSCs, 0.05%), halo-like pluripotent stem cells (HLSCs, 0.2%), corona- like pluripotent stem cells (CLSCs, 0.2%), pluripotent stem cells (PLSCs, 0.3%), germ layer lineage pluripotent stem cells (GLSCs, 0.2%), embryonic lineage-specific ectodermal stem cells (EctoSCs, 3%), lineage-specific mesodermal stem cells (MesoSCs, 3%), and lineage-specific endodermal stem cells (EndoSCs, 3%) (Fig. 1) [1,2,4].

Adult telomerase positive stem cells do not lose the telomerase enzyme at birth, rather they maintain the telomerase enzyme if they have not committed to a particular progenitor cell lineage [4]. This gives them unlimited proliferation potential [6-8]. However, once they commit to becoming a progenitor cell, they lose the telomerase enzyme and assume all characteristics of progenitor cells, e.g., progression into differentiated cells and a defined lifespan, which is a biological clock of 70 population doublings from commitment to a progenitor cell lineage until programmed senescence and cell death [1,2,4-8].

The normally hibernating quiescent aTPSCs are present throughout all the connective tissue stroma of the body [15-27]. When damage occurs to the body, the connective tissue niche resident "Maternal" aTPSCs become activated and proliferate. With continued proliferation, Daughter aTPSCs are released from their connective tissue niches, and migrate to the damaged tissue. At the damage site, the **D**aughter stem cells respond to local cues (in the form of tissue-specific exosomes), and repair/replace damaged progenitor cells and differentiated cells to restore body function [4,28-35]. In the 48+ years (1975-present) that I have been studying TPSCs I have NEVER seen them form teratomas or cancerous tissue of any kind when naïve undifferentiated aTPSCs were transplanted into an individual, animal or human [34-60].

Naturally occurring adult TPSCs were discovered in 1975 while studying complete limb regeneration in adult terrestrial salamanders [28-34]. I have been studying them ever since. I began by learning everything that I could about these cells as single cell clones from avians, mice, rats, and humans, derived by repetitive single cell clonogenic analysis. Their unique characteristics were examined as cells grown outside the body (*ex vivo*) in cell culture. These characteristics included their unique sizes, cell surface markers, expressed genes, phenotypic expression markers, nutrition requirements, freeze/thaw, growth characteristics, proliferation potential, differentiation potential, reactivity to human recombinant inductive factors, reactivity to tissue specific exosomes, reactivity to progression agents, reactivity to inhibitory agents, presence throughout the life span of the individual, and presence in different organs. The results were tested and validated in 15 species of animals, including (newborn, pre-puberal, mature, and geriatric) humans [2,4,15,34-36,41,61-84]. These results suggested the hypothesis that aTPSCs would make excellent candidates for use in regenerative medicine.

2. Materials and Methods

Methodologies to harvest aTPSCs from humans using minimally invasive fresh isolate procedures were devised and patented [84,85]. Institutional Review Board (IRB)-approved human clinical studies began to ascertain their ability to affect change in human diseases and injuries. Informed consent guidelines [81] are explained to each participant. In brief, the differentiation capabilities of TSCs, PSCs, MesoSCs, EctoSCs, and EndoSCs are explained. The isolation, segregation, *ex vivo* activation, and treatment protocols are explained. The participants are cautioned to adhere to the Informed Consent Guidelines to maximize the effect of the telomerase positive stem cells to treat their respective problems. These included NO alcohol, NO smoking or vaping nicotine, NO recreational drugs, NO lidocaine, and NO chemotherapeutic drugs, during their treatment because these agents KILL the telomerase positive stem cells. Limit caffeine to $\leq$ 95 mg per day (the amount in 8 oz. of regular coffee) because dosages greater than 95 mg per day prevent the telomerase positive stem cells from differentiating. Zero use of corticosteroids in individuals where either ectodermal lineage cells or endodermal lineage cells are wanted, because corticosteroids pre-commit the TSCs and PSCs to the mesodermal germ line lineage. The participants are required to ingest 'Nutra' (Dragonfly Foundation for Research and Development, DFRD, Macon, GA) for a minimum of two months prior to procedure to stimulate the "Maternal" aTPSCs to proliferate in situ, making the individual their own bioreactor. Eighteen hours before the procedure the participant is instructed to ingest two glacial caps (GC, DFRD) to mobilize the proliferated "Daughter" aTPSCs into their blood stream [44-59,83-85].

At harvest, the participants are weighed. The volume of blood removed is equal to 2-ml of blood per pound of body weight. For a person weighing 200 lbs., 400-ml of blood is withdrawn via venipuncture and placed into multiple 10-ml purple top EDTA vacuum tubes (Becton-Dickinson, Franklin Lakes, NJ). The tubes are inverted 3-4 times during the blood draw to mix EDTA contents with the blood to prevent clotting. When the harvest is finished and all blood transferred to the 10-ml EDTA tubes, the blood is then transferred to 50-ml polypropylene centrifuge tubes (Falcon,

Becton-Dickinson). The 50-ml tubes containing the EDTA/blood mixture are placed into a 4 ℃ refrigerator for a minimum of 12-18 hours [85].

After 12-18 hours at 4 ℃ (activation step), the hematocrit formed is a whole blood pellet (containing RBCs, Platelets, large exosomes**,** and serum), a buffy coat (containing serum, WBCs, platelets, and intermediate exosomes), and supernatant (containing serum, small exosomes, and aTPSCs) [86]. The supernatant (containing serum, small exosomes, and aTPSCs) is withdrawn into separate 50-ml centrifuge tubes (40-ml maximum per tube), discarding the blood pellet and buffy coat. Then, a series of centrifugation steps at 4 ℃ begin using a 5810R Eppendorf centrifuge (Eppendorf 5810 Refrigerated centrifuge with rotor FA-45-6-30, aerosol tight lid, Eppendorf, Hamburg, Germany) is performed with a serum gradient at 1,000, 2,000, 4,000, 6,000, 8,000, 10,000, 14,000, and 16,000 RCF, to separate MesoSCs, EndoSCs/EctoSCs, GLSCs, PSCs, CLSCs/HLSCs, small exosomes, and TSCs from each other [85]. After each centrifugation step, the supernatants are decanted to a separate tube and centrifuged at the next higher centrifugation speed. The respective pellets for each category of aTPSCs from each centrifugation step are pooled and processed to further purify their constituents. Purification entails resuspending pellet contents in 30-ml of sterile saline and spinning at 1/10th the original centrifugation speed. To further purify, if necessary, the pellet is resuspended in sterile water and spun at 1/10th original centrifugation speed [water will lyse any remaining progenitor cells, differentiated cells, and exosomes, leaving aTPSCs intact] [86,87]. The supernatant is discarded, and the pellet resuspended in 5-ml of sterile saline. Once the TSCs (usTSCs/sTSCs), HLSCs/CLSCs, PSCs, GLSCs, EctoSCs, MesoSCs, EndoSCs, are segregated into individual cell groups, they are purified, and the aTPSCs are then recombined for respective treatments.

For neurological treatments, the pooled TSCs are used for intranasal delivery, while the remaining pooled aTPSCs are used for systemic intravenous delivery. The TSCs are centrifuged and then resuspended in 2-ml of sterile saline. The mucus in the nose is washed out with 0.065% sterile saline (Nasal Spray, [CVS, Walgreen, Target, etc.] Pharmacy) and the participant placed into the Trendelenburg (nostrils pointing upward) position. One ml of TSC solution is dropped onto the olfactory epithelium of each nostril, using a tuberculin syringe (syringe only, NO needle). The patient is held in the Trendelenburg position for an additional five minutes, then placed into the upright position. The remaining aTPSCs are resuspended in 250-ml of heparin/sterile saline and given systemically by intravenous infusion through the median cubital vein for 30-45 minutes [50-57,59,83,84].

For cardiovascular treatments, the pooled TSCs are used for primary intravenous delivery, while the remaining pooled aTPSCs are used for secondary systemic intravenous delivery. TSCs are resuspended in 250-ml of heparin/sterile saline and given systemically by a very slow (180-240 minutes) intravenous infusion, through the median cubital vein. [There are channels through the ventricular heart muscle from the inner chambers to the outer layer of the heart, called the *vena communicantes minimi*. These channels are roughly 3-5 microns in diameter, which is too small for the passage of RBCs or WBCs. Serum moves back and forth through these channels during systole and diastole.] The aTSCs are sufficiently small size (0.1-2.0 microns) to easily traverse these channels, where they contribute to repairing cardiomyopathic cells and tissue, forming new cardiac myocytes, new vasculature, and repairing the cardiac skeleton [34,35]. The remaining aTPSCs are resuspended in 250-ml heparin/sterile saline and given systemically by regular (30-45 minutes) intravenous infusion through the median cubital vein [43,49].

For pulmonary treatments, the TSCs, HLSCs, CLSCs, and PSCs are polled for nebulization, while the remaining GLSCs, EctoSCs, MesoSCs, and EndoSCs are pooled for systemic intravenous delivery. The aTPSCs pooled for nebulization are centrifuged, resuspended in 2-3-ml of sterile saline, and nebulized (deep breathing into the lungs through the mouth) using a nebulizer. The remaining pooled aTPSCs are resuspended in 250-ml heparin/sterile saline and given systemically by regular (30-45 minutes) intravenous infusion through the median cubital vein [46,47].

For systemic delivery (e.g., autoimmune, renal, metabolic, systemic issues), all aTPSCs are pooled for systemic intravenous delivery. They are resuspended in 250-ml heparin/sterile saline and given systemically by intravenous infusion through the median cubital vein for 60 minutes [45,48,58].

For orthopedic issues, the EctoSCs and EndoSCs are pooled and added to the second aliquot. All other aTPSCs are pooled, then divided into two separate aliquots (first and second). The first aliquot is resuspended in sterile saline, centrifuged to pellet cells, and then resuspended in 2-5-mls of sterile saline, depending on number of injections into joints. The second aliquot is combined with the pooled EctoSCs and EndoSCs, resuspended in 250-ml heparin/sterile saline and given systemically by regular (30-45 minute) intravenous infusion through the median cubital vein [44].

3. Results

Table 1 Results from IRB-Approved Clinical Study Protocols of Fresh Isolate Telomerase Positive Stem Cell Technologies

Ref #	Clinical Trial	Sample Size, n=	Adverse Events	Description	Efficacy
44	Osteoarthritis	6	None	Initially Bone on bone; decreased pain, increased joint space, increased ambulation	100%
45	Systemic Lupus Erythematosus	1	None	Rescued from death, increased organ functioning from less than 25% to ~70%, 12+ years	100%
42, 46	Idiopathic Pulmonary Fibrosis	2	None	Increased pulmonary function in one participant from 14% to 27%, and then stabilized at 25% for 8+ years. In other participant from <25% to ~70% for 12+ years	100%
42, 47	Chronic Obstructive Pulmonary Disease	51	None	48 participants demonstrated an increase in lung function (FEV_1), one participant for 8+ years. Three participants showed no effect to treatment, but did not follow informed consent guidelines	94%
48	Celiac Disease	1	None	Completely reversed symptoms of celiac disease during donor transplantations, went from 1:73 titer to 1:<1 gliadin titer during treatment period. Reverted when donor stem cell treatments stopped	100%
43, 49	Cardiovascular Disease	2	None	One participant had myocardial infarction six years prior to treatment initiation. 1st Txt raised cardiac output from <25% to 35%, 2nd Txt from 35% to 45%; Other participant raised cardiac output from <25% to ~70%	100%
50	Dry Age-Related Macular Degeneration	4	None	Two participants completely reversed symptoms, restoring complete vision to individuals. Other two participants the Txts did not work, one had heart problems that were treated instead, while the other did not follow informed consent guidelines	50%
51	Alzheimer's Disease	4	None	Two participants completely reversed symptoms. Other two participants the Txts did not work, they did not follow informed consent guidelines	50%
40, 81, 82	Parkinson's Disease	12	None	2/12 – no response, did not follow informed consent guidelines; 10/12 showed reversal of symptoms 1st month after Tx. At 7 & 14-months post-Tx 2/12 regressed at slower rate than before treatments began; 4/12 remained in stasis; 4/12 normal or near normal.	66%
52	Traumatic Blindness	1	None	From completely blind to shades of black and gray (partial restoration of 'night' vision) after two Txts.	100%
53	Traumatic Spinal Cord Injury	1	None	From complete paraplegia from T12 and below, to regain of bladder/bowel function after two successful Txts.	100%
54	Chronic Inflammatory Demyelinating Polyneuropathy	3	None	Inability to walk prior to treatments. 2/3 demonstrated ability to walk unassisted post-Txts; 1/3 demonstrated no change – did not following informed consent guidelines	66%

55	Stroke	1	None	Decreased cognition and decreased ambulation pre-Txt. Post-Txts showed increasing gain of cognitive function and ambulation	100%
56	Traumatic Brain Injury	1	None	Decreased cognitive function, no movement of limbs on ipsilateral side of body. After two Txts showed increased cognition & ability to move all limbs	100%
57	Multiple Sclerosis	3	None	1st participant pre-Txt: decreased cognitive function, motorized wheelchair and on ventilator 24/7; Post x 2 Txts – increased cognitive function, walk with leg braces, drove vehicle, breathing own for 4 years. Five years after Txts ceased began to regress, passed away due to respiratory infection. 2nd & 3rd participants – no effect, did not follow informed consent guidelines	33%
59	Amyotrophic Lateral Sclerosis	2	None	Two participants – one showed stasis to slow decline for 5 years, regressed after Txts stopped, passed away due to respiratory infection; the other is currently in stasis for 10+ years	100%
58	Chronic Kidney Disease	1	None	Reversed symptoms of kidney failure and restored kidney function for 3 years. Once Txts stopped, slow regression of symptoms leading to kidney failure.	100%
	Treated Problem Did not follow IC. Something Else **Totals**	83 11 2 **96**	Safety* **Safety***	Average Efficacy = Average Efficacy = Average Efficacy = **Average Efficacy =**	100% 11.5% 2.1% **86.4%**

Legend to Table 2. FEV_1, forced expiratory volume in 1-second; Txt(s), treatment(s); IC, Informed Consent Guidelines; *Safety, we have lost 7 people from the clinical studies due to their demise. One was lost to a traffic accident, one was lost due to intentional 3rd party poisoning, and five were lost due to death incurred by pneumonia from 4-8 years after completing the last of their stem cell treatment(s). The remaining participants are still alive. All participants signed the Informed Consent Guidelines [80][80]. There are 11 individuals (11.5%) where their treatments failed to produce any effect. They acknowledged smoking and drinking during their treatments, contrary to signed Informed Consent Guidelines. In two individuals, their aTPSCs healed something other than what the stem cells were proposed to treat.

4. Discussion

We use inherent size and differentiative capabilities to define where the aTPSCs are placed for treatment. For example, MSCs have been used to treat neurological problems [88-90]. I am at a loss to understand that rationale, since MSCs derived from single cell clones will only form white fat, cartilage, and bone [4]. Nevertheless, MSCs are also given intranasally, but due to their large size, a high osmolarity compound, such as mannitol, needs to be used. Mannitol shrinks-the olfactory epithelial cells forming channels to allow the MSCs to gain entrance to the brain past the blood brain barrier. For those individuals past puberty, if there is only a single application of mannitol on the nasal mucosa (olfactory epithelium), no harm, no foul. Two or more applications of nasal mannitol create permanent channels through the olfactory epithelium through the blood brain barrier, potentially allowing free access for bacteria and viruses to the meninges (causing meningitis) and beyond [91-95].

Alternatively, we utilize TSCs for directed neurological treatments. The rationale is based on their size, differentiation potential, and migratory ability. The size of TSCs is 0.1-2.0 microns. Because of their inherently small size, the use of mannitol or other high osmolarity substances is not necessary. TSCs can easily slide between the olfactory epithelia (mucosal cells) to bypass the blood brain barrier. The TSCs will form all the cells of the brain and spinal cord, e.g., various types of neurons, glial cells, meninges, blood vessels, etc., from their naïve undifferentiated state. One concern is that TSCs may be trapped by the nasal mucus before they ever reach the olfactory epithelium. Therefore, the nasal mucus is washed out of the nose prior to application of the aTSCs onto the olfactory epithelium [39,40,50-57,59].

Clinical studies paralleling our animal model systems for Parkinson's Disease [39,40], Myocardial Infarction [34,35], and Pulmonary Fibrosis [22] began and showed a 100% safety record for aTPSC transplant and 100% efficacy for reversing signs and symptoms of these diseases [22,34,35,39,40]. Additional human clinical studies are added for

multiple diseases and/or traumatic injuries to determine which would or would not be affected with the aTPSC treatments. The diseases/injuries selected are Osteoarthritis of hips, knees, and ankle joints [44], Rheumatoid Arthritis, Systemic Lupus Erythematosus [45], Pulmonary Fibrosis [46], Chronic Obstructive Pulmonary Disease [47], Celiac Disease [48], Cardiovascular Disease [49], Dry-Age-Related Macular Degeneration [50], Alzheimer's Disease [51], Traumatic Blindness [52], Traumatic Spinal Cord Injury [53], Chronic Inflammatory Demyelinating Polyneuropathy [54], Stroke [55], Traumatic Brain Injury [56], Multiple Sclerosis [57], Sciatica, Neuropathies, Amyotrophic Lateral Sclerosis [58], and Chronic Kidney Disease [59] (Table 1).

Cumulative results thus far have shown a 100% safety record for transplanting *ex vivo* activated fresh isolates of aTPSCs and a cumulative 86.4% efficacy for reversing signs and symptoms of their respective problems in 96 individuals (Table 1). There are 13 individuals where their respective aTPSCs treatment(s) failed to demonstrate a positive result for the condition the aTPSCs are supposed to treat. Eleven individuals (11.5%) acknowledged smoking, ~~and~~ drinking, or other non-approved activities during their treatments, thus not following Informed Consent Guidelines. In two individuals (2.1%), their aTPSCs treated something other than what the stem cells were supposed to treat, thus giving a negative response.

For example, we treated four individuals with Dry Age-Related Macular Degeneration (Dry-AMD) [50]. While medications can be given to treat Wet-AMD to slow its progression, there is no current treatment available for individuals with Dry-AMD. Following six fresh isolate treatments with aTPSCs (i.e., TSCs intranasal and remaining aTPSCs via systemic intravenous infusion) two participants had their Dry-AMD completely reversed, restoring complete vision to the individuals. The other two participants did not show any resolution of their vision problems, even after six treatments. One participant had severe heart problems that their body treated instead. The other individual did not follow informed consent guidelines, with respect to NOT drinking alcohol and NOT smoking cigarettes during their stem cell treatments. The final efficacy for an n=4 sample size for age-related Dry-AMD was 50% [60] (Table 1). We have seen similar negative results occur in participants treated for COPD [47], CVD [49], AlzD [51], TSCI [53], CIDP [54], and MS [57].

In clinical trials ESCs, iPSCs, and MSCs have shown far less efficacy than aTPSCs for treating diseases and trauma [88-90,96-123]. We attribute this decrease in efficacy to the pre-differentiation of ESCS and iPSCs into progenitor cells to prevent teratoma formation when implanted into an individual in the undifferentiated naïve state. Once committed, ESCs and iPSCs assume all the characteristics of progenitor cells, including a limited lifespan, and are relegated to only forming the cell types within the lineage in which they are pre-committed.

With respect to MSCs, while having a limited lifespan, the isolation protocols routinely used commercially in stem cell clinics do not purify the MSCs from other cell types that are present. For example, the general commercial stem cell clinic rationale is that bone marrow is composed of hematopoietic stem cells, their respective downstream cell types, and marrow stroma/MSCs. Therefore, the isolation protocols used, after a bone marrow harvest, are to separate out the blood elements and consider everything else as stroma/MSCs. Unfortunately, other types of cells are present in bone marrow as well. These other cell types include endosteal cells, osteoblasts, chondroblasts, chondrocytes, adipoblasts, unilocular adipocytes, endothelioblasts and endothelial cells (for arterial system, venous system, and lymphatic system), smooth muscle myoblasts, smooth muscle cells, fibroblasts, fibrocytes, and a very small fraction of aTPSCs [1-4]. Adipose tissue, another location for MSC harvest, suffers from the same isolation rationale as MSCs from bone marrow. The current stem cell clinic rationale is that adipose tissue is composed of unilocular adipocytes and stroma/MSCs. Unfortunately, there are also adipoblasts, endothelioblasts and endothelial cells (for arterial system, venous system, and lymphatic system), tripotent myoblasts, smooth muscle myoblasts, smooth muscle cells, nerve fibers with associated Schwann cells, neuroblasts, sensory nerve endings, fibroblasts and fibrocytes (for epineurium, perineurium, and endoneurium), and a very small fraction of aTPSCs [1-4]. Besides non-MSC-contaminating cells in their preparations, the original MSCs identified by Caplan [12] and verified by Pittenger et al. [13], will only form white fat, hyaline cartilage, and endochondral bone, as demonstrated in MSCs cloned from single cells [4]. There have been reports of other cell types formed from MSCs. These other cell types were either from a 'pluripotent' MSC or that the MSCs transdifferentiated into other germ layer lineage cells. I would propose that cell types other than fat, cartilage, and bone, were from contaminating cells within their isolate preparations, either by differentiated cells, progenitor cells, and/or aTPSCs.

Our choice to split the stem cell fractions into two groups, one for directed treatment and one for systemic delivery was not arbitrary, but rather based on real world conditions. We discovered that no matter where the *ex vivo* activated aTPSCs were placed within the body for directed treatment, the body would re-direct them for treating the most life-threatening conditions first. This occurred multiple times throughout the aforementioned clinical studies with respect

to Parkinson Disease/Cardiovascular disease [49], T12 Paraplegia/Restoration of Bladder & Bowel function [53], Dry-AMD/Heart Repair [50], and SLE/Repair & Restoration of tissues following a brown recluse spider bite [45].

From these and other occurrences we have learned that no matter where the stem cells are placed, the body will re-direct the *ex vivo* activated stem cells to be the most life-threatening injuries/diseases first. After that, if one splits the stem cell populations into directed and systemic, the body will allow the directed stem cells to remain in place and using both directed and systemically placed aTPSCs heal injuries/diseases in reverse order of occurrence, from the most recent to distant past.

A comparison and contrast literature review were performed to determine the pros and cons of using aTPSCs, MSCs, iPSCs, and/or ESCs for regenerative medicine (Table 2).

Table 2 Potential of Various Stem Cells for Regenerative Medicine

Attributes	**Telomerase Positive Stem Cells (TSCs, PSCS, MesoSCs, EctoSCs, EndoSCs)**	**Telomerase Negative Progenitor Cells (MSCs)**	**Telomerase Positive induced Pluripotent Stem Cells (iPSCs)**	**Telomerase Positive Embryonic Stem Cells (ESCs)**
% in Adults[1]	10%	40%	50%	NA
Telomerase[2]	Positive	Negative	Positive	Positive
Native Location in the Body[3]	Connective Tissue Matrices Throughout	Organ-Associated	Throughout the Body	2-Cell Stage to Blastocyst
Age Range[4]	Newborn to Geriatric	Newborn to Geriatric	Newborn to Geriatric	2-Cell Stage to Blastocyst stage
Numbers With Aging[5]	Remain Constant	Decline with age	Remain Constant	End at Blastocyst stage
Native Naïve State	Quiescent	Quiescent	Spontaneous Differentiation	Spontaneous Differentiation
Teratoma Formation *In Vivo*[6]	Absent	Absent	Present	Present
Responsive to Inhibitory Factors[7]	Yes	Yes	Yes	Yes
Increase in Cell Numbers[8]	In Situ & *Ex vivo*	*Ex vivo*	*Ex vivo*	*Ex vivo*
Growth in Culture[9]	Suspension & Adherent	Adherent	Adherent	Adherent
Responsive to Proliferation Factors[10]	Yes	Yes	Yes	Yes
Proliferation Potential[11]	Unlimited	Hayflick's Limit 50-70 Doublings	Unlimited	Unlimited
Responsive to Inductive Factors[12]	Yes	Only in Committed Lineage	No	No
Responsive to Local Cues[13]	Yes	Only in Committed Lineage	No	No

Cell Types Formed[14]	All Cells Types	Lineage Committed Cell Types	All Cell Types	All Cell Types
Time Period Fresh Isolate to *In Vivo* Use[15]	24 hours	4 hours	1-2 years	1-2 years
Time Period Isolation to *Ex vivo* Use[16]	5-10 days	10-20 days	1-2 years	1-2 years
Treatment Number Potential[17]	Millions To Trillions	Millions	Millions	Millions
Ability to migrate to tissue damage[18]	Yes	Yes	Unknown	Unknown
Express MHC Class-I markers[19]	TSCs & PSCs No MesoSCs, EndoSCs, EctoSCs Yes	Yes	NA	No
Immuno-Protected[22]	Yes	No	Yes	No
Autologous Treatments[20]	Yes	Yes	Yes	No
Allogeneic Treatments[21]	TSCs & PSCs Yes MesoSCs, EctoSCs, EndoSCs No	Yes	Yes	Yes

Legend to Table 2. Comparison of attributes of telomerase-positive stem cells (TSCs, PSCs, and MesoSCs) to telomerase-negative progenitor cell (MSC), induced pluripotent stem cells (iPSCs), and embryonic stem cells (ESCs), i.e., 1, percentage of stem cells present in adult individual [1,4]; 2, presence or absence of the enzyme telomerase; 3, their native location within the body; 4, age range of the individual from which the cells can be removed; 5, their numbers with respect to aging of the individual; 6, native naïve state in vitro; 7, presence or absence of teratoma (cancerous tissue) formation *In Vivo*; 8, responsiveness to inhibitory agents such as Leukemia Inhibitory Factor (LIF) or Anti-Differentiation Factor (ADF); 9, location of where induced increase in cell numbers can occur: in situ (in the body) or *ex vivo* (in culture); 10, responsiveness to proliferation factors; 11, proliferation potential; 12, responsive to inductive factors; 13, responsive to local environmental cues; 14, cell types formed; 15, time period required from isolation to use *In Vivo*; 16, time period required from isolation to finished *ex vivo* expansion; 17, treatment number potential; 18, ability to migrate to tissue damage; 19, Express MHC Class-I markers; 20, Immuno-protected from the recipient's; 21, Autologous treatment; and 22, Allogeneic treatment [1,4,13-27,34-60,64-79,82,83,85,88-90,96-122].

From their inherent attributes (Table 2), we hypothesize that endogenous adult telomerase-positive stem cells, e.g., TSCs, PSCs, EctoSCs, MesoSCs, and EndoSCs, would make excellent stem cell candidates for regenerative medicine.

Preliminary studies with limited numbers of individuals demonstrated their safe application (100%) as well as demonstrating efficacious (cumulative 86.4%) treatment for Osteoarthritis of hips, knees, and ankle joints [44], Rheumatoid Arthritis, Systemic Lupus Erythematosus [45], Pulmonary Fibrosis [46], Chronic Obstructive Pulmonary Disease [47], Celiac Disease [48], Cardiovascular Disease [49], Dry-Age-related Macular Degeneration [50], Alzheimer's Disease [51], Traumatic Blindness [52], Traumatic Spinal Cord Injury [53], Chronic Inflammatory Demyelinating Polyneuropathy [54], Stroke [55], Traumatic Brain Injury [56], Multiple Sclerosis [57], Sciatica, Neuropathies, Amyotrophic Lateral Sclerosis [58], and Chronic Kidney Disease [59] (Table 1).

5. Conclusion

Regenerative medicine is rife with studies using ESCs, iPSCs, and MSCs to treat various deleterious diseases and trauma. Unfortunately, in controlled clinical studies the reported results have been less than the hype associated with them. If ESCs and iPSCs are used in their naïve state, they form cancerous tissue (teratomas) when implanted. To prevent teratoma formation ESCs and iPSCs have to be pre-committed to cell-specific progenitor cells or differentiated cells. At this point ESCs and iPSCs lose the ability to propagate indefinitely and lose the ability to form any cell type of the body. Autologous MSCs have been shown to be 100% safe for transplant in clinical trials. However, their efficacy for treating conditions, other than fat, cartilage, and/or bone related, has been abysmal at best, averaging 1-5% efficacy. There is a fourth category of stem cells for regenerative medicine, i.e., the healing aTPSCs. The healing aTPSCs are few in number within the body as hibernating quiescent "Maternal" stem cells. Upon stimulation, the aTPSCs proliferate symmetrically to large numbers of naïve "Daughter" stem cells due to the presence of the telomerase enzyme. Coupled with their ability

to differentiate into any progenitor or differentiated somatic cell in the body from their naïve state under the direction of locally released exosome cues, make them an ideal candidate for regenerative medicine. When tested in clinical studies, both autologous and allogeneic aTPSCs, demonstrated that they were 100% safe to transplant and demonstrated a cumulative efficacy of 86.4% for reversing signs and symptoms in 20 separate chronic diseases or traumatic injuries in 96 individuals (Table 1). The results suggested that the aTPSCs retain all the positive aspects of ESCs, iPSCs, and MSCs, while exhibiting none of their negative aspects. Therefore, I propose that aTPSCs be an added category of stem cells for use in regenerative medicine to increase efficacy of proposed healing treatments.

Compliance with ethical standards

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Disclosure of conflict of interest

There is a conflict of interest with respect to enrolling participants in our IRB-approved open access open enrollment Phase-0 (everyone gets treated) clinical studies of *ex vivo* activated aTPSCs fresh isolates for treating chronic diseases, traumatic injuries, and orthopedic disorders. If you would like to learn more about healing cells and their ability to affect a positive repair and restorative response; apply for acceptance into our IRB-approved open access open enrollment Phase-0 (everyone gets treated) clinical studies for *ex vivo* activated fresh isolates of aTPSCs; and/or become a collaborator, you can email Dr. Henry E. Young PhD at young.hey1@yahoo.com. In the Subject line of the email state your interest.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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