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Advances in Cardiovascular Regenerative Medicine

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Abstract

Cardiovascular disease (CVD) continues to be the leading cause of global morbidity and mortality, despite major therapeutic advances over the past decades. A better understanding of the molecular and cellular mechanisms of CVD, as well as improved therapeutic strategies for its management and treatment, are urgently needed. Cardiovascular regenerative medicine is a rapidly evolving field providing novel approaches including cell-based, cell-free, tissue engineering, human-induced pluripotent stem cells (iPSCs), and hybrid therapies, which are changing the standard of care for millions of people. These treatments include regenerative medicine therapeutic strategies for the management of cardiovascular complications associated with severe COVID-19 infection and Post-Covid Syndrome (AKA Long-Hauler's Syndrome). The field of cardiac regenerative medicine must fill the void that exists between the bench and the clinic if physicians are to be properly informed and receive the necessary tools to treat the growing threat of CVD in the US and around the world. This review outlines how regenerative medicine is advancing the study and practice of cardiology and which procedures are currently in place to guide physicians in the use of this technology for the regeneration of lost or damaged cardiac tissue.

Keywords: Regenerative medicine; extracellular vesicles; exosomes; cardiovascular disease; cardiology; Covid-19; long-haulers syndrome

Demand for Regenerative Medicine Therapies in Cardiology

Within all societies across the globe, cardiovascular diseases (CVDs) are the leading cause of morbidity and mortality. An estimated 17.9 million people died from CVDs in 2019, representing 32% of all global deaths [1]. Of these deaths, 85% were due to heart attack and stroke [1]. According to data collected by the National Health and Nutrition Examination Survey (NHANES), the prevalence of CVD, comprising Congenital Heart Defects (CHD), Heart Failure (HF), stroke, and hypertension, equals 49.2% or 126.9 million adults worldwide [2]. In adults, 20 years of age and older, the incidence of disease is positively correlated with an increase in age for both males and females [2]. Currently, many cardiac treatments are available, including drug-based revascularization [3,4], medical device implantation [5], engineered heart tissue transplantation [6], and surgery (e.g., heart transplantation) [7]. However, for conditions that are difficult to treat effectively, such as stroke resulting from ischemic heart disease and congestive HF, more pragmatic treatments are still of urgent need.

Over the past two decades, the emergence of many regenerative technologies and cell-based therapies has offered potential solutions to these troubling medical conditions. Regenerative medicine is an innovative, interdisciplinary method for rebuilding, replacing, or repairing damaged tissues throughout the body [8-9]. Currently, one of the most attractive features of regenerative medicine is the potential for drug-free and surgery-free options for the treatment of patients with chronic pain and severe injuries [10]. The focus of regenerative medicine has heavily favored the delivery of differentiated stem and progenitor cells [11], using these matured cells to stimulate the replacement of lost and damaged cells [12]. In cardiology, these therapies have focused on stimulating endogenous tissue-resident epicardial cells to promote improved cardiac function and healing of damaged cardiac tissue [12,13]. Cell-based therapies are currently the most studied clinical regenerative medicine therapy and are changing how we approach human genetics, drug discovery and clinical efforts to harness regenerative cell biology for heart diseases [14]. Unfortunately, regenerative medicine within the field of CVD has been riddled with controversies following the discovery of nefarious activities of one of the pioneers of the field and the subsequent retraction of several high-profile publications [15].

Despite these challenges, interest in regenerative strategies for cardiac regeneration continues to gain momentum and cardiac regenerative therapies are now closer to reality than ever before. This is quite an achievement considering that it was originally believed that cardiac tissue was unable to regenerate in the adult human. While there is a clear deficiency in adequate regenerative potential, our group and others have shown that the potential to repair does persist in mammalian hearts past the neonatal stage [16,17]. In a recent publication from our lab we describe a method to prolong epicardial-mediated regeneration capacity of the neonatal mouse [16]. By supplying explanted hearts with exogenous cardiac developmental growth factors, we were able to promote cardiac repair after inducing injury. Repair was realized via epicardial cell activation, proliferation, and migration to the injury site, resulting in recruitment of cardiomyocytes and subsequent heart tissue regeneration [16].

In the example above we stimulated ex-vivo cardiac repair thought the activation of several endogenous cell populations. However, regenerative biologists continue to design innovative approaches to directly reprogram non-cardiomyocytes, such as cardiac fibroblasts, into functional cardiac myocytes using induced pluripotent stem cell (iPSC) technology [9,18,19]. Genetic modification, including fluorescent iPSC-derived cardiomyocyte reporter systems, allows specific cell lineages to be labelled, facilitating cell isolation for

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downstream applications including drug testing, disease modeling and cardiac regeneration [20]. As this manuscript is finalized for publication a new and exciting technology was described in the journal *Science*, where the authors were able to use iPSC technology to dedifferentiate postmitotic adult cardiomyocytes into dividing fetal-like cardiomyocytes and drive cardiac regeneration, in vivo [21].

As growth continues in research and innovation, the field of regenerative medicine has been included in the academic curriculum at some major medical institutions, including the Wake Forest Institute for Regenerative Medicine, University of Pittsburgh McGowan Institute of Regenerative Medicine, Harvard Stem Cell Institute's Medical Scientist Training Fellowship, Duke Hematology-Oncology Fellowship program, Institute of Stem Cell Biology, Regenerative Medicine at Stanford School of Medicine [22] and the Ansary Stem Cell Institute, Division of Regenerative Medicine at Weill Cornell University. Despite these efforts, there remains limitations, including the lack of available economic support and conflicting interests, which severely hinder the growth of knowledge and the advancement of medical innovation [23]. Although work in cardiac regenerative medicine has positively impacted the lives of many patients, we have only begun to scratch the surface of what the field of regenerative medicine has to offer patients. Below, we will discuss how regenerative medicine is advancing the study and practice of cardiology.

Regenerative Medicines Used in the Treatment of Cardiovascular Disease

Cell transplantation-induced repair

Cell-based therapy has shown potential benefit in a variety of human diseases. In cardiac regenerative medicine, one goal of cell transplantation, yet to be adequately realized, is for transplanted cells to engraft, differentiate and replace nonfunctional cells with cardiomyocytes and vasculature. A parallel goal is to utilize paracrine signaling by the transplanted cells to induce regeneration of the endogenous myocardium [24]. The types of cells originally used in these early transplantation experiments were skeletal muscle progenitor cells or myoblasts and bone marrow-derived cells. More recently cell types including cardiomyocytes, stem cells (i.e., embryonic and iPSCs), umbilical cord blood cells [25], tissue-derived fibroblasts, smooth muscle cells and adipose tissue-derived cells are being used [24-26]. However, a lack of engraftment of transplanted cell populations within the infarcted myocardium is a major limitation observed across all cell types attempted. One explanation for such poor cellular retention is attributed to the harsh inflammatory response mounted following myocardial infarction (MI). Another contributing factor is the heart's natural limited ability to mount a reparative response following injury and very limited ability for cellular turnover [17]. As such, the indirect effects of cell transplantation such as paracrine signaling, immunomodulatory interactions and improvement of the microenvironment have far exceeded the direct effects upon myocardium repair, and it is currently the consensus within the field that these indirect effects are what is driving the reported improvements [15-27]. As our understanding of the molecular biology of tissue regeneration has evolved, novel therapies and therapeutic targets have emerged, including secretory factor-based therapies such as growth factors, cytokines and extracellular vesicles (including exosomes) and microRNAs (miRNAs). Experimental studies to support the significance of paracrine signaling demonstrate that the administration of conditioned media from cardiac cells (likely exosomes) was able to confer cardio-reparative effects without requiring the physical transplantation of the cells within the infarcted heart [28]. There is also growing focus on cellular reprogramming, genetic and epigenetic modifications, and inflammatory response modulation, which may have a role in myocardial repair and regeneration [12].

Paracrine-mediated repair

The secretome is the repertoire of factors secreted by transplanted cells which, through paracrine-mediated signaling, can impact the activities of other cells within the local microenvironment as well as systemically. Up to 80% of the therapeutic effect of adult Mesenchymal Stromal/Signaling Cells (MSCs) may occur through such paracrine-mediated actions [29-31]. The secretome of MSCs has also been shown to enrich the microenvironment of the infarcted myocardium. It can accomplish this by improving cell survival, enhancing angiogenesis and reducing inflammation and adverse remodeling, resulting in recruitment and activation of endogenous resident cells for cardiac repair [16-28]. The secretome from heart-derived cells contains a mixture of many cytokines implicated in angiogenesis (angiopoetin-1, angiogenin, and vascular endothelial growth factor [VEGF]), myogenesis (hepatocyte growth factor), and stem cell recruitment (stromal cell-derived factor 1α [SDF1α] and stem cell factor [SCF]) [32]. Furthermore, in addition to classical cytokines, Tseliou *et al.* demonstrated that the surface marker *endoglin* (CD105), which is universally expressed on all heart-derived cells, is liberated *in vivo* and inhibits transforming growth factor beta (TGFß) induced fibrosis by cardiac fibroblasts [33]. These insights have opened opportunities to explore whether manipulating the cytokine profile of heart-derived cells would improve cell-treatment outcomes. Mechanistically, Tilokee *et al.* found that increasing SDF1α recruited circulating bone marrow cells, reduced apoptosis and induced the generation of new cardiomyocytes [34]. Additionally, SDF1α secreted by allogeneic MSCs was able to improve endothelial function in dilated cardiomyopathy patients [35].

Besides being released as soluble proteins, these paracrine factors can also be carried as cargo within extracellular vesicles (EVs) such as microvesicles or exosomes, which are of particular interest to the field [36]. These nano-sized EVs (50-150 nm in diameter), carry a range of bioactive factors such as cytokines, growth factors, sugars, lipids, mRNAs and microRNAs. They can be isolated from cultures of MSCs or from umbilical cord, cord blood and bone marrow and have shown to convey protective effects in the hearts of both ischemia and reperfusion models [37], raising the possibility that exosomes might be exploited as alternative, non-cellular therapeutic approaches for cardiac repair and regeneration. Studies have shown that intramyocardial injection of MSC-derived exosomes from distinct tissue sources efficiently reduced the infarct size and enhance cardiac function, preserving cardiac systolic and diastolic performance, in ischemic models [38-39]. In addition to decreasing initial infarct size, exosomes are able to stimulate angiogenesis, reduce fibrosis and remodeling, alter immune cell function and improve long-term cardiac contractile function [40]. Moreover, exosomes can transfer these multiple factors to other cells by fusing with the plasma membrane and expelling their cargo therein [41]. These exosomes are also thought to be capable of transferring mitochondrial and non-mitochondrial cargos, contributing to improved intracellular energetics [42]. A recent study showed that mitochondria-rich extracellular vesicles (M-EVs), collected from induced pluripotent stem cell-derived cardiomyocyte (iCM)-conditioned medium after intramyocardial injection, can restore intracellular bioenergetics and contractile properties of cardiomyocytes [42].

Roles of exosome-derived miRNAs in cardiac regeneration

Many studies have focused on the microRNA content of exosomes to explain their function in regenerative therapies. One such study

by Khan *et al.* shows how mouse embryonic stem cell (ESC)-derived exosomes can promote endogenous repair and preserve cardiac function when injected intramyocardially after left anterior descending (LAD) ligation in a murine model of myocardial infarction. The authors conclude that the beneficial effects observed with ESC-derived exosomes are mediated, at least in part, by the transfer of miR-294 [43]. miR-294 is known for modulating cellular reprogramming, proliferation and cell survival. Wang *et al.* described cardioprotection, including cell survival and angiogenesis, by human endometrium-derived MSCs (EnMSC). This study identified miRNAs contained in EnMSC-derived exosomes, in particular miR-21, as potential mediators of EnMSC therapy [44]. In addition, cardioprotective actions attributed to miR-210 released by exosomes have also been reported in infarcted mouse models [45].

In a study of myocardial ischemia/reperfusion, intramyocardially injected Wharton's Jelly-derived MSC EVs (WJ-MSC-EV) led to a remarkable reduction in infarct size and considerably alleviated undesirable inflammatory traits in both the heart and serum of EV-treated animals [46]. Furthermore, MSC-EVs engineered to overexpress miRNA-181a dramatically influenced inflammatory responses after myocardial ischemia-reperfusion injury, potentially by downregulating pro-inflammatory cytokines TNF-α and IL-6 and significantly upregulating expression of the anti-inflammatory cytokine IL-10 [47]. MSCs have been shown to possess broad immunomodulation capabilities and can influence both adaptive and innate immune responses [48]. These benefits are considered multifactorial, since a joint action of antiapoptotic, pro-survival and anti-inflammatory effects occurs [49-50].

Anti-inflammatory effects of MSCs in cardiac tissue

In the cardiovascular system, MSCs can protect the myocardium by reducing the level of inflammation, promoting the differentiation of myocardial cells around infarct areas and inducing angiogenesis [48-51]. Chiossone *et al.* demonstrated that MSCs are capable of alternatively activating macrophages resulting in macrophage suppression of both innate and adaptive immune responses [52]. They achieve this by enhancing the polarization of M2, anti-inflammatory, macrophages through a prostaglandin E2-dependent mechanism which inhibits the proliferation of $CD8^{(+)}$ T cells and induces the expansion of regulatory T cells (Tregs) [52]. Another example of alternatively activated macrophages is shown when high expression levels of CD206 (a marker of alternatively activated macrophages) results from the interactions between MSCs and macrophages [53]. This high expression of CD206, and the anti-inflammatory cytokine IL-10 in macrophages, inhibits the inflammatory response in vitro. Furthermore, co-culturing macrophages with MSCs resulted in low levels of pro-inflammatory cytokines IL12 and TNF-alpha and high macrophage expression of the anti-inflammatory cytokine IL6, promoting tissue repair [53]. In addition to regulating inflammation by altering cytokine levels, MSC are also capable of mediating inflammation via soluble factors like Nitric oxide (NO). Not only is NO known to induced T-cell suppression by inhibiting T-cell proliferation, but it has also been shown that MSC production of NO is one of the major mediators of T-cell suppression [54-56]. Furthermore, it has been demonstrated that MSC with eNOS/NOS3 over-expression enhance cardiac repair when injected into the myocardium of rats with MI [57], and that iNOS activity is required for the anti-fibrotic therapeutic properties of MSCs [58].

iPSC therapy for cardiac regeneration

Pluripotent stem cells have become a major focus of cardiovascular regeneration therapies, particularly after the discovery of iPSCs. Unlike embryonic stem cells (ESCs), iPSCs are not encumbered by ethical dilemmas and concerns. These cells are adult somatic cells that are reprogrammed to a pluripotent state and can subsequently be differentiated into functional cardiomyocytes and endothelial, as well as, vascular smooth muscles [59]. Cardiac repair was induced in a rat MI model using cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs) and human MSC-loaded patches (hMSC-PA) [60]. Epicardial implant of hMSC-PA provides a complimentary microenvironment, which enhances vascular regeneration through prolonged secretion of paracrine factors, but more importantly it significantly improves the retention of intramyocardially injected hiPSC-CMs, restoring cardiac function. Notably, the majority of injected hiPSC-CMs display adult CM-like morphology suggesting that the secretomic milieu of hMSC-PA constitutes pleiotropic effects *in vivo*. A dual approach may be a promising means to enhance cardiac repair for post-MI hearts [60]. Zha *et al.* (2020) utilized the CRISPR/Cas9 system to target one MHC class I molecule, β2 microglobulin (B2M), to generate "less immunogenic" iPSC-derived MSC (iPSC-MSC) lines for allogenic transplantation [61]. B2M-knockout (KO) iPSC-MSCs could more efficiently escape immune response-mediated killing by peripheral blood-derived monocytes (PBMCs) compared to control cells. The loss of B2M did not alter the innate immunosuppressive feature of MSCs.

Progression of regenerative medicine guidelines for health practitioners in cardiology

Regenerative medicines, while offering the promise to treat of a variety of diseases, continue to experience push-back from regulatory entities. For many years various cellular therapies endured similar hurtles, however in the last decade we have witnessed impressive results following administration of chimeric antigen receptor (CAR) T cell therapy [62]. The Food and Drug Administration (FDA) has mandated that these cells must be manufactured under an Investigational New Drug (IND) approval, and that the cells be manufactured according to current Good Manufacturing Practices (cGMP). Several sections of Title 21 of the Code of Federal Regulations (CFR) contain regulatory guidelines established by the FDA regarding cGMP of these therapies. Manufacturing of CAR T cells is specifically covered within Parts 210 and 211, with some additional information available in Part 600 of title 21 CFR [62].

Market approval requirements, such as compliance with cGMP and the performance of pivotal pre-clinical and clinical trials with robust study designs and sufficient patient populations for efficacy and safety, is costly and takes time. Therefore, as a result many companies have failed to consider these issues when marketing regenerative medicines. Despite the slow nature of market approval, the use of unauthorized regenerative medicine from private clinics has continuously increased in recent years raising concerns by the FDA over safety. In the United States, the FDA is the presiding regulatory body, and regulates these therapies under a comprehensive policy framework announced in November of 2017, which was subsequently updated in 2019 regarding the use of Cellular, Tissue, and Gene Therapies. This policy allowed the use of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/P's) by physicians to gather pre-clinical and clinical data regarding safety and efficacy.

In addition to the standard pathway for approval, the FDA also has expedited pathways for market approval of regenerative medicines. For example, in 2019, the FDA approved the drug Zolgensma (onasemnogene abeparvovec-xioi) under an accelerated drug designation. This drug was the first gene therapy approved to

treat children less than two years of age with spinal muscular atrophy. Although some skeptics question the safety of therapies approved using accelerated programs, the FDA remains committed to establishing the safety and efficacy of all products. Despite many labs currently exploring biologics for cardiac injury, no therapies are approved by the FDA for regenerating cardiac tissue at this time. Nevertheless, there are currently over 1,200 ongoing clinical trials for regenerative medicine worldwide (statista.com). As of 2021 there are 383 reported phase 1, 685 phase 2 and 152 phase 3 trials currently being conducted. Greater than 50% of these trials are in the fields of Orthopedics and Dentistry, followed by Immunology and Oncology, but at least 8% are predicted to be focused on cardiology by the year 2025.

COVID-19 and Long-COVID (AKA Long Hauler Syndrome)

Alleviation of "Cytokine Storm"

The recent (ongoing) COVID-19 pandemic has infected over 207 million people and has led to >4.5 million deaths worldwide as of years end 2021. A hallmark of more severe cases in patients with SARS-CoV-2, (the virus that causes COVID-19) is acute respiratory distress syndrome (ARDS), an inflammatory condition produced primarily by an overactive immune response. The so termed "cytokine storm," features elevated levels of pro-inflammatory cytokines such as IL-2, IL-6, IL-7, IL-22, CXCL10, and TNFα [63]. Whilst the lungs are the primary site of infection for SARS-CoV-2, in more severe cases, the effects can be detected in multiple organ systems [64]. Indeed, many COVID-19 positive patients develop cardiovascular complications, such as myocardial injury, myocarditis, cardiac arrhythmia, and thromboembolism, which are associated with higher mortality [65]. In early case reports from Wuhan, China, patients with adverse outcomes, including ICU admission and mortality, had significantly higher levels of cardiac TnI (troponin I) a marker of myocardial heart muscle injury [66]. BNP (Brain-type natriuretic peptide) levels were also elevated among ICU admissions in Washington DC and appeared more universal than troponin elevations [67]. Myocardial injury was found in 22% of hospitalized patients and nearly 60% of deceased patients [68]. Moreover, cardiac arrhythmias were shown to be present in 44% of patients with COVID-19 in the ICU [69]. Although cardiac cells express high levels of the major SARS-CoV-2 entry-receptor angiotensin-converting enzyme 2 (ACE2), it remains unclear whether these cases constitute direct or indirect injury, perhaps by damaging the cardiac vasculature [70-71]. Given the significant deleterious effect of the virus on the heart, effective therapy to treat COVID-19 patients is in urgent need.

Regenerative medicine offers various cell-tissue therapeutics and related products, such as natural killer (NK) cell, CAR T cell, EV/exosome, purified amniotic fluid therapies, as well as other tissue products [64]. MSCs have been studied as a treatment for inflammatory conditions and immunologic diseases for some time and the immunomodulatory effect of MSCs has been well characterized [48]. Included in the repertoire of MSC immunomodulatory activities are: inhibition of proliferation and function of T cells, B cells, dendritic cells, and natural killer cells, polarization of monocytes to anti-inflammatory macrophages and increased production of anti-inflammatory IL-10 and decreased production of pro-inflammatory TNF-α and IL-12 [48] MSCs, with their immune-moderating properties, as well as their regenerative potential and antimicrobial properties, were assessed for their efficacy against the inflammatory effects of ARDS [72]. An analysis of those assessments suggested minimal risk and significant potential benefit using MSC therapy for the treatment of COVID-19 patients with symptoms of ARDS. In addition, MSCs have antifibrotic effects that may alleviate lung fibrosis in some COVID-19 patients.

The studies over the past decade provide good preliminary evidence for researchers and clinicians alike to further investigate the use of cellular therapies in COVID-19 patient cohorts. Several phase I/II clinical trials involving the infusion of allogenic MSCs have been performed in 2020/21 and proved successful in reducing mortality and other effects related to SARS-CoV-2 infections [72]. To date, there are ~80 clinical trials registered on *clinicaltrials.gov* designed to evaluate the use of MSCs in COVID-19 patients [73]. However, most of these trials are still recruiting patients, are not yet complete or the outcomes are yet to be published.

Promising results from evidence-based medicine

In a case-study detailing the progress of a 51-year-old male patient with multi-organ involvement due to SARS-CoV-2 infection, who experienced cardiac arrest, MSCs were administered both systemically (IV) and intrathecally [74]. Briefly, after the first MSC transplantation, blood laboratory values indicative of heart damage decreased (*i.e.* AST, ALT, LDH, CK, pro-BNP, ferritin, triglyceride, fibrinogen, ammonia, and myoglobin). After the second injection, C-reactive protein (CRP), a measure of inflammation, reached normal values. This patient also exhibited very low ejection fraction (EF; 25%) prior to systematic administration of MSCs, however post administration EF increased to 60%. The authors concluded that MSCs provided a therapeutic effect on the heart. After the fourth MSC dose, the patient's heart functions returned to normal[74]. In another clinical study, five patients with severe COVID-19 were treated with Wharton's jelly-derived MSCs (150×10^6 cells per injection) [75]. These patients were subjected to three intravenous injections 3 days apart, and monitoring was performed on days 0, 3, 6, and 14 in routine tests for inflammatory cytokines, and flow cytometry of CD4 and CD8 markers. The results showed that IL-10 and SDF-1 increased after cell therapy, while VEGF, TGF-β, IFN-γ, IL-6, and TNFα decreased. Additionally, myocardial enzyme tests (CPK, LDH, and Troponin I) performed for all patients before and after cell therapy indicated the improvement of tested metrics over time [75]. In a separate study of ten patients with COVID-19-mediated pneumonia, seven patients were administered MSCs, while the remaining three served as a placebo control [76]. MSC transplantation was safe, with no reports of infusion-related reactions or delayed hypersensitivity. One critically ill patient with severe pneumonia receiving MSC treatment exhibited increased oxygen saturation and a significant decrease in plasma CRP, which also serves as a biomarker of myocardial damage[76]. Compared to the placebo-treated controls, patients in the MSC-treated group experienced normalization of immune cell populations, reduced serum TNF-α, and increased IL-10 [77]. A proposed clinical study that aims to deliver MSC-derived exosomes via the inhalation route to COVID-19 patients (NCT04276987) will potentially constitute a paradigm shift. In the light of a recent study that demonstrated efficacy of inhaled stem cell-derived therapy in both *ex vivo* and animal models of pulmonary fibrosis [78], the inhalation route may become more routinely investigated along with the conventional intravenous route. Although there is significant promise in the use of MSCs for cellular therapy to treat cardiovascular conditions, their efficacy for use in treating COVID-19-related cardiac dysfunction and injury is inconclusive, but promising.

Next phase treatment of chronic patients with "long-COVID"

Although the majority of patients appear to recover from COVID-19 and resume normal activities, some develop a variety of post-acute sequelae of SARS-CoV-2 infection. Patients can develop

chronic damage to the lungs [79], heart [80-81], kidneys [82], brain [83], or extremities through either a cytopathic effect of viral replication, an exuberant immune response or thromboembolism. The resulting tissue injury can lead to organ dysfunction, and promote symptoms include fatigue, shortness of breath, and cognitive impairment [84]. These symptoms can present in up to 80% of patients who recover from COVID-19 and is referred to as "post-COVID-19 syndrome", also known colloquially as 'long-COVID'. Patients diagnosed with the syndrome are referred to as "long-haulers" [84]. As research into long-COVID moves forward, investigators are tracking patients over time, gathering samples for analysis, and correlating analytical results with symptoms to better define this syndrome [85].

Conclusions

An increased interest in the therapeutic application of MSCs is a promising sign that the COVID-19 pandemic, and the years 2020/2021, may be the dawn of the new therapeutic era of cellular regenerative medicine, not only for COVID-19 sufferers, but also for lethal infectious, autoimmune and inflammatory diseases, including CVDs. It is perhaps time for funding agencies to invest more heavily in accelerating both pre-clinical and clinical research utilizing cellular and acellular therapies, and combinations of regenerative medicine with other therapeutics. Regenerative medicine therapies may turn out to be an important contribution to bringing about an end to the COVID-19 pandemic and alleviate the burden that long-COVID will surely pose moving forward in a world already revenged by debilitating and deadly CVDs.

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