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Combinatorial Nutraceutical Supplement Pill (CNSP) activates naïve adult telomerase positive stem cells *in-situ* to heal cardiomyopathies

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Abstract

Thirty percent of all deaths worldwide can be attributed to cardiovascular diseases. Myocardial infarction is due to one or more blocked coronary vessels. Following a myocardial infarction, the damaged myocardium is often replaced with non-functional scar tissue, resulting in heart failure. The prognosis of patients with heart failure is poor, with failure rates approaching 50%. Lack of blood flow is a major contributor. Multiple strategies have been developed to restore blood flow to the damaged myocardium. These strategies include pharmacological treatments, reopening the blockage with stents, bypassing the blockage with coronary arterial bypass grafts, and/or forming new blood vessels with stem cells. Since none of these strategies have proved to be permanent fixes, *ex vivo* activated adult telomerase-positive stem cells (aTPSCs) were examined to restore the damaged myocardium and vasculature of the heart. Individuals with cardiac outputs ≥25% were treated with *ex vivo* activated aTPSCs. Following treatment, cardiac output increased from 20-45% for 12+ years in these individuals. These results suggest that *ex vivo* activated aTPSCs are a viable option for the treatment of individuals with cardiovascular disease. Unfortunately, some individuals with cardiac outputs at or below 10% are too fragile to undergo the *ex vivo* activated aTPSC procedures. Therefore, a nutraceutical formulation (CNSP) was developed to activate the aTPSCs *in-situ* for cardiac repair. In an individual with a cardiac output of less than 10%, cardiac output increased by 25% following six months ingestion of CNSP. And increased cardiac output of 10-15% occurred after an additional 12-36+ months of CNSP.

Keywords: Cardiomyopathy; CNSP; aTPSCs; Stem cells; Myocardial infarction; Regenerative medicine

1. Introduction

Cardiovascular diseases (CVD) have been the leading cause of mortality worldwide, responsible for 30% of all deaths. CVD is responsible for more than 7.5 million in-patient cardiovascular disease procedures in the USA. Heart disease costs in the USA are approximately $219 billion dollars per year. This total includes medications, health care services, and premature death [1]. Contributing risk factors such as obesity, hypercholesterolemia, hyperlipidemia, and hypertension result in atherosclerosis, which results in cardiovascular disease [2].

In the USA, coronary artery disease (CAD) results in ischemic heart disease that manifests itself as angina pectoris and myocardial infarction (MI). Thus, CAD is a major cause of disability and death in this country. Myocardial infarction is due to one or more blocked coronary vessels [2-5]. Following an MI, the myocardium is often replaced with non-functional scar tissue [6]. Such scarring and damage to the myocardium often results in left ventricular systolic dysfunction, ventricular aneurysm, decreased cardiac output, and heart failure [7-9]. Mortality and morbidity following myocardial infarction remain high despite current pharmacological treatments, utilizing angiotensin converting enzyme inhibitors (ACE-I) and/or angiotensin-receptor blockers (ARBs) [8]. Myocardial infarction and the consequent

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loss of functional myocardium is a major factor in the etiology of heart failure [10]. Heart failure is a common, disabling, and lethal condition [5,11]. The prognosis of patients with heart failure is poor, with an increasing failure rate approaching 50%.

Once the myocardium dies after an MI, it is replaced with scar tissue. The location, size, structure, composition, and mechanical properties of the scar tissue are all critical determinants for the fate of patients who survive the initial infarction [6]. An additional complication is that the insulating scar tissue can disrupt the electrical properties of the myocardium, predisposing the patient to arrhythmias, which can prove fatal. Ventricular arrhythmias are likely to require cardioversion in order to prevent fatal events [9]. Such scarring and damage to the myocardium often results in left ventricular systolic dysfunction, ventricular aneurysm, and heart failure [8-10].

Since one of the major contributors to mortality in individuals with cardiovascular disease is lack of blood flow to the ischemic myocardium, multiple therapeutic regimens have been designed to address this issue. One therapeutic regimen is to physically re-open blocked vessels with angioplasty followed by (drug eluting) stents. A second therapeutic regimen is to bypass blocked vessels with coronary arterial bypass grafts (CABG). A third therapeutic regimen is to regrow new blood vessels by the placement of stem cells, such as embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), cardiac stem cells (CSCs), mesenchymal stem cells (MSCs), or skeletal myoblasts, at the openings of the coronary arteries in the ascending aorta. The rationale for using these stem cells is the generation of new vasculature, via angiogenesis and/or vasculogenesis, to restore blood flow to the existing and damaged myocardium [12-18].

Since none of these strategies have proved to be permanent fixes, an alternative approach was chosen. This alternative approach was to use adult telomerase positive stem cells (aTPSCs) to restore the histoarchitecture of damaged heart tissues, i.e., restoring functional myocardium, cardiac skeleton, and vasculature. There are five subpopulations of aTPSCs within adult animals, including humans, e.g., 0.1-2-micron totipotent stem cells (TSCs), 6-8-micron pluripotent stem cells (PSCs), and 10-12-micron ectodermal stem cells (EctoSCs), mesodermal stem cells (MesoSCs), and endodermal stem cells (EndoSCs) [19].

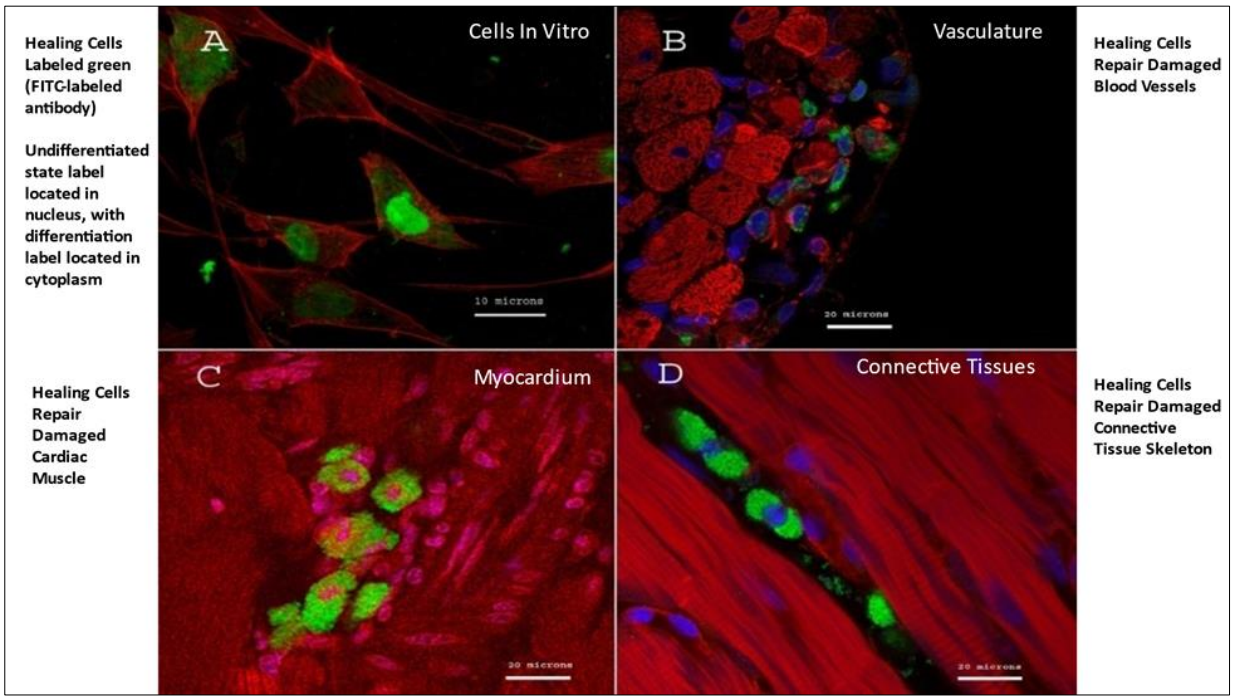

Figure 1. Adult rat model of myocardial infarction demonstrated that PSC clone, Scl-40-beta, repaired a damaged heart by regeneration/repair of myocardium, cardiac skeleton, and vasculature. *Reprinted with permission from Young et al. Adult reserve stem cells and their potential for tissue engineering. Cell Biochemistry Biophysics. 2004; 40(1):1-80 [21*]

Pre-clinical animal studies in an adult rat model of myocardial infarction. A genomically labeled PSC clone (Scl-40-beta) of Healing Cells (aTPSCs) in culture (A) and infused into damaged myocardium (B-D). Fig. 1A. Note presence of the (green) label in the nucleus of undifferentiated healing cells. With differentiation of the stem cells the genomic label

translocates to the cytoplasm. Fig. 1B. Within the coronary vessels. Fig. 1C. Within the myocardium. And Fig. 1D. Within the connective tissue cardiac skeleton. These results demonstrated that a PSC clone repaired a damaged heart by regeneration/repair of myocardium, cardiac skeleton, and vasculature [20,21].

In contrast to ESCs, iPSCs, CSCs, MSCs, or skeletal myoblasts deposited at the openings of the coronary vessels to generate new blood vessels, aTPSCs were chosen to repair and/or replace ALL damaged tissues of the heart, e.g., myocardium, cardiac skeleton, and vasculature, resulting in a functional increase in cardiac output. *Ex vivo* (outside the body) activated aTPSCs were utilized based on their unique sizes, differentiation capabilities, the histoarchitecture of the heart, and a protracted systemic delivery [22]. The aTPSCs isolation, activation, and use in human clinical studies followed FDA-mandated guidelines for minimal manipulative procedures [22,23]. The patented technologies for *ex vivo* activation of aTPSCs [24] involve proliferation of aTPSCs inside the body (*in-situ*), mobilization of aTPSCs into the bloodstream *in-situ*, removal of whole blood by venipuncture, *ex vivo* activation of aTPSCs, separation of aTPSCs from blood, discarding the blood pellet and buffy coat, segregating aTPSCs into individual populations, and recombining the populations for subsequent use, dependent on disease or disorder to be treated. The step-by-step sequence for *ex vivo* activated aTPSCs in CVD repair entails an individual to ingest 'Nutra' (Dragonfly Foundation for Research and Development [DFRD], Macon, GA), which causes aTPSCs to proliferate *in-situ*. This is necessary because TSCs make up such a small fraction (0.1%) of all the stem cells in the body [25,26]. By stimulating the aTPSCs to proliferate *in-situ*, this creates an environment whereby the individual being treated becomes their own sterile bioreactor, propagating autologous aTPSCs, including the TSCs, to large numbers prior to harvest. Eighteen hours before harvest, the individual ingests 'Glacial Caps' (DFRD). Glacial Caps stimulate the connective tissue resident proliferated aTPSCs *in-situ* to mobilize into the blood stream. At time of harvest whole blood, containing blood elements and aTPSCs, is removed by venipuncture, i.e., 2-ml (cc's) of whole blood for every pound body weight and placed into 10-ml EDTA purple top blood collection tubes (Becton-Dickinson, Franklin Lakes, NJ) to prevent clotting. The blood/EDTA mixture is transferred to 50-ml centrifuge tubes (Falcon, Becton-Dickinson) and placed at 4℃ for 12-18 hours. During this activation step, the blood elements and aTPSCs self-separate. Self-separation is based on time, temperature, gravity, differential precipitation, and zeta potential. The hematocrit that forms consist of a blood pellet, containing red blood cells (RBCs), platelets, large exosomes, and serum; a buffy coat, containing white blood cells (WBCs), intermediate-sized exosomes, and serum; and the supernatant, containing aTPSCs, small exosomes, and serum. The blood pellet and buffy coat are discarded. Differential density gradient centrifugation is performed using serum, saline, and sterile water [26]. These protocols remove the small exosomes and serum from the aTPSCs and separate the aTPSCs into TSCs, PSCs, EctoSCs, MesoSCs, and EndoSCs. The TSCs are aliquoted in sterile saline as an individual population and the PSCs, EctoSCs, MesoSCs, and EndoSCs are pooled in sterile saline to form a mixed population. The TSCs are suspended in 250-ml of sterile heparinized saline and, using intravenous infusion through the median cubital vein, infused systemically into the individual using a protracted 180–240-minute duration. Following TSCs infusion, the pooled PSCs, MesoSCs, EctoSCs, and EndoSCs are suspended in 250-ml of sterile heparinized saline and infused into the individual using a 30-45-mintue duration [26].

The very small TSCs and the inherent histoarchitecture of the heart, including the thebesian veins (*vena communicantes minimi)*, were used to heal the heart from within without the necessity of trying to pass larger stem cells through blocked coronary vessels. The thebesian veins consist of multiple small 3 to 5-micron endothelial-lined channels that traverse the cardiac musculature from inside the four chambers to the visceral pericardium covering the outside of the heart. The endothelial-lined channels are too small for the passage of RBCs, WBCs, platelets, and large and intermediate-sized exosomes, but are of sufficient size for the back-and-forth passage through the cardiac musculature of serum and small exosomes during systole and diastole. Because of their small size, the 0.1-2.0-micron TSCs can also traverse the 3 to 5-micron thebesian veins, during systole and diastole. Their inherent size, coupled with the extended time frame in which the TSCs are infused (180-240-minutes), allows the TSCs to effectively migrate to areas of damage within the heart and repair the heart from the inside out. This was proven in individuals demonstrating an increase in their functional cardiac output from 25% to ≥ 50% (Fig. 2) [22,26]. For example, a Systemic Lupus Erythematosus (SLE) patient's cardiac output dropped precipitously, 90% to 30%, during time of ingestion of hydroxychloroquine to slow progression of SLE. At time of his first aTPSC transplant his cardiac output was slightly below 25%. Autologous *ex vivo* activated aTPSCs transplants entailed a long systemic infusion of TSCs followed by regular infusion of PSCs, MesoSCs, EctoSCs, EndoSCs. Gender-matched and ABO blood group-matched allogeneic *ex vivo* activated aTPSCs transplants entailed a long systemic infusion of TSCs followed by a regular infusion of PSCs only. The first *ex vivo* activated aTPSC (autologous) transplant raised his cardiac output to 25%. The second *ex vivo* activated aTPSC transplant was from an allogeneic 42-year-old A+ male donor, which raised his cardiac output to approximately 40%. The third *ex vivo* activated aTPSC transplant was from an allogeneic 73-year-old O-negative male donor and raised his cardiac output to approximately 70%. A total of 20 autologous and nine allogeneic *ex vivo* activated aTPSC transplants thus far have maintained cardiac output at approximately 70% for over twelve years and counting (Fig. 2) [22].

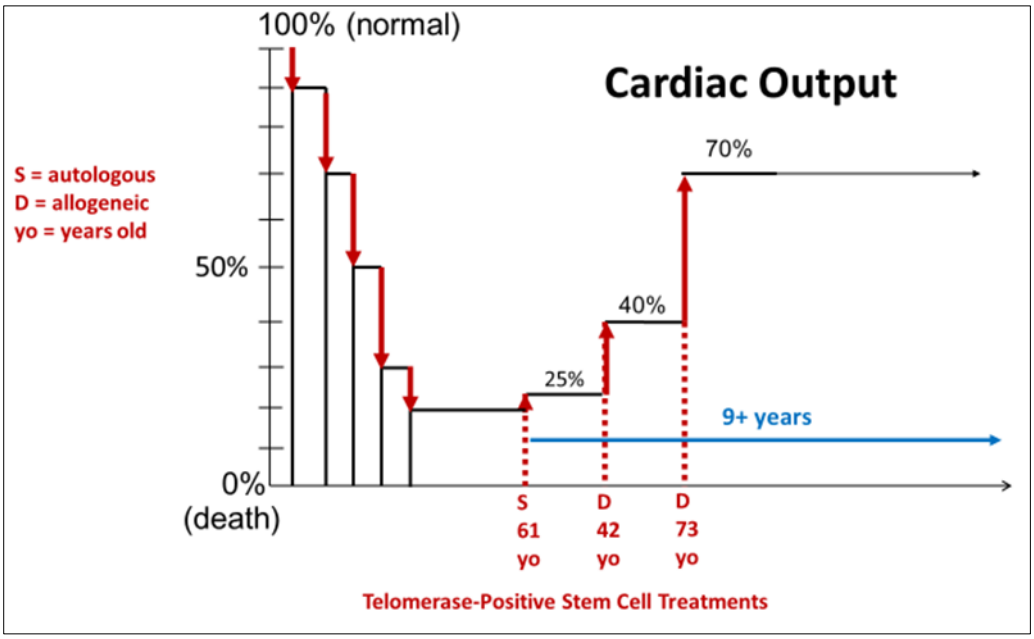

Figure 2 A Systemic Lupus Erythematosus (SLE) patient was treated with multiple autologous and allogeneic (matched for gender and ABO blood group) *ex vivo* activated aTPSCs. ~~'s~~ Cardiac output was raised from for over twelve years and counting. *Reprinted with permission from Young HE, Speight MO. Cardiovascular disease treated with telomerase-positive stem cells. Stem Cells and Regenerative Medicine. 2020; 4(2):1-8 [22].*

Use of *ex vivo* activated aTPSCs procedures to increase cardiac output by repairing myocardium, cardiac skeleton, and vasculature work well with individuals having a cardiac output ≥25%. Those individuals with cardiac outputs of 10% or less may be too fragile to withstand the blood harvesting procedures necessary to isolate, *ex vivo* activate, and infuse aTPSCs. The *ex vivo* activation procedure includes removal of 2-ml (cc's) of blood for every pound of body weight to harvest the aTPSCs. For example, a two-hundred-pound individual would require 400-ml of oxygen-carrying blood to be removed and processed to isolate the aTPSCs. The RBCs, WBCs, Platelets, and exosomes are destroyed during processing. The hypothesis being tested is whether a formulation could be developed to stimulate the proliferation of adult telomerase positive stem cells *in-situ*, stimulate their mobilization into the blood stream *in-situ*, and activate the adult telomerase positive stem cells *in-situ* to heal the damaged heart, thereby increasing functional cardiac output, without the aTPSCs ever having to leave the body.

2. Material and methods

The formulation developed was modeled on a series of steps that occurred during complete limb regeneration in adult terrestrial salamanders [27-33].

- Step 1 is amputation of a limb that initiates the restoration/regeneration process.
- Step 2 is a purse string closure of epidermis surrounding the wound site.
- Step 3 is a thickening of the epidermis at the wound site forming an apical epidermal ridge (AER).
- Step 4 is the debridement and removal of the damaged tissues by macrophages, NK-cells, and neutrophils (innate immune system).
- Step 5 is release of glycoproteins from the apical epidermal ridge (AER) into the area between the proximal uninjured tissues and the AER.
- Step 6 is the proliferation of connective tissue-resident stem cells within the proximal uninjured tissues of the limb and body.
- Step 7 is the migration of stem cells from the proximal uninjured tissues to an area adjacent to the AER.
- Step 8 is the arrival of similar stem cells via the vasculature to an area between the proximal uninjured tissues and the AER.
- Step 9 is continued release of glycoproteins from the AER resulting in stem cell proliferation.
- Step 10 is the release of glycoproteins from the uninjured proximal tissues.
- Step 11 is the formation of zones that recapitulate the embryonic lineage pathways to begin stem cells differentiating into the missing tissues of the limb. From distal to proximal:

- Zone-1 is the glycoprotein-secreting AER.
- Zone-2 contains proliferating undifferentiated stem cells.
- Zone-3 contains proliferating mesodermal stem cells and ectodermal stem cells.
- Zone-4 contains mesodermal progenitor cells and ectodermal progenitor cells beginning to transform into differentiated cell types.
- Zone-5 contains newly differentiated skeletal muscle, cartilage, bone, tendons, ligaments, blood vessels, epidermis, and nerve fibers. And
- Zone-6 contains proximal uninjured differentiated cells.

- Step 12 is outgrowth of the forming appendage to its original length, while maintaining the zonation patterns. And
- Step 13 is complete restoration/regeneration of the histoarchitecture of the missing limb.

The key steps above for complete restoration/regeneration of the histoarchitecture of a missing limb that are relevant to development of the formulation are Steps 4-11, and 13. With respect to the formulation developed, the following steps were deemed important. 1. All steps needed to occur *in-situ*, there would be no removal from the body, *ex vivo* activation, or infusion of aTPSCs from or to the individual. 2. The proliferation of adult telomerase positive stem cells would occur symmetrically within their connective tissue niches throughout the body. 3. A continuous mobilization of the aTPSCs into the vasculature would occur 24/7, rather than a bolus release, as long as the compound was routinely being ingested. 4. Increased circulation to all organs and tissues throughout the body was necessary to ensure delivery of the stem cells to damaged tissues. 5. Activation of the stem cell's homing receptors to damaged tissue needed to occur. 6. Activation of the stem cell's surface receptors for inductive, progressive, and/or inhibitory agents (locally released exosomes) needed to occur. 7. Support a strong innate immune system (e.g., macrophages, NK cells, and neutrophils), which is key critical for proper wound healing to occur. And 8. To prevent the newly differentiated cells from overgrowing the existing tissue.

The formulation started with 1/4th organic 'Nutra' (DFRD) and 1/16th reverse osmosis filtration microcystin-free, EDTA/Sepharose column heavy metal-depleted 'Glacial Caps' (DFRD) as the starting base. Additional constituents were added to fulfil criteria 4-8. The proprietary formulation, consisting of multiple organic dark fruits and vegetables, zinc gluconate, and folinic acid, was patented [34]. Once the formulation constituents were tested in large animals under IACUC approval, the proprietary formulation, termed 'combinatorial nutraceutical supplement pill' (CNSP), under IRB approval with Informed Consent, was given to an individual with less than 10% cardiac output. The dosage was based on body weight. Healing dose was set at 1 capsule of CNSP per 50-pounds body weight, and accelerated healing dose was set at 1 capsule per 25-pounds body weight. A ramp up procedure was used to attain the accelerated healing dose. Week one, 1 capsule of CNSP per 100 pounds body weight; weeks 2-4, 1 capsule of CNSP per 50 pounds body weight; and thereafter, 1 capsule of CNSP per 25 pounds body weight.

3. Results

A sixty-three-year-old male one week after open heart surgery (consisting of five coronary arterial bypass graft procedures and 15 drug eluting stents) had a massive acute myocardial infarction leaving him with less than 10% cardiac output at discharge from hospital. He was placed on the national registry for a heart transplant. By four months on CNSP, his cardiac output had risen to 35% and his name was removed from the heart transplant list. An additional 12-36+ months on CNSP and his cardiac output rose to ~50%. He is continuing to ingest CNSP daily (Fig. 3) [22].

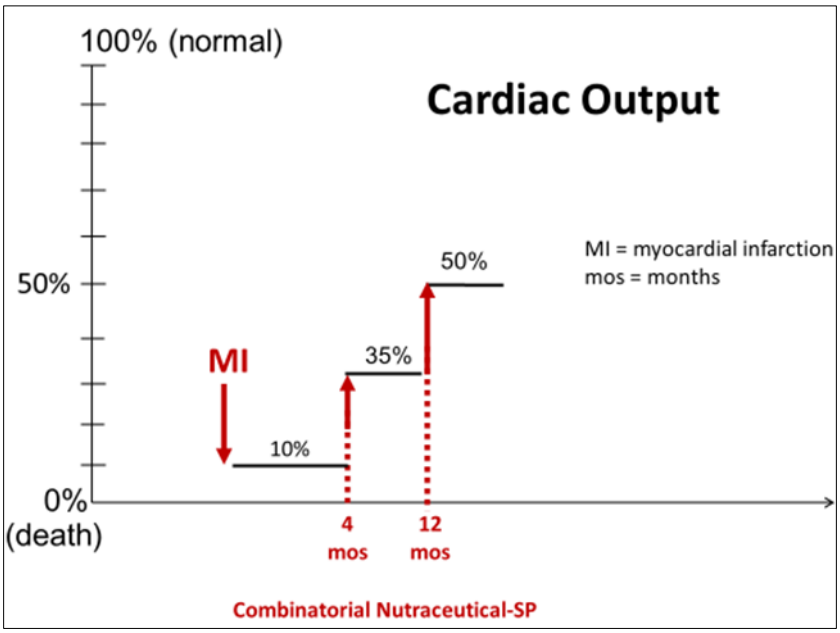

Figure 3 Ramp up ingestion of CNSP over a four-month time frame caused an increase in functional cardiac output from <10% to 35%. Additional CNSP caused a further increase in cardiac output from 35% to approximately 50% for 36+ months. *Reprinted with permission from Young HE, Speight MO. Cardiovascular disease treated with telomerase-positive stem cells. Stem Cells Regen Med. 2020; 4(2):1-8 [22]*

4. Discussion

Cardiovascular disease, especially ischemic heart disease, is one of the major causes of death and disability in the United States. Myocardial infarction due to one or more blocked coronary arteries or their tributaries present unique challenges to the field of regenerative medicine [2-5]. Following an MI, the myocardium is often replaced with non-functional scar tissue [6]. Myocardial infarction and the subsequent loss of fully functional myocardium is a major factor in the etiology of heart failure [10]. The prognosis of patients with heart failure is poor, with a failure rate approaching 50% [5,11].

Once the myocardium dies, it is replaced by scar tissue over several weeks. The size, location, composition, structure and mechanical properties of the scar tissue are all determinants of the fate of patients who survive the initial infarction [6]. An additional complication is that scar tissue can disrupt the electrical properties of the myocardium, predisposing the patient to arrhythmias, which can prove to be fatal. Ventricular arrhythmias are particularly likely to require cardioversion in order to prevent future fatal events [9]. Such scarring and damage to the myocardium often results in left ventricular systolic dysfunction, ventricular aneurysm, and heart failure [7-9]. Mortality and morbidity following myocardial infarction remain high despite current pharmacological treatments, utilizing angiotensin converting enzyme inhibitors (ACE-I) and/or angiotensin-receptor blockers (ARBs) [8]. The loss of cardiac tissue underlies heart failure, and current pharmacological treatments do not address this problem [22].

Inflammatory changes of the coronary arteries can cause fibrosis and blockage of these blood vessels. Opening the blocked coronary arteries with balloon angioplasty intuitively may initially appear to be an attractive strategy. But even with the subsequent use of drug-eluting stents, the opened blood vessels tend to form fibrous connective tissue in about 3-6 months within the area of the stent that causes re-blockage of these blood vessels [22].

Coronary artery bypass grafting has been shown to be an effective surgical therapy short term. CABG has been demonstrated to be superior to the use of drug-eluting stents. CABG utilizes the great saphenous vein, or sometimes the internal thoracic vein. One end of the graft is implanted into the aorta, and the other into the blocked artery distal to the blockage. It is important to orient the venous graft properly so that the valves do not block the blood flow. Arterial grafts from the internal thoracic artery can also be used [13,35]. In this therapy, the distal end of the internal thoracic artery is grafted to the blocked coronary artery distal to the blockage. However, current therapeutic approaches still present significant risks to the patient [35].

In contrast, if one could form new pristine arteries in the patient's heart, by either angiogenesis and/or vasculogenesis using stem cells, one could avoid the risks associated with drug-eluting stents or CABG procedures. Indeed, stem cells are the current "holy grail" of regenerative medicine [36]. The use of stem cells to regenerate the patient's coronary arteries could offer hope to patients suffering from cardiovascular disease, even those who have experienced a myocardial infarction, resulting in dead or damaged myocardium. The source of stem cells for such purposes can present problems. Currently ESCs, iPSCs, CSCs, MSCs, skeletal muscle myoblasts, are being used for these purposes. The use of embryonic stem cells is very controversial due to moral and ethical concerns. Teratoma formation occurred after infusing either naïve undifferentiated ESCs or naïve undifferentiated iPSCs for cardiac repair [36-38]. The use of donor allogeneic stem cells would require immunosuppressive therapy to avoid tissue rejection. However, immunosuppressive therapy can cause increased morbidity and mortality due to a graft versus host disease (GvHD) response [6,39,40]. Moreover, the use of partially differentiated stem cells presents difficulties as well. Some reports have appeared concerning the use of partially differentiated MSCs to affect cardiac vascular regeneration [11,41,42]. As reported, more primitive stem cells, such as the very small embryonic-like stem cells (VSELs), a telomerase negative multipotent progenitor cell, may be more effective than the more differentiated telomerase negative tripotent progenitor MSCs for forming new vasculature [43]. Studies with more differentiated stem cells (cardiac stem cells and skeletal muscle myoblasts) have produced very limited and inconsistent improvements in cardiac structure and function [16-18]. Further study is needed to document which of these stem cells are most effective in the treatment of cardiac disease. This is particularly pressing since failure of therapeutic modalities results in the need for a heart transplant and there is a waiting list for heart transplants [22].

An alternative to the above therapeutic modalities is the use of aTPSCs. In IACUC-approved animal models of cardiovascular disease [20,21], a PSC clone from adult rat aTPSCs (Scl-40-beta), formed functional components of the heart, e.g., cardiac muscle, cardiac skeleton, and cardiac blood vessels, after transplantation following induced heart damage (Fig. 1). In addition, under IACUC approval, TSCs and PSCs were found as resident populations within the hearts of adult rats and adult pigs [44,45]. Based on these results, a hypothesis was generated that *ex vivo* activated aTPSCs could form the basis for therapeutic approaches for repair and regeneration of functional heart tissue, e.g., myocardium, cardiac skeleton, and vasculature [22]. The hypothesis was tested in IRB-approved clinical studies of cardiovascular repair using *ex vivo* activated aTPSCs [22,24,26,46]. Rather than placing a bolus of the stem cells at the openings of the coronary vessels in the ascending aorta, as had been done for ESCs, iPSCs, CSCs, MSCs, VSELs, or skeletal muscle myoblasts to generate new blood vessels**,** an alternative approach was undertaken. This alternative approach entailed restoration/regeneration of the damaged myocardium, cardiac skeleton, and vasculature of the ischemic heart muscle to restore functional cardiac output to the heart. The unique size of the TSCs (0.1-2.0 microns) along with the fact that the heart actually has two vascular systems, not just the coronary arterial/venous system, was used. The heart also contains the thebesian veins (*vena communicantes minimi)*. Thebesian veins are small endothelial-lined vascular channels without valves of less than 5 microns in diameter. Their size is too small for blood cells, either RBCs (7 microns) or WBCs (10+ microns), or mesenchymal stem cells (10-20+ microns) or MesoSCs (10-12 microns) or even PSCs (6-8 microns) to traverse [19,20]. Thebesian veins are found in all four chambers of the heart and run from inside the chambers, through the myocardium, to the visceral pericardium lining the outside of the heart. When the heart undergoes systole (contraction) fluid, consisting of serum and small exosomes, is pushed from the inside chambers through the thebesian veins in the myocardium to the pericardium lining the outside layer of the heart. During diastole (relaxation) fluid returns to the inside chambers via the thebesian veins through the myocardium from the visceral pericardium of the heart. It was hypothesized that by giving the heart TSCs (0.1-2 microns) over an extended period of time (180-240-minute systemic infusion), that these very small stem cells could migrate to areas of tissue damage within the walls of the heart to repair the damaged myocardium, repair the cardiac skeleton, and revascularize the heart, as they traverse back and forth through the thebesian veins [22,26].

This rationale was utilized for treating a 60-yer-old male that was diagnosed as two-week terminal stage-IV Systemic Lupus Erythematosus (SLE). His cardiac output was slightly less than 25% at time of first treatment (Fig. 2) [22,47]. His first adult telomerase-positive stem cell treatment consisted of autologous naïve TSCs given by slow (120-180-minute) initial intravenous infusion, followed by pooled naïve PSCs, EctoSCs, MesoSCs, and EndoSCs, given by regular (30-45-minute) secondary intravenous infusion. By two weeks his cardiac output had risen to 25%. His second transplant utilized gender-matched, ABO-blood group-matched allogeneic naïve TSCs (initial 120-180-minute infusion) and allogeneic naïve PSCs (secondary 30-45-minute infusion) from a 42-year-old A+ male donor. One month later his cardiac output had risen to 40%. His third transplant utilized allogeneic naive TSCs (initial 180-240-minute infusion) and naïve PSCs (secondary 30-45-minute infusion) from a 73-year-old O-negative male donor. One month later his cardiac output had risen to 70%. He has had an additional 19 autologous and seven allogeneic naïve aTPSC transplants from gender-matched A+ (positive) or O- (negative) donors. These 29 naïve aTPSC transplants have maintained their cardiac output at approximately 70% for twelve years and counting (Fig. 2) [22,34,47]. Only allogeneic naïve TSCs and PSCs were used from the gender-matched, ABO blood group-matched donors because these aTPSCs are immunoprotected. TSCs and

PSCs do not express MHC-Class-I markers on their cell surfaces in their naïve states. In contrast, MesoSCs, EctoSCs, and EndoSCs do express MHC-Class-I markers in their naïve states [19]. The MHC-Class-I markers allow the body to recognize self from non-self. Hence, there is the potential for allogeneic naïve MesoSCs, EctoSCs, and EndoSCs to be rejected by the recipient's immune system in a graft versus host disease response if the allogeneic stem cells are utilized [39,40]. The results from a clinical study [22] suggested that *ex vivo* activated autologous naïve aTPSCs, *ex vivo* activated allogeneic naïve TSCs, and *ex vivo* activated naïve PSCs could be given by intravenous infusion to positively affect cardiac repair by increasing functional cardiac output.

Two other cardiac patients with heart problems that had resulted from decreased cardiac outputs utilized the same protocol by regular IV infusion, i.e., naïve TSCs by slow IV infusion followed by pooled naïve PSCs, MesoSCs, EctoSCs, and EndoSCs. Only a modest increase in their functional cardiac output was noted and was not as dramatic as seen with the individual with SLE. In these other instances, only autologous naïve aTPSCs were utilized for their treatments [22].

A fourth individual wanted entrance into our ex vivo activated aTPSCs IRB-approved clinical studies. These studies were to test the effectiveness of *ex vivo* activated adult telomerase positive stem cells for reversing the signs and symptoms of various disease states, e.g., neurodegenerative, cardiovascular, pulmonary, autoimmune, systemic, renal, and orthopedic diseases or disorders [22,26,46-63]. From his medical history it was noted that he had been treated successfully with chemotherapy for leukemia during the previous three years. Unfortunately, the adverse side effects from the chemotherapy regimens stenosed his coronary vessels and caused severe cardiomyopathy. One week following open heart surgery during which five arterial bypass graft procedures and balloon angioplasty with 15 drug eluting stenting procedures were performed, he suffered a massive myocardial infarction that left him with less than 10% cardiac output. His name was placed on the national heart registry to receive a heart transplant by his cardiologist. It was determined that he was too fragile to undergo the stem cell harvest procedure of *ex vivo* activation of aTPSCs [22]. In this 200-pound individual, over 400-ml of oxygen-carrying red blood cells would have had to be removed and destroyed to isolate the aTPSCs. Thus, a hypothesis was proposed that formulation of nutraceuticals, starting with Nutra and Glacial Caps, could be created to effectively mimic the effects of *ex vivo* activated aTPSCs. For all functions to be relegated to inside the individual, those functions needed to meet the following eight criteria: 1. All steps needed to occur *in-situ*, there was to be no removal of whole blood from the individual, no removal of aTPSCs from the individual, no *ex vivo* activation, and no subsequent transplantation into the individual. 2. The proliferation of adult telomerase positive stem cells would occur symmetrically within the individual in their connective tissue niches throughout their body. 3. A continuous mobilization of the aTPSCs into the bloodstream would occur. 4. It would be necessary to increase circulation to all organs and tissues throughout the body. 5. It would be necessary to activate the stem cell's homing receptors to damaged tissue. 6. It would be necessary to activate the stem cell's surface receptors for inductive, progressive, and/or inhibitory agents (locally released exosomes). 7. It would be necessary to support a strong innate immune system (e.g., macrophages, NK-cells, and neutrophils), which is key critical for proper wound healing to occur. And 8. It would be necessary to prevent the newly formed tissue from overgrowing the existing tissues.

A proprietary formulation, designated CNSP (Combinatorial Nutraceutical-Supplement Pill, DFRD) was developed based on a combination of organic dark fruits and vegetables; reverse osmosis filtration to remove microcystins and passage over a Sepharose-EDTA column to deplete heavy metals from the mobilization agent; zinc gluconate; and folinic acid. The individual with <10% cardiac output (Fig. 3) and on the heart transplant list began ingesting CNSP, using the ramp-up procedure, e.g., 1 capsule of CNSP per 100 pounds body weight for the first week, 1 capsule of CNSP per 50 pounds body weight for the next 2-4 weeks, and 1 capsule of CNSP per 25 pounds body weight thereafter. Four months after he started ingesting CNSP, his cardiac output rose from <10% to 35% and his name was removed from the heart transplant registry. By 12 months on CNSP and his cardiac output rose an additional 15% (Fig. 3). Since being on CNSP following his massive myocardial infarction, his quality of life has improved significantly. Before hospital discharge, he could barely walk ten steps without passing out. Now, he is playing nine holes of golf daily, weather permitting.

There are distinct differences between naïve aTPSCs activated *ex vivo* versus naïve aTPSCs activated *in-situ*. The *ex vivo* activated aTPSCs procedures require 2-ml (cc's) of blood per pound body weight to be removed and destroyed to isolate the aTPSCs. The *ex vivo* activated aTPSCs treatment occurs as a bolus of stem cells that can be given once every two months if autologous cells are used; and more often if allogeneic donor cells, matched for gender and ABO-blood group, from multiple donors are used. It was noted that the longer the initial infusion occurred with the infused TSCs, e.g., 180-240-minutes versus 120-180-minutes, the better the results. The *in-situ* activated aTPSCs treatment requires no removal and no destruction of oxygen-carrying blood. The CNSP treatment releases *in-situ* activated autologous naïve aTPSCs that are delivered continuously, 60 minutes per hour, 24 hours per day (1,440 minutes), for as long as the CNSP is ingested.

An alternative to ESCs, iPSCs, CSCs, MSCs, VSELs, or skeletal muscle myoblasts to revascularize the heart, or even *ex vivo* activated aTPSCs to repair the heart is offered for healing cardiomyopathies. The alternative is the use of ingestible CNSP to activate naïve aTPSCs *in-situ* for healing cardiomyopathies. IRB-approved clinical studies are currently underway to determine if *in-situ* activation of naïve aTPSCs with CNSP will have a similar effect as *ex vivo* activated naïve aTPSCs at reversing signs and symptoms of diseases or disorders other than those of cardiovascular origin, e.g., neurodegenerative, pulmonary, autoimmune, systemic, renal, or orthopedic.

5. Conclusion

Cardiovascular disease is responsible for 30% of all deaths worldwide. Following a myocardial infarction, the myocardium is often replaced with non-functional scar tissue, resulting in heart failure. Heart failure is a common, disabling, and lethal condition. Since lack of blood flow is a major contributor to CVD, multiple strategies have been developed to restore blood flow to the damaged myocardium. These strategies include pharmacological treatments, reopening the blockage with balloon angioplasty followed with drug eluting stents, bypassing the blockage with CABG procedures, and/or use of stem cells, e.g., ESCs, iPSCs, CSCs, MSCs, VSELs, or skeletal myoblasts, to revascularize the heart. Since none of these strategies have been proven to be a permanent fix, we tested activated naïve aTPSCs to restore/regenerate ALL the damaged tissues of the heart, e.g., myocardium, cardiac skeleton, and vasculature, to increase functional cardiac output. *Ex vivo* activated aTPSCs worked for individuals with cardiac outputs near or above 25%, increasing functional cardiac output long term for 12+ years and counting. The increased cardiac output suggested the restoration of the histoarchitecture of the heart, i.e., restoration of functional cardiac myocytes, vasculature, and the cardiac skeleton. Individuals with cardiac outputs of 10% or less would probably not survive destruction of oxygen-carrying blood to isolate *ex vivo* activated aTPSCs. Therefore, an alternate strategy was needed. This strategy entailed activation of naïve aTPSCs *in-situ* for restoration of the histoarchitecture of the heart resulting in an increase in functional cardiac output. CNSP was developed to fulfill that role. Use of CNSP only for the *in-situ* activation of naïve aTPSCs worked for an individual with an initial cardiac output below 10%. During their ingestion of CNSP, their cardiac output increased from <10% to 35% (six months) and to ~50% for 3+ years and counting. These results suggested restoration of functional cardiac myocytes, vasculature, and cardiac skeleton occurred with a resultant increase in functional cardiac output. These results also suggested that *in-situ* activation of naïve aTPSCs with CNSP could be used with individuals too fragile to withstand the *ex vivo* activated naïve aTPSCs procedures. Clinical studies are underway to determine if continual release of *in-situ* activated autologous naïve aTPSCs by CNSP will have a similar effect as bolus treatments of *ex vivo* activated (autologous or allogeneic) naive aTPSCs at reversing signs and symptoms and thereby increase quality of life in individuals with diseases or disorders, other than those that are cardiovascular in origin.

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 clinical studies of CNSP *in-situ* activated naïve aTPSCs and *ex vivo* activated fresh isolate autologous and allogeneic naïve aTPSCs for treating chronic diseases, traumatic injuries, and orthopedic disorders. If you would like to learn more about the healing naïve aTPSCs and their ability to affect a positive repair and restorative response; apply for acceptance into our IRB-approved open access open enrollment clinical studies for either the *ex vivo* activated autologous fresh isolate aTPSCs, the *ex vivo* activated allogeneic fresh isolate aTPSCs, or the CNSP *in situ* activation of autologous 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 ethical approval

All research performed has been conducted under the oversight of Institutional Animal Care and Use Committees (IACUC) for animal studies and Institutional Review Board (IRB) for human clinical studies.

Statement of informed consent

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

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