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Cardiovascular Disease Treated with Telomerase-Positive Stem Cells

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ABSTRACT

Cardiovascular disease is responsible for 30% of all deaths worldwide. Myocardial infarction, due to blocked coronary artery(ies) or their tributaries, presents unique challenges to the field of regenerative medicine. Following a myocardial infarction (MI), the myocardium is often replaced with non-functional scar tissue, resulting in heart failure, which is a common, disabling, and lethal condition. The prognosis of patients with heart failure is poor, with a failure rate approaching 50%. The loss of cardiac tissue underlies heart failure. Since lack of blood flow is a major contributor, multiple strategies have been developed to restore blood flow to the damaged myocardium. These strategies include reopening the blockage, bypassing the blockage, biomaterials as a storage depot for factors controlling repair, and use of stem cells. Since none of these strategies have been proven to be a permanent fix, we tested telomerase-positive stem cells to restore the damaged tissues of the heart. Individuals with cardiac outputs at or below 25% were treated with these stem cells. After treatment, cardiac output, as a measure of function, increased from 20-45% in these individuals. These results suggest that telomerase-positive stem cells are a viable option for the treatment of individuals with cardiovascular disease.

Keywords

Stem Cell, Adult, Telomerase, Cardiovascular Disease, Myocardial Infarction, Regenerative Medicine.

Introduction

For decades, the broad class of cardiovascular diseases (CVD) has been the leading cause of mortality worldwide, responsible for 30% of all deaths (about 17 million annually). CVD is responsible for more than 7.5 million in-patient cardiovascular disease procedures in the USA. CVD places a significant economic burden on patients and health care systems. In 2010, the direct medical costs of CVD totaled US $272 billion (USD) in the US alone. The direct medical expenses caused by cardiovascular disease in the European Union may reach 106 billion pounds per annum. The aging population is an additional factor resulting in increases in cardiovascular disease. Contributing risk factors such as obesity, hyperlipididemia, hypercholesterolemia, and hypertension result in atherosclerosis, which then causes cardiovascular disease [1].

In the United States, coronary artery disease (CAD) results in ischemic heart disease that manifests itself as angina pectoris and myocardial infarction. Thus, CAD is a major cause of disability

and death in this country. Myocardial infarction due to one or more blocked coronary arteries or their tributaries present unique challenges to the field of regenerative medicine [1-4]. Following a myocardial infarction (MI), the myocardium is often replaced with non-functional scar tissue [5]. Such scarring and damage to the myocardium often results in left ventricular systolic dysfunction, ventricular aneurysm, decreased cardiac output, and heart failure [6-8]. Myocardial infarction and the consequent loss of fully functional myocardium is a major factor in the etiology of heart failure [9]. Heart failure is a common, disabling, and lethal condition [4,10]. The prognosis of patients with heart failure is poor, with a failure rate approaching 50%. The loss of cardiac tissue underlies heart failure, but current pharmacological treatments do not address this problem.

Once the myocardium dies after a myocardial infarction, it is replaced over several weeks by scar tissue. The size, location, composition, structure and mechanical properties of the healing scar are all critical determinants of the fate of patients who survive the initial infarction [5]. 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 likely to require cardioversion in order to prevent fatal events [8]. Such scarring and damage to the myocardium often results in left ventricular systolic dysfunction, ventricular aneurysm, and heart failure [6-8]. Thus, mortality and morbidity following myocardial infarction remain high despite current pharmacological treatments, including treatment with angiotensin converting enzyme inhibitors (ACE-I) and/or angiotensin-receptor blockers (ARBs) [7].

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. These regimens include physically re-opening blocked vessels, bypassing blocked vessels, placement of a bio-scaffold patch to act as a reservoir for bioactive factors to remodel the myocardium, and application of stem cells, e.g., embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), cardiac stem cells (CSCs), mesenchymal stem cells (MSCs), and others, to restore blood flow to the myocardium [11-19].

We propose the use of an alternate population of stem cells, rather than ESCs, iPSCs, or MSCs, as a treatment modality for myocardial ischemia. We have extensively characterized endogenous adult-derived telomerase-positive stem cells [20]. Collectively, their characteristics include the presence of the telomerase enzyme when the stem cells are in their native quiescent undifferentiated state; loss of the telomerase enzyme during their differentiation; in vitro differentiation into a minimum of 66 distinct cell types of all three embryonic germ layer lineages, e.g., ectoderm, mesoderm, and endoderm, spermatogonia and notochord [20,21]; induced formation of functional cells in vitro, e.g., neurotransmitter-secreting neurons [22], cardiomyocyte contraction regulated by propranolol and isoproterenol [23], and insulin secretion in response to a glucose challenge in pancreatic islet organoids [24]. The telomerase-positive stem cells demonstrated their ability to regenerate/repair the appropriate damaged tissues in induced animal models of Parkinson disease [25], cortical brain trauma [26], myocardial infarction [27], and lung fibrosis [28]. And in our human clinical studies we have shown increases in organ functioning in individuals treated with naïve telomerase-positive stem cells for Parkinson disease [29], idiopathic pulmonary fibrosis [30], chronic obstructive pulmonary disease [31], celiac disease [32], and systemic lupus erythematosus [33]. Based on those studies, we hypothesize that adult-derived telomerase-positive stem cells, e.g., totipotent stem cells (TSCs), pluripotent stem cells (PSCs), and mesodermal stem cells (MesoSCs), would increase heart function, expressed as an increase in cardiac output, in post-myocardial infarction participants.

Materials and Methods

Autologous and allogeneic adult-derived telomerase-positive stem cells (TSCs, PSCs, and MesoSCs) were tested in IRB-approved study protocols for autoimmune diseases and cardiovascular disease. Two males were assessed in this particular clinical study. The first was a 61-year-old male with systemic lupus erythematosus (SLE) of 31 years duration, demonstrating a cardiac output of less than 25% at time of initial treatment [33]. The second was a 63-year-old male previously having undergone five coronary arterial bypass graft surgeries and 15 stents. Following a massive acute myocardial infarction, his cardiac output was less than 10% at discharge and he was placed on the list to receive a heart transplant.

At age 30, the SLE individual was diagnosed as stage-I, genetically inherited along his maternal germ line. Progressing from stage-I SLE (age 30) to stage-IV SLE (age 61) he stated that he tried every prescribed AMA-approved treatment for SLE recommended by his rheumatologists, but the treatments either did nothing or accelerated the progression of his diseases [32,33]. Physician notes showed that he exhibited a significant drop in cardiac output, from 90% to 30%, coinciding with taking hydroxychloroquine to slow the progression of his SLE.

Since past pharmacological treatments did not perform as expected, an alternate experimental therapy was attempted, i.e., the use of autologous and allogeneic telomerase-positive stem cells. Recipients and donors were mandated to follow the informed consent guidelines for telomerase-positive stem cells for clinical therapy [30-33]. These guidelines consisted of a defined protocol to maximize the number of telomerase-positive stem cells for harvest and subsequent repair of the tissues, and included avoidance of alcohol, tobacco products, vaping, recreational drugs, lidocaine, and chemotherapeutic agents because they kill telomerase-positive stem cells; limit use of caffeine and corticosteroids because they alter the differentiative capabilities of telomerase-positive stem cells; ingestion of combinatorial nutraceuticals (CN) (DFRD, Macon, GA) daily for a minimum of 30 days prior to initial harvest and then throughout subsequent treatments of recipient (and donor harvests) to increase proliferation of telomerase-positive stem

cells within the person's own connective tissues, thus making the person their own bioreactor for stem cell proliferation; drink plenty of fluids two weeks before stem cell harvest; limit moderate to excessive exercising during a two-week window around stem cell harvest/treatment to maximize directed repair responses; and to ingest glacial caps (DFRD) 18 hours before stem cell harvest to mobilize stem cells into the blood stream. Donors were screened for gender, ABO-blood group, infectious diseases, genes for autoimmune diseases, and genes for any other deleterious genetic mutations. Donors were also given the option to have their activated mesodermal stem cells returned to them [33].

Harvesting of telomerase-positive stem cells occurred using venipuncture, withdrawing 210 to 420cc's of blood, based on body weight of the individual. The telomerase-positive stem cells were separated from the blood cells utilizing 'FDA-mandated minimal manipulative procedures', segregated into individual populations of TSCs, PSCs, and MesoSCs, and activated [30-33]. Allogeneic mesodermal stem cells from donors were not used due to their expression of self-recognition MHC Class-I molecules on their cell surface [20]. Since MHC Class-I molecules might induce a graft versus host disease (GvHD) response [34,35], it was felt that it was too great a risk for potential detriment to the recipient. Neither TSCs or PSCs display either MHC Class-I or HLA-DR molecules on their cell surface [20,36] and therefore were utilized in the treatment protocol from the allogeneic donors.

Just before his first treatment with autologous telomerase-positive stem cells the individual was diagnosed as two-week terminal stage-IV SLE with his organs functioning at or less than 25%, including two non-functional organ systems. He was on 384 mg of hydromorphone every 24 hours for neuropathic pain, brain fog, pain sensitivity to touch/pressure, L1 to S4 spinal rootlets were fibrosed to his spine, bilateral sciatica, polyarthralgia, generalized muscle aches and pains, skeletal muscle cramping and twitching, almost continuous cluster headaches alternating with occasional migraines, photosensitivity, insomnia, narcolepsy, severe fatigue, cardiac arrythmias, pericarditis, painful breathing, bilateral pleuritis, difficulty breathing, anemia, recurrent low grade fever, hepatitis, jaundice, nephritis, pancreatitis, mouth ulcers, nose ulcers, night sweats, fever, chills, dry eyes, dry 'alligator' skin, abdominal pain, gastritis, nausea, vomiting, alternating diarrhea and constipation, enlarged lymph nodes, increase in numbers and severity of allergies, urticaria, mastocytosis, "red leopard spots" (IgG reactivity to IgM's), adult respiratory distress, idiopathic pulmonary fibrosis, cold insensitivity, vasculitis, complete absence of hair below his clavicles, sparse thinning white hair on his head, anti-nuclear antibodies (ANA), etc., and in his own words "basically a living hell" [33].

During the nine years since his first (autologous) stem cell treatment, the SLE participant has undergone 28 telomerase-positive stem cell treatments, 18 additional autologous and nine allogeneic, thus far. Allogeneic TSCs and PSCs were obtained once from a 42-year-old A-positive male, twice from a 53-year-old O-negative male, twice from a 50-year-old A-positive male, and four times, age at time of donation of 73, 75, 77, and 80-year-old O-negative male. Due to the many and varied symptoms expressed by the SLE recipient, multiple treatment regimens were performed. Pooled autologous and/or allogeneic TSCs only for intranasal infusion for neurogenic issues; pooled autologous and/or allogeneic TSCs only diluted in 250-ml of 0.9% sterile saline for slow intravenous infusion using the Thebesian venous system for cardiovascular issues; pooled allogeneic and/or autologous TSCs and PSCs in 2-3-ml of 0.9% sterile saline for nebulization for breathing issues; and pooled allogeneic and/or autologous TSCs and PSCs and autologous MesoSCs diluted in 0.9% sterile saline for regular intravenous infusion for other systemic organ and associated autoimmune issues [30-33].

Because we felt that the 63-year-old post-MI patient was too fragile to withstand blood draws to isolate telomerase-positive stem cells for his treatment, we attempted a different strategy. We mixed the combinatorial nutraceuticals with ¼ dosage of the glacial caps (CN-SP, DFRD, Macon, GA) to simulate both the proliferation of his endogenous telomerase-positive stem cells and mobilization of the stem cells into his vasculature 24 hours a day, seven days a week. He was instructed to ingest CN-SP every day.

Results

Sixty-one-year-old two-week terminal stage-IV SLE male had a cardiac output less than 25%. First transplant with autologous TSCs raised his cardiac output to 25%. Second transplant with allogeneic TSCs from a 42-year-old A+ male raised his cardiac output to approximately 40%. Third stem cell transplant with allogeneic TSCs from a 73-yrear-old O-negative male raised cardiac output to approximately 70%. Remaining 18 autologous and 7 allogeneic telomerase-positive stem cell transplants have maintained his cardiac output at approximately 70% through 9+ years and counting (Figure 1).

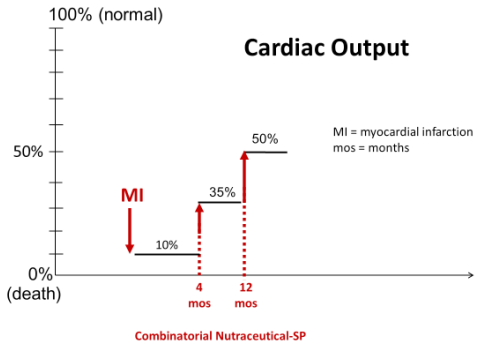

Figure 1: SLE patient's cardiac output dropped precipitously, 90% to 30%, during time period of ingestion of hydroxychloroquine to slow progression of SLE. At time of first stem cell transplant, cardiac output was below 25%. First stem cell transplant (autologous) raised cardiac output to 25%. Second stem cell transplant from allogeneic 42-year-old A+ male raised cardiac output to approximately 40%. Third stem cell transplant from allogeneic 73-yrear-old O-negative male raise cardiac output to approximately 70%. A total of 28 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.

Sixty-three-year-old male following massive myocardial infarction, at hospital discharge his cardiac output was 10% and he was put on waiting list for a heart transplant. After four months ingesting CN-SP, his cardiac output rose to 35% and his name was removed from heart transplant list. Eight additional months on CN-SP and his cardiac output rose an additional 15% (Figure 2).

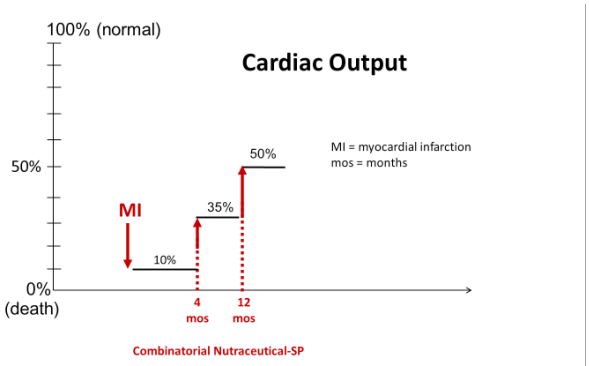

Figure 2: Sixty-three-year-old male with five previous coronary arterial bypass graft surgeries and 15 stents had a massive acute myocardial infarction leaving him with a 10% cardiac output at discharge from hospital and placed on heart transplant list (March 2019). By four months on CN-SP, his cardiac output had risen to 35% and his name was removed from heart transplant list. Eight additional months on CN-SP and his cardiac output rose an additional 15% (March 2020) (Figure 2). He is continuing to ingest CN-SP on a daily basis.

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 [1-4]. Following a myocardial infarction (MI), the myocardium is often replaced with non-functional scar tissue [5]. Myocardial infarction and the subsequent loss of fully functional myocardium is a major factor in the etiology of heart failure [9]. The prognosis of patients with heart failure is poor, with a failure rate approaching 50%.

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 [5]. 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 [8]. Such scarring and damage to the myocardium often results in left ventricular systolic dysfunction, ventricular aneurysm, and heart failure [6-8]. Thus, 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) [7]. The loss of cardiac tissue underlies heart failure, but current current pharmacological treatments do not address this problem.

One of the major contributors to mortality in individuals with cardiovascular disease is lack of blood flow to the ischemic myocardium. Alternative therapeutic regimens have been developed to restore of blood flow to the ischemic heart muscle. These therapeutics include physically re-opening blocked vessels, bypassing blocked vessels, a bio-scaffold patch as a reservoir for agents to direct remodeling of the myocardium, and application of stem cells, e.g., ESCs, iPSCs, CSCs, MSCs, etc., to restore blood flow to the myocardium [11-19].

Opening the blocked vessels with balloon angioplasty intuitively may initially appear to be an attractive strategy. But even with the subsequent use drug eluting stents, the opened vessels had a tendency to form fibrous connective tissue in about 3-6 months within the area of the stent that caused re-blockage of these vessels.

Inflammatory changes of the coronary arteries cause fibrosis and blockage of these vessels. Coronary artery bypass graft (CABG) has been shown to be an effective surgical therapy. 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 thee 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 be used [12,36]. Current therapeutic approaches still present risks to the patient. If one could regenerate the patient's arteries, one could avoid these risks. Indeed, stem cells are the current "holy grail" of regenerative medicine [37]. The use of stem cells to regenerate the patient's arteries could offer hope to patients suffering from cardiovascular disease, even those who have experienced a myocardial infarction. The source of stem cells for such purposes can present problems. 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 [37-39]. The use of allogeneic stem cells would require immunosuppressive therapy to avoid tissue rejection. However, immunosuppressive therapy can cause increased morbidity and mortality due to a GvHD response [5,34,35]. Moreover, the use of partially differentiated stem cells presents difficulties. Some reports have appeared concerning the use of partially differentiated mesenchymal stem cells to effect cardiac vascular regeneration [10,40,41]. Apparently, the more primitive stem cells, such as the very small embryonic-like (VSEL) stem cells may be more effective than the more differentiated mesenchymal stem cells [42]. Studies with more differentiated stem cells have produced very limited and inconsistent improvements in cardiac structure and function [17-19]. Further study is needed to document which 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.

The results from using adult-derived telomerase-positive stem cells in animal models in the treatment of neurodegenerative diseases, heart disease, and pulmonary diseases, suggest that these stem

cells might be excellent candidates for the repair of heart muscle by intravenous delivery [25-28].

Since adult-derived telomerase-positive stem cells can form the functional components of heart tissue and cardiac blood vessels [20,21,23,27], they might also form the basis for therapeutic approaches for repair/regeneration of functional heart tissue. Previous studies noted that both telomerase-positive totipotent stem cells and telomerase-positive pluripotent stem cells were resident populations in the connective tissue stroma of skeletal muscle [43-45], blood [45-47], lung [28], adipose tissue [48], dermis [48], kidney [49], and bone marrow [50], of multiple species of adult mammals, including humans [20]. Therefore, we hypothesized that similar telomerase-positive stem cells may be present in adult rat and porcine hearts. Utilizing immunocytochemical staining of sectioned tissues with cell surface markers for these stem cells, e.g., CEA-CAM-1 for TSCs and SSEA-4 for PSCs addressed this hypothesis. The results demonstrated that cells positive for stage-specific antigen-4 (SSEA-4) (PSCs) and cells positive for carcinoembryonic antigen-cell adhesion molecule-1 (CEA-CAM-1) (TSCs) were identified within the intramural myocardium of the adult porcine heart at some distance from the epicardium of the heart, thus both totipotent and pluripotent stem cells were located in adult rat hearts and adult porcine hearts [51,52].

Next, we used a pre-clinical animal model of induced myocardial infarction to determine if adult telomerase-positive stem cells, delivered to ischemic heart muscle, would participate in tissue repair. We tested two hypotheses 1) would telomerase-positive stem cells repair ischemic heart muscle, and 2) could cardiac repair occur using systemic delivery of the stem cells. We used two different methods to cause myocardial ischemia in a pre-clinical animal model. The first technique was to freeze the apex of the heart with liquid nitrogen and then directly inject genomically-labeled PSCs (Scl-40β) into the frozen heart muscle to determine if repair was even possible. The second technique utilized transient ligation of the left anterior descending (LAD) coronary artery followed by systemic delivery of the genomically-labeled telomerase-positive stem cells by tail vein injection [23,27], to simulate intravenous infusion.

As shown, [23,27] an undifferentiated genomically-labeled telomerase-positive pluripotent stem clone incorporated into damaged myocardial tissues of the heart and assisted in the repair of those tissues by differentiating into vasculature, cardiac myocardium, and the connective tissue skeleton [23,27]. Our second model utilized rat hearts that had had their left anterior descending (LAD, "widow maker") transiently ligated followed by injection of the genomically-labeled telomerase-positive pluripotent stem cell clone into the tail vein of the rat to mimic systemic delivery of the stem cells to the heart. The results show incorporation of the genomically-labeled cells into the myocardium, vasculature, and connective tissue cardiac skeleton [23,27].

The pre-clinical animal models were followed by studies in an IRB-approved clinical trial protocol utilizing telomerase-positive stem cells as treatment modalities for chronic diseases. Our first person was actually treated for Parkinson disease. After the stabilization of his Parkinsonian symptoms, the next telomerase-positive stem cell treatment that did not reverse any of his Parkinson symptoms. And we could not understand why this had occurred. Six months after that treatment we learned for the first time that the individual had had a myocardial infarction six years previously that left him with a six-year sustained cardiac output of 25%. During his semi-annual visit with his cardiologist, where they routinely measured his cardiac output, his cardiac output had risen to 35%. We gave him another intranasal treatment for his Parkinson disease, and again no reversal in Parkinson symptoms. But six months later his cardiac output had risen to 45%. It seemed that the body was "stealing" stem cells from the site of placement (intra-nasal infusion for Parkinson disease) and sending them to his heart instead to fix a diseased heart. His long-standing heart problem apparently was more life threatening to the patient than his Parkinson disease [52]. These results coupled with the migratory ability to repair damaged cortical tissue that we saw in the pre-clinical animal model of Parkinson disease [26] led us to theorize that the body could circumvent directed treatments with telomerase-positive stem cells once given access to activated stem cells.

Rather than placing a bolus of the stem cells at the apex of the coronary vessels as had been done for ESCs, iPSCs, CSCs, and MSCs, we took an alternative approach to revascularizing the ischemic heart muscle. We used the unique size of the TSCs (0.1-2.0 μm) along with the fact that the heart actually has two vascular systems, not just the coronary arterial/venous system. The heart also contains the vena communicantes minimae (small communicating veins), also known as the Thebesian veins. The Thebesian veins are small vascular channels without valves of less than 5 μm in diameter. Their size is too small for blood cells, either RBCs (7 μm) or WBCs (10+ μm), or mesenchymal stem cells (10-20+μm) or MesoSCs (10-12 μm) or even PSCs (6-8 μm) to traverse [20]. The Thebesian veins are found in all four chambers of the heart and run from inside the chambers, through the myocardium, to the pericardium lining the outside of the heart. When the heart undergoes systole (contraction) fluid is pushed from the inside chambers through the Thebesian veins in the myocardium to the outside layer of the heart. During diastole (relaxation) fluid returns to the inside chambers through Thebesian veins through the myocardium from the outside layer of the heart. We hypothesized that by giving the heart TSCs (0.1-2 μm), these very small stem cells could repair the damaged myocardium and revascularize the heart as they traversed back and forth through the Thebesian system of vessels.

We utilized this rationale for treating a 61-yer-old individual that was two-week terminal stage-IV SLE with a cardiac output of less than 25%. His first telomerase-positive stem cell treatment consisted of autologous TSCs given by intravenous infusion, followed by PSCs and MesoSCs, also given by intravenous infusion. By two weeks his cardiac output had risen to 25%. His second transplant utilized allogeneic TSCs and PSCs from a 42-year-old A+ male. One month later his cardiac output had risen

to 40%. His third transplant utilized allogeneic TSCs and PSCs from a 73-year-old O-negative male. One month later his cardiac output had risen to 70%. He has had an additional 18 autologous and seven allogeneic transplants that have maintained his cardiac output at approximately 70% for nine years and counting (Fig. 1).

We treated two other cardiac patients with heart problems that had resulted from decreased cardiac outputs, utilizing the same protocol, i.e., TSCs only by slow IV infusion followed by pooled PSCs and MesoSCs by regular IV infusion. We noted modest increases in their cardiac output, although not as dramatic as with the SLE individual. In these other instances, only autologous TSCs, PSCs, and MesoSCs were utilized for treatment.

We have another individual we are currently working with (Figure 2) that had five CABG surgeries and 15 stents before suffering a massive myocardial infarction in March of 2019. The acute MI left him with barely 10% cardiac output and his name was placed on the waiting list to receive a heart transplant by his cardiovascular surgeon. Our normal stem cell transplant procedure entails withdrawing 400-ml of blood to isolate and activate the telomerase-positive stem cells before re-infusion. Because of his fragile health status and the fact that he probably could not withstand our standard isolation procedure, we tried a different approach. We modified our combinatorial nutraceutical mixture (CN) that induced proliferation in situ of telomerase-positive TSCs, PSCs, and MesoSCs, and added ¼ dose of our glacial caps, which we use to mobilize the telomerase-positive stem cells into the blood stream prior to harvest. We hypothesized that the mixture, termed CN-SP, should proliferative and mobilize his TSCs, PSCs, and MesoSCs into his blood stream 24 hours a day, seven days a week to give his damaged heart a continuous supply of autologous telomerase-positive stem cells for repair. By four months after discharge, with an initial cardiac output of barely 10%, on just the CN-SP ingestion alone he gained 25% cardiac output and was removed from the heart transplant list. As of March 2020, he gained an additional 15% cardiac output and his quality of life has significantly improved. The results from the first patient [52], the SLE individual, two other cardiac patients, and the CN-SP individual (n=5), suggest that following a myocardial infarction patients can benefit from telomerase-positive stem cells, either by intravenous infusion or by CN-SP to repair their damaged tissues and increase their cardiac output.

To increase our sample size and verify the capabilities of the telomerase-positive stem cells, we propose two Phase-II randomized double-blinded placebo-controlled studies.

First, we propose using an expanded population of post-MI patients who are on the list to receive a heart transplant and randomly have ½ population ingest CN-SP and ½ population ingest placebo. Then measure cardiac output in both sets of the patients every two months for one year. If a HLA-matched heart becomes available for an enrollee, as long as they are still on the transplant list, they will receive the donor heart.

Second, we propose using an expanded population of post-MI patients who have a cardiac output of less than 50%, comparing autologous telomerase-positive TSCs, PSCs, and MesoSCs to telomerase-negative autologous MSCs to determine which population, TSCs/PSCs/MesoSCs versus MSCs, is better suited to repair damaged heart tissue and increase cardiac output. Cardiac output will be measured in both sets of patients every two months for one year and outcomes compared.

Conclusion

Cardiovascular disease is responsible for 30% of all deaths worldwide. Following a myocardial infarction (MI), the myocardium is often replaced with non-functional scar tissue, resulting in heart failure, which is a common, disabling, and lethal condition. Since lack of blood flow is a major contributor, multiple strategies have been developed to restore blood flow to the damaged myocardium. These strategies include reopening the blockage, bypassing the blockage, biomaterials as a storage depot for factors controlling repair, and use of stem cells. Since none of these strategies have been proven to be a permanent fix, we tested telomerase-positive stem cells to restore the damaged tissues of the heart. Individuals with cardiac outputs at or below 25% were treated with Telomerase-positive stem cells. After treatment, cardiac output, as a measure of function, increased from 20-45% in these individuals. These results suggest that telomerase-positive stem cells are a viable option for the treatment of individuals with cardiovascular disease.

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