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# Unique Aspects of the Design of Phase I/II Clinical Trials of Stem Cell Therapy

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## Abstract

This chapter will review the unique aspects and limitations of the design of phase I/II (safety and efficacy) clinical trials of stem cell therapy. Although the classical pharmacologic principles applicable to drugs are not applicable to biologic (live cell) therapeutic agents, an important stage in the development of any new therapeutic agent is the establishment of an optimal dosage and delivery route. This can be particularly challenging when the treatment is a biologic agent, such as stem cells, that may exert its therapeutic effects via complex or poorly understood mechanisms. To date, clinical studies have shown inconsistent findings regarding the relationship between cell dose and clinical outcomes. This can be at least partially attributed to variations in donor cell type, source, characteristics, dosing/concentration, delivery route, underlying mechanisms of action, and efficacy endpoints tested. The current recommendations will be reviewed herein to give new investigators a general understanding of the unique issues that need to be considered and addressed when designing a stem cell therapy phase I/II clinical trial.

**Keywords:** phase I/II clinical trial, regenerative medicine, stem cells

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## 1. Introduction

The past decade has witnessed the exciting development of novel stem cell therapies aimed at regenerating or restoring organ function. Preclinical and pilot studies using stem cells derived from a variety of tissue sources have led to the conduct of phase I/II clinical trials for chronic diseases, formerly thought to be incurable. Systems currently targeted for stem cell therapy

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include cardiovascular, neurologic, pulmonary, autoimmune and liver diseases, as well as diabetes, frailty, and cutaneous wounds, among others [1–20]. In cardiovascular diseases, preclinical studies have served to provide feasibility, safety, and, importantly, mechanistic insights [21–27], whereas phase I/II studies have provided evidence, in the short-term, of the safety and efficacy of autologous and allogeneic bone marrow-derived mesenchymal stem cells (MSCs) [1, 2, 6, 18, 28, 29], autologous bone marrow and peripheral CD34+ stem cells [30, 31], and autologous cardiac-derived stem cells [32–34] in humans. Nevertheless, studies are lacking comparing the efficacy and sustainability of the various different cell types, as well as identifying the most effective dose, time of delivery, and route of administration. Other important questions that remain to be investigated are whether concurrent pharmacologic treatments beneficially or adversely interact with the various cell therapies and whether cell therapy increases the risk for opportunistic infections or malignancy development or progression [27, 35, 36]. Only through the rigorous conduct of large, multicenter clinical trials that include well-defined clinical endpoints and outcomes, a longer duration of follow up (years) and larger number of patients can these questions be addressed [37]. Of note, new biological therapeutic strategies, such as stem cell therapy, necessitates new evaluations tools that elucidate mechanisms of action and measure clinically relevant outcomes. From cell type to dosing, timing, and delivery as well as evaluating safety and clinical efficacy, stem cell therapy provides both unique opportunities and challenges in our quest to develop effective and sustainable therapeutic strategies for cardiovascular diseases as well as other chronic and disabling conditions [37].

Successful stem cell based therapy involves a complex orchestration of events, including engraftment and differentiation as well as secretion of bioactive molecules that inhibit apoptosis and fibrosis and stimulate neovascularization and endogenous stem cell recruitment, proliferation, and differentiation [35, 38, 39]. Notably, existing mechanistic studies support the importance of cell–cell interactions between MSCs and host cells within stem cell niches, which provide structural support and produce the soluble signals that regulate stem cell function in tissues [21, 24, 25, 39, 40]. This enhanced phenotypic and mechanistic understanding of the underpinnings of stem cell based therapy can be harnessed for improved clinical trial design as well as for development of newer generations of cellular as well as new molecular products that have greater efficacy and sustainability [36, 37].

This chapter will provide a general understanding of the unique issues that need to be considered and addressed when designing a phase I/II (safety and efficacy) stem cell therapy clinical trial for cardiovascular disease. The concepts are applicable to other chronic diseases for which stem cell therapeutic approaches are being developed and investigated [19]. For instance, the use of cells as therapeutic agents differs in significant ways from the established principles of pharmacokinetics and pharmacodynamics utilized in pharmacology. An important stage in the development of any new therapeutic agent is the establishment of an optimal dose and route of administration [29, 36]. Biologic therapies create unique challenges in this regard because they exert their therapeutic effects via complex or undefined mechanisms. Indeed, although clinical trials of stem cell therapy for various diseases began over a decade ago, specification of optimal dosage and delivery has not been established. The available clinical studies have shown inconsistent findings regarding the relationship

between cell dose and clinical benefit, due, at least in part, to variations in donor cell characteristics, cell types, cell dosing/concentration, and route (intravenous, intra-arterial, intra-tissue) and timing of administration [29, 36]. We will also review the unique aspects of the selection of clinically relevant endpoints, donors and donor cell characteristics, and autologous versus allogeneic cell therapy.

## 2. Description and regulatory aspects

The customary first-in-human study or phase 1 investigation is used to determine the dose and timing of an investigational drug or biologic agent, as well as identify adverse events associated with agent administration in a dose-dependent fashion. Prior to designing a phase I study, it is critical that appropriate preclinical studies are conducted. Moreover, the clinical study must be conducted with appropriate ethical and quality standards, which includes protocol approval by the institutional review board (IRB), also known as an independent ethics committee (IEC), ethical review board (ERB), or research ethics board (REB). These committees review the methods proposed for the research study and monitor the study, in parallel with the data safety monitoring board (DSMB), for adherence to the protocol and adverse event reporting throughout the study period until completion. An important goal of a standard phase I clinical trial is to determine the maximally tolerated dose and/or recommended dose for further testing in larger phase II efficacy trials. Phase II studies aim to provide further information on dosing, tolerability, and major safety concerns, and potential for efficacy in the target patient population. These data are then utilized by researchers and sponsors to estimate the chance of success in achieving important clinical endpoints, such as mortality and hospitalization risk reduction, in phase III trials, obtain drug approval by the regulatory agencies, and bring the intervention into the market for use by clinicians as standard of care.

In the United States, in order to obtain approval, sponsors of drugs or biologic products not previously authorized for marketing in the United States must submit an Investigational New Drug (IND) application to the Food and Drug Administration (FDA) [41]. IND applications must contain sufficient information about the drug or biologic agent, investigators, clinical protocol, and nonclinical toxicologic data. Safety and efficacy must be supported by evidence from controlled studies of adequate size with disease-appropriate endpoints. The conventional approach to obtaining favorable consideration for a marketing license for a new drug or biologic agent is to do 2 or more large scale clinical trials designed to establish clinical benefit directly, often including a comparison between the new drug and a control drug to show improvement in survival, quality of life, or an existing surrogate endpoint for one of the outcomes.

At the time of submission of an IND or as an amendment to an existing IND, a request for regenerative medicine advanced therapy (RMAT) designation can be made. The twenty-first Century Cures Act describes the criteria required for RMAT designation ([www.FDA.gov](http://www.FDA.gov)). According to the FDA, the criteria include that, (a) “the drug be a regenerative medicine therapy, which is defined as a cell therapy, therapeutic tissue engineering product, human cell and tissue product, or any combination product using such therapies or products, except for

those regulated solely under Section 361 of the Public Health Service Act and part 1271 of Title 21, Code of Federal Regulations”; (b) “the drug is intended to treat, modify, reverse, or cure a serious or life-threatening disease or condition”; and (c) “preliminary clinical evidence indicates that the drug has the potential to address unmet medical needs for such disease or condition” ([www.FDA.gov](http://www.FDA.gov)).

The process of obtaining an IND usually requires many years and vast financial resources. As part of the 1997 FDA Modernization Act, three fast-track FDA approval programs were enacted into law to allow for accelerated approval of certain eligible agents. The FDA fast-track program reduced the review period needed to bring first-in class agents to market and quickened the approval of agents that combat serious or life-threatening illnesses that lack standard treatments. With this addition of alternative paths to marketing approval that eased some of the stringent FDA requirements, designing proper phase I trials became even more important to help make early decisions about the potential efficacy of a drug or biologic agent.

The FDA Amendments Act of 2007 (FDAAA) reviewed, expanded, and reaffirmed several existing pieces of legislation regulating the FDA. These changes allowed the FDA to perform more comprehensive reviews of potential new drugs and devices. The FDAAA extended the authority to levy fees to companies applying for approval of drugs, expanded clinical trial guidelines for pediatric drugs, and created the priority review voucher program to expedite the review process for drugs that are expected to have a particularly great impact on the treatment of a disease. The program grants a voucher for use of priority review to a drug developer as an incentive to develop treatments for neglected diseases. The voucher can be used for future drugs that could have wider indications for use, but the company is required to pay a fee to use the voucher. The FDA Safety and Innovation Act of 2012 (FDASIA) is a piece of regulatory legislation that provides the FDA the authority to collect user fees from the medical industry to fund reviews of innovator drugs, medical devices, generic drugs, and biosimilar biologics. It also created the breakthrough therapy designation program and extended the priority review voucher program to make rare pediatric diseases eligible. Breakthrough therapy was designed to further expedite drug development, and was not meant to require that the drug be an actual “breakthrough” [42]. The goal was to facilitate and prioritize the FDA review of new drugs for serious or life-threatening diseases for which early phase clinical trials demonstrated significant treatment benefits over the existing therapeutic options [41].

Another critical regulatory aspect of the design and implementation of a clinical trial is ensuring subject protection and data quality to make certain that the study and its conclusions are robust and can support future trials as well as potential regulatory submissions for marketing approval. Good clinical practice (GCP) is the internationally recognized quality standard used to maintain safeguards on quality, safety, and efficacy. GCP represents ethical and scientific quality standards for designing, recording, and reporting trials that involve the participation of human subjects. Successful implementation of GCP reduces or obviates the need to duplicate the testing carried out during the research and development of novel agents. The International Council for Harmonization of Technical Requirements provides guidelines for GCP for Pharmaceuticals for Human Use (ICH). This is a project that assembles the regulatory authorities and pharmaceutical industry experts of Europe, Japan and the United States to review and

deliberate the scientific and technical aspects of pharmaceutical product registration [43]. The mission and goal of the ICH is to streamline the research and development of new treatments by minimizing or removing testing duplication and producing greater harmonization in the interpretation and application of technical guidelines and requirements for product registration. The process of harmonization aims to develop a more efficient use of human, small and large animal, and material resources and to accelerate the global development and availability of new therapeutic strategies. Importantly, this needs to be achieved without reducing quality, safety, and efficacy and regulatory obligations to protect public health. The ICH guidelines have been adopted as law in several countries, but in the United States they remain only as guidance for the FDA [43].

As part of GCP, a detailed data safety and monitoring plan is implemented for all clinical trials. The plan should include a reporting system to the data coordinating center (DCC) as well as to the independent data and safety monitoring board (DSMB). The DSMB is tasked with the responsibility for safeguarding the interests of study participants, assessing the safety and efficacy of study procedures, and for monitoring the overall conduct of the trial. Web-based computing systems for data collection and data management must be compliant with current federal regulations, specifically, Title 21 of the CFR parts 210–211 (GMP), 820 (Quality System Regulation for Medical Devices), and 11 (Electronic Records and Electronic Signatures). Electronic Case Report Forms (eCRFs) are used to capture the data appropriate to address study objectives by the DCC. The clinical trial coordinators undergo the appropriate training by the DCC in order to have continuous access to enrollment, randomization, and data submission. Randomization for the clinical trials is performed centrally by the DCC. For stem cell clinical trials, a randomized treatment assignment is generated and sent to the study team and cell-manufacturing laboratory. The protocol coordinator, through regular site visits, monitors the quality and timeliness of data submission, as well as compliance with the study protocol, and works with the clinical center to address any deficiencies or discrepancies. A site visit report is distributed to the investigative team and the trial's sponsoring agency. In regard to adverse events (AE), standard operating procedures (SOPs) are developed that outline the reporting requirements for both the FDA and DSMB. For example, automatic emails are generated each time an AE is reported and serious adverse events (SAEs) require an independent medical monitor review that is located at the DCC. Back-up mechanisms are employed in the form of a weekly summary that is provided to the safety and regulatory group to ensure that the AEs receive the proper attention. If an AE requires expedited reporting to the FDA or the DSMB, the DCC prepares a detailed report based on the medical monitor's adjudication and source documentation received by the center. DCCs normally have internal tracking mechanisms to ensure meeting regulatory reporting requirements and to document DSMB responses.

Stem cell therapy clinical trials require a good manufacturing practice (GMP) cell manufacturing facility. These facilities are accredited by the foundation for the accreditation of cellular therapy (FACT). The GMP facilities are expected to have a Quality Assurance (QA) team that is responsible for the documentation system. This typically includes the Standard Operating Procedures (SOP) documents, Certificates of Analysis, documents with specifications for critical materials, supplies, and reagents, and master batch production records. The GMP facility is expected to have cell-manufacturing rooms that are supplied with all the necessary equipment, such as

biosafety cabinets, incubators, bench top centrifuges, and microscopes. The laboratories in the facility must be HEPA filtered, under appropriate air handling (positive pressure), and must meet class 10,000 specifications in the manufacturing rooms and class 100,000 in the general laboratory, liquid nitrogen freezer rooms, storage rooms, and gown in/out areas. Standard operating procedures require that all laboratory equipment is cleaned and maintained according to established quality control schedules. Internal and external audits of the quality systems ensure compliance with current FDA requirements (21 CFR Part 1271 & 21 CFR Part 210 & 211) and other applicable standards from AABB, FACT, and JCAHO (CLIA). The goal of the comprehensive quality systems is to monitor the daily operational and manufacturing activities of the GMP facility in order to prevent, detect and correct flaws or inadequacies that could adversely impact the safety of patients and/or the safety, purity, potency, or efficacy of the manufactured cell therapy products.

### **3. Limitations of phase I/II trials of stem cell therapy for CVD**

#### **3.1. Cell types**

Various different cell types are currently undergoing investigation in phase I/II clinical trials, including mesenchymal stem cells, cardiac-derived stem or progenitor cells, and bone marrow derived mononuclear cells. The cell characteristics, secretomes, and mechanisms of action of these various stem cells are under intense investigation but have not been completely elucidated. The modes of delivery utilized also vary according to the specific disease process and these include intravenous, intracoronary, and intramyocardial or transendocardial [27, 36], as will be discussed in a separate section.

Growing evidence shows the potential of bone marrow derived MSCs as a safe, durable, sustainable, and novel cell-based biologic therapeutic for a diverse range of clinical applications aimed at preventing or reversing organ injury and promoting tissue regeneration. There are numerous advantages to using MSCs as a therapeutic strategy. MSCs are relatively easy to isolate and expand; they exhibit multilineage differentiation capacity, immunomodulatory, anti-inflammatory, anti-fibrotic, and trophic effects; they home to injury sites; and they have an excellent safety profile in both autologous and allogeneic transplantation [2, 6, 8, 21, 22, 44, 45]. Importantly, the use of MSCs engenders few ethical issues since they originate from adult tissues. Preclinical models employing large animals have been instrumental in advancing phenotypic and mechanistic insights underlying MSC therapy for heart disease [21–24]. Furthermore, the growing human phenotypic data supports the notion that MSC therapy is safe [1, 2, 8, 18, 19, 46] and has the capacity for repair of diverse organ systems and amelioration of multiple disease processes [1–17, 19, 29]. The field is advancing rapidly and numerous MSC sources, including bone marrow, adipose tissue, umbilical cord blood, umbilical cord, and amniotic membranes/placenta are under investigation. Successful MSC therapy involves a complex orchestration of events, including MSC engraftment, differentiation, and, perhaps more importantly, secretion of bioactive molecules that inhibit apoptosis and fibrosis and stimulate neovascularization and endogenous stem cell recruitment, proliferation, and differentiation [35, 38]. Notably, existing mechanistic studies support the importance of cell–cell

interactions between MSCs and host cells within stem cell niches, which provide structural support and produce the soluble signals that regulate stem cell function in tissues [21, 24]. This enhanced phenotypic and mechanistic understanding of the underpinnings of MSC-based therapy can be harnessed for improved clinical trial design as well as for development of newer generations of MSC products that have greater efficacy and sustainability.

Cardiac-derived stem or progenitor cells are adult resident multipotent stem cell population(s) identified by characteristic cell markers, including *c-kit* (CD117), *sca-1*, *Isl1*, and Wilms tumor 1, and by the ability to form cardiospheres in vitro [47–49]. Substantial evidence demonstrates that cardiac stem cells (CSCs) reside in stem cell niches in the heart and not only participate in myocardial homeostasis but also proliferate and differentiate in response to myocardial injury [21, 49, 50]. CSCs can differentiate into cardiomyocyte, endothelial, and smooth muscle cell lineages [49, 51, 52], although their degree of contribution to the generation of new cardiomyocytes is controversial [52–55]. Despite ongoing controversy [53–55], multiple preclinical studies, including a recent meta-analysis [56], have demonstrated that injection of CSCs into animal models of ischemic heart disease slowed the progression of pathological cardiac structural changes and improved cardiac function [24, 25, 49, 56–59].

Phase I/II clinical trials are building upon these promising preclinical results. Bolli and colleagues demonstrated the safety and efficacy of *c-kit* + autologous CSCs in patients with heart failure scheduled to undergo Coronary Artery Bypass Graft surgery [32, 60, 61]. With regards to efficacy, the study showed improvement in cardiac function as well as reduction in myocardial infarct size at the 4-month and 1 year time points. Takehara and colleagues evaluated the safety and therapeutic efficacy of autologous CSCs in combination with a sustained release hydrogel matrix producing a controlled release of basic fibroblast growth factor (bFGF) to augment the effect of the cells in patients with heart failure due to ischemic heart disease [62]. This study demonstrated that tissue engineering offers the potential to improve the poor cell survival post-injection, one of the major obstacles limiting the effectiveness of cell therapy, irrespective of the cell type.

The other major cardiac-derived cell therapy currently under clinical investigation is the “cardiosphere.” Cardiospheres are undifferentiated cells isolated from subcultures of atrial or ventricular biopsy specimens. They grow as self-adherent clusters [47] and have been described as clonogenic, expressing stem and endothelial progenitor cell markers, and having properties of adult cardiac stem cells, including long-term self-renewal and differentiation into cardiomyocyte (demonstrating contractile activity and/or expressing cardiomyocyte markers), endothelial, and smooth muscle cell lineages in vitro and in vivo [47]. Cardiospheres are a mixture of both early-stage committed and primitive cells, comprised of a core of *c-kit* + stem cells, layers of differentiating cells, and an outer cell layer of mesenchymal stromal cells [48]. Preclinical models demonstrate that cardiosphere-derived cells (CDCs) are able to reduce scar size after myocardial infarction, improve cardiac function, and increase the viability of myocardium [63]. A Phase I clinical trial of autologous CDCs delivered by intracoronary infusion in patients with impaired cardiac function 2–4 weeks after myocardial infarction demonstrated both cell safety and cell efficacy, reported as increased viable myocardium, improved regional contractility, and reduced scar mass post treatment [34].

Bone marrow is a source of heterogeneous stem cells and progenitors that have the capacity to differentiate into various cell lineages. Clinical trials employing autologous bone marrow mononuclear cells (BM-MNCs) have evaluated the impact of timing of cell delivery after acute myocardial infarction [64–69]. Although BM-MNC therapy has repeatedly been shown to be safe, delivery in the immediate environment and up to 4 weeks after myocardial infarction has not been consistently or conclusively effective for improving cardiac function or structure.

In patients with chronic ischemic heart failure, a phase II trial investigated the efficacy of transendocardial delivery of BM-MNCs on cardiac performance and perfusion at 6 months [70]. Although the study showed no significant effect on cardiac structural or functional parameters, exploratory (post-hoc) analyses demonstrated significant improvement in cardiac function that was associated with higher counts of bone marrow CD34<sup>+</sup> and CD133<sup>+</sup> progenitor cells. These findings suggest that the bone marrow's cellular composition dictates clinical efficacy and that specific cell populations yield a larger regenerative benefit [71].

BM-MNCs have also been tested in clinical trials of refractory angina, a condition characterized by frequent angina attacks unresponsive to maximal medical therapy, and obstructive coronary artery disease not amenable to coronary revascularization [72]. A recent meta-analysis found that cell-based therapy produces improvement in measures of cardiac function and use of anti-anginal medications, and a decreased risk of major adverse cardiovascular events [73]. Notably, an improvement in myocardial perfusion, assessed by single photon emission computed tomography (SPECT), was also noted and there were significantly fewer atrial and ventricular arrhythmias in the cell therapy group. Previous meta-analyses [74, 75] reported similar results of decreased angina frequency and myocardial infarction rate and improved exercise tolerance.

Together the above clinical trials established the safety profile of the BM-MNCs in acute myocardial infarction, chronic ischemic heart failure, and refractory angina. Some of these trials [67–69] also emphasized the need to further optimize clinical trials with the goal of determining the ideal therapeutic time, cell administration route, cell population, and cell dose after acute myocardial infarction [36]. It is worth noting that two meta-analyses [76, 77] provided evidence of efficacy, indicating that BM-MNC therapy prevents pathologic cardiac structural changes, which continue during long-term follow-up, specifically by decreasing infarct size and left ventricular enlargement. In addition, one of these meta-analyses reported that administration of BM-MNCs in patients with ischemic heart disease reduced mortality, recurrent myocardial infarction, and stent thrombosis.

Collectively, the multiple clinical trials using BM-MNCs suggest that despite the benefits of easy accessibility, ability to obtain large quantity of cells without a need for ex vivo expansion, vast preclinical and clinical bone marrow transplantation experience, and a positive safety profile [76, 77], there are significant concerns regarding the efficacy of BM-MNCs for acute myocardial infarction and chronic ischemic heart failure [1, 67, 68, 70]. It is important to note that these completed phase I trials were primarily focused on establishing the safety profile of BM-MNCs, and efficacy results may have been limited by the small number of patients. The multicenter, randomized, controlled, phase III study entitled “The Effect of Intracoronary Reinfusion of BM-MNC on All Cause Mortality in Acute Myocardial Infarction (BAMI) trial” (NCT01569178) is designed to test efficacy and is currently ongoing.

### **3.2. Donors and donor cell characteristics: age, comorbidities, carcinogenic potential, and sex differences**

The proper use of stem cells for clinical applications requires a general understanding of the stem cell aging process [78]. For instance, as MSCs age, their multilineage differentiation, homing, immune modulation and wound healing properties gradually become compromised [78, 79]. Indeed, aging has detrimental effects on stem cells [78, 80, 81], with recent evidence suggesting a “quiescence-to-senescence switch” [82]. These age-related declines in stem cell therapeutic efficiency may be due to intrinsic stem cell aging and age-related changes in the local (tissue) environment, including extracellular matrix components and the stem cell niche [81, 83, 84]. Together these changes produce a decline in stem cell self-renewal, maintenance and therapeutic potential. Thus, the ability of MSCs to function therapeutically likely depends on the age and health status of the donor.

Although the effects of aged MSCs on cardiac repair have not been measured directly, studies have compared the effects of age and comorbidities on human bone marrow cell “angiogenic potency.” Aging, renal failure, C-reactive protein and other health factors correlated significantly with poor angiogenic potency of bone marrow cells [85, 86]. Similarly, the number and migratory capacity of endothelial progenitor cells was reduced in hypertensive patients [87] and those suffering with ischemic cardiomyopathy [88]. Extrapolating these findings to stem-cell therapy for heart disease, suggests that the therapeutic potential of autologous MSCs obtained from patients with ischemic heart disease would allow for only limited recovery, whereas a more robust cardiac repair would occur if allogeneic MSCs from young, healthy donors were used instead. However, while it seems that age and/or comorbidities have a negative impact on the cardiac therapeutic potential of MSCs, such a direct comparison has not been conducted. Alternatively, a study on recipient age and stem cell therapy by Golpanian et al. showed that older (>60 years old) patients respond just as effectively as younger (<60 years old) patients when administered MSC therapy for chronic ischemic cardiomyopathy [89]. This is of great significance as the majority of the population with heart disease in need of cell-based therapy is comprised of aged individuals.

There is conflicting evidence regarding the potential of MSC therapy to promote carcinogenesis [90–93]. Whether the MSCs act as cancer cells themselves by undergoing spontaneous malignant transformation or they interact with surrounding tumor stromal elements remains unclear [94]. Rosland et al. [91] demonstrated spontaneous malignant transformation of human bone marrow-derived MSCs grown in long-term cultures. These cells proliferated more rapidly, were unable to undergo complete differentiation, and exhibited an altered morphology and phenotype compared to normal human MSCs. Additionally, when these transformed cells were injected into immunodeficient mice, histologic examination revealed rapid-growing tumor deposits found throughout the lung tissue. In contrast, in a study by Bernardo et al. [92], isolated human bone marrow-derived MSCs were grown in culture until they reached senescence or passage 25. Subsequently, cells were assessed genetically at different time points and various tumor-related proteins were measured. The majority of MSCs displayed a progressive decrease in proliferative capacity with shortened telomeres until reaching senescence. Importantly, cultured MSCs did not express telomerase activity or human

telomerase reverse transcriptase transcripts and no chromosomal abnormalities or alternative lengthening of telomeres were noted. These data lend support to the safety of *ex vivo* MSC expansion and use in regenerative cell therapy. Nevertheless, careful attention to the functional, phenotypic, and genetic characterization of culture-expanded MSCs as well as other types of stem cells should still be given [94].

Sex differences exist in many disease states and particularly in cardiovascular disease [95, 96]. Post-menopausal women are at a higher risk of coronary artery disease, myocardial infarction, and atherosclerosis compared to pre-menopausal women and age matched men. Based on these findings, disparities in cardiovascular disease outcomes between women and men have been attributed to differences in sex steroid expression, predominantly estrogen. Sex differences also exist with respect to the roles of stem cells in organ repair and regeneration after injury. Female MSCs exhibit decreased apoptosis, decreased interleukin-6, decreased tumor necrosis factor, increased endothelial growth factor, and increased vascular endothelial growth factor expression compared to male donor MSCs [97]. Moreover, in a mouse myocardial infarction model, treatment with female MSCs produced greater recovery of cardiac functional parameters compared to male MSC treatment [98]. The effect of estradiol on MSCs contributes to these differences [99]. Understanding how stem cells are influenced by donor sex and recipient hormonal environment may help account for sex-related disparities in clinical outcomes as well as utilize the beneficial effects of these hormones to optimize transplanted stem cell function and survival.

### 3.3. Autologous versus allogeneic cell therapy.

An important issue in this new field is whether stem cells can be used as an allograft [2, 18, 28]. One potential advantage of allogeneic stem cells is their potential use as an “off-the-shelf” therapeutic agent, avoiding the need for bone marrow aspiration or cardiac biopsy and tissue culture delays prior to treatment. In addition, the function of autologous stem cells may be impaired in patients with comorbidities and/or advanced age, as described in the previous section [78, 79, 81, 84]. Regarding the most studied cell type, MSCs, the absence of major histocompatibility class (MHC) II antigens [100–102] and the secretion of T helper type 2 (TH2) cytokines characterize MSCs as immunoprivileged and immunosuppressive [102, 103], although there is some evidence that allogeneic MSCs may be cleared to a greater extent than autologous cell preparations possibly via formation of alloreactive antibodies [104]. Indeed, a meta-analysis of 82 preclinical studies [105] demonstrated that allogeneic therapy is equally as safe and effective as autologous therapy with MSCs, further suggesting that allogeneic MSCs are characteristically immunomodulatory.

The safety and therapeutic benefit of intravenous administration of allogeneic MSCs versus placebo has been demonstrated in patients after acute myocardial infarction [6, 106, 107]. Moreover, our group conducted phase I/II clinical trials comparing allogeneic and autologous MSCs delivered by transendocardial stem cell injection into patients with chronic ischemic cardiomyopathy and non-ischemic, dilated cardiomyopathy and showed that both MSC types are safe and clinically effective [2, 18, 29, 108]. These studies are paving the way for the development of allogeneic cell-based regenerative therapies for structural and functional disorders of the myocardium as well as other organs and disease processes [19, 20].

Other stem cell types may have similar immunologic properties. Regarding cardiac-derived stem cells, it has been reported that human CSCs may have immunomodulatory capacity *in vitro* [109], resembling the properties described for MSCs. A recent preclinical study using a porcine model of ischemic cardiomyopathy showed safety and efficacy of allogeneic CSCs alone and in combination with MSCs [110]. These preclinical findings require testing in future clinical trials. In this regard, the “ALLogeneic heart STem cells to Achieve myocardial Regeneration” (ALLSTAR; NCT01458405) clinical trial is investigating the safety and efficacy of allogeneic cardiospheres [48], in the absence of immunosuppression, after reporting positive preclinical findings [111, 112]. It is important to note that emerging evidence supports the idea that cardiospheres share the immunomodulatory properties of MSCs, since they express the classic markers, including CD105, CD90, and CD73 [113–115], and as such may be cardiac-specific stromal or mesenchymal cells. If these cardiac-derived CD105<sup>+</sup> cells are successfully used as an allograft, it would further support the notion that allogeneic cell therapy may be broadly applicable.

### 3.4. Selection of dose and delivery

As with any traditional new drug, establishing the optimal dose and delivery method is a critical part of the development of new stem cell therapies [36, 37, 116]. In a phase II-a study, drug development normally comprises an estimate of a non-effective dose and the highest tolerated dose, whereas in a phase II-b the objective is to determine the dose–response relationship by testing doses ranging from clinically non-effective to the highest tolerated. This paradigm is problematic in stem cell therapy development. Unlike traditional pharmacology, where pharmacokinetic and pharmacodynamics principles and methods are effectual, different principles and assumptions underlie the assessment of the correct dosing regimens in the field of stem cell therapy [36]. Stem cell therapies tested in phase I/II studies are not usually titrated to any specific pharmacodynamic effect targeting a particular physiological marker or pathway, although secondary assessments of dose on various biomarkers and/or cardiac functional parameters are done. The total number of cells administered is not necessarily proportional to the clinical effect, at least using the traditional clinical parameters, such as cardiac structure, functional capacity, and quality of life measurements. Indeed, the small number of preclinical [117–120] and clinical studies [2, 29, 121–125] that have examined cell dose have so far demonstrated conflicting results regarding the relationship between the quantity of cells delivered and clinical efficacy. The variability in cell types and delivery methods as well as the heterogeneous within and between-patient pathophysiology of cardiovascular disease contribute, at least in part, to the challenges in cell dose optimization [36, 116]. Thus, there is a need to design studies that compare both cell dose and delivery methods to determine which combination provides the best clinical outcome in a particular disease state (e.g., acute myocardial infarction vs. chronic heart failure). Other important factors that should be addressed in the field include the need to standardize the growing variety of stem cell sources (e.g. bone marrow, adipose tissue, placenta, umbilical cord, heart, etc.) and production methods and develop adequate methods for measuring the quality, potency, and/or biologic activity of stem cell preparations. This includes investigating concentration-dependent stem cell aggregation or clumping [126], which impacts cell viability and homing or engraftment in injured tissue,

effects of culture media used for therapeutic stem cell preparation on tissue receptors or effector sites [127, 128], and effects of needle bore-induced shear forces on stem cell integrity [129, 130]. Moreover, given that the injected cells must survive and interact with the surrounding tissue microenvironment, the disease state must be consistent in order to compare cell dosing and clinical efficacy. Investigators planning to initiate clinical trials of stem cell therapy using a particular cell type and delivery method in a particular disease state should be mindful of any assumptions being made based on studies of other cell types and/or delivery methods and disease states and ensure that adequate attention has been paid to all of these as of yet incompletely understood variables.

### 3.5. Safety

Assessment of safety in phase I/II trials of stem cell therapy for cardiovascular disease is currently targeted to detect major concerns, including severe end-organ damage, such as myocardial infarction, stroke, or lung injury, severe allergic reactions, laboratory abnormalities, hemodynamic instability, or death. However, it is important to note that, as with all drugs and therapeutic agents, clinical safety can only be assessed with adequately powered long-term studies. This concern is particularly relevant with the cardiovascular end points usually assessed, such as functional capacity and quality of life. Although small phase I/II studies are useful to demonstrate improvement in these patient-centered outcomes, they may miss important safety signals and thus provide limited overall safety information.

### 3.6. Efficacy

Phase II trials are intentionally designed with a small number of patients and relatively short duration of follow up, and therefore do not have the power to assess the effect of the therapeutic agent on clinical outcomes such as mortality or hospitalization risk. The efficacy outcomes used in phase II trials are usually surrogate end points and translation biomarkers that correlate with mortality and/or hospitalization risk and can be assessed in the period of time of the trial. Therefore, the rationale for phase II clinical trials is to identify the potential clinical benefits of the novel therapy being tested, with minimal regard to statistical significance, on which phase III trials can then be based to confirm the findings in a larger population over a longer period of time.

The surrogate end points and translational biomarkers used for efficacy assessment in cell therapy phase II trials for cardiovascular disease create several challenges when designing phase III trials, which measure mortality or other clinical outcomes such as cardiac function. In other words, the efficacy end points in cell therapy phase II and phase III trials of cardiovascular disease are usually different. This uncertainty around the translatability and predictive value of changes in the phase II surrogate end point or biomarker to future changes in phase III clinical outcomes is a major issue for researchers and sponsors. There is evidence suggesting that although a potential surrogate marker or biomarker may have a strong association with clinical outcomes, it does not necessarily translate into a strong correlation with clinical outcomes in a phase III trial setting even if a favorable trend was observed in previous studies. For instance, improvement in left ventricular remodeling correlates significantly with

clinical outcomes, such as mortality, in patients with heart failure. However, whereas some therapeutic agents that improve left ventricular remodeling also reduce mortality, other agents shown to reduce mortality have not been found to improve remodeling. Therefore, multiple surrogate endpoints and biomarkers are usually assessed in phase II trials in order to identify any signs of potential clinical efficacy.

## **4. Recommendations for the Design of Phase I/II trials of stem cell therapy for CVD**

### **4.1. Identifying novel markers of clinical improvement**

The development of novel surrogate markers of clinical benefit is crucial for the design of successful clinical trials [116]. An important issue is the identification of markers for subpopulations of patients with cardiovascular diseases based on the pathophysiology and the mechanism of action of the therapeutic agent. Novel applications such as genomics, proteomics, and bioengineering applications and devices can be utilized in this regard and to develop targeted therapies.

### **4.2. Matching end point selection with mechanism of action**

In order to more accurately and precisely determine clinical efficacy in a trial, surrogate markers and biomarkers must be mechanistically affected by the studied drug or biologic agent [116]. It is important to recognize that repairing or regenerating injured cardiovascular tissues is a complex task involving various mechanisms of action that will have a different impact on different end points. For example, the antifibrotic effects of MSCs lead to a reduction in infarct scar size whereas the pro-angiogenic effects lead to neovascularization and increased perfusion, both of which improve cardiac function and structure. Therefore, future clinical trials must connect biological pathways, drug mechanisms of action, and underlying pathophysiology to be successful in developing efficacious novel therapies [116].

### **4.3. Use of a combination of efficacy and surrogate marker endpoints**

As stated previously, in a phase II clinical trial the measure for success should not be linked to the achievement of statistical significance for a small number of primary endpoints in an effort to reduce the likelihood of a false positive finding [37, 116, 131]. The metric of reaching statistical significance for clinical endpoints is the goal of phase III trials. In this regard, the current recommendations for Phase II studies [37, 116, 131] are that many primary endpoints should be assessed, with each prospectively declared and its findings reported; the goal being to identify novel clinical benefits of the new therapy. In order to properly design phase III trials, investigators need to know all of the endpoint results as set out in the phase II study protocol. Moreover, to assess the consistency of the findings, the phase II investigators should select efficacy endpoints from different categories [37]. In cell therapy studies for cardiovascular disease, the important categories to evaluate include, (a) cardiac structure and function, such as infarct size, ventricular sphericity, ejection fraction, ventricular volumes, measures of

contractility and diastolic performance, (b) biomarkers, such as atrial and brain natriuretic proteins, cardiac enzymes, TNF-alpha, C-reactive protein, micro-RNAs, and transcriptomic-based biomarkers, (c) physical functional capacity, such as 6 minute walk distance, peak walking time, and maximal oxygen consumption, and (d) quality of life, such as Minnesota Living with Heart Failure questionnaire, the Short Form-36, need for revascularization and recurrent myocardial infarction or heart failure exacerbations [37].

#### **4.4. Development of novel analytical methods and guiding principles**

The use of a combination of endpoints representing various different categories is expected to improve the development of cell-based therapies, but new analytical methods many need to be developed to manage these large quantities of data [37, 116, 131]. The data from the various categories needs to be evaluated using statistical methods that can generate a cumulative assessment of the impact of the intervention. The analytical methods utilized will need to be adapted based on the directionality and stratification of data, the disease process or clinical setting, specific patient population, and any potential discordant information that arises. These methods may help provide the power needed to detect differences in efficacy endpoints in phase II studies. However, as discussed previously, power is not as important as understanding the multitude of data points to avoid not translating a clinical benefit observed in a phase II study into a phase III trial. This approach highlights the importance of proper endpoint selection and consideration of individual components and decision-making guidelines.

Certain guiding principles for the assessment of phase II studies of cell-based therapies have been recommended [37]. These include “strength of association,” “consistency and concordance,” “coherence,” “dose response,” and “safety.” Strength of association refers to whether the cell therapy being evaluated provides a greater clinical benefit than the control group. If the cell therapy has been beneficial compared to control in other studies involving different patient populations and/or clinical protocols, thus showing consistency and concordance, this benefit would support causality. In contrast, any differences in results between studies would need to be evaluated for possible biological reasons, such as differences in stem cell dose, manufacturing, delivery method, etc. Coherence is also an important principle as it links the observed clinical endpoints with the underlying physiologic effects of the cell-based therapy. For example, a therapy that improves cardiac structure and/or function and improves physical functioning or quality of life provides coherence to the results. Finally, the importance of dose response, sustainability of effect, and safety for cell-based therapies is similar to that of any pharmacologic drug, although there are unique aspects of cell-based therapies as discussed in the previous sections.

### **5. Need for regenerative medicine training programs and patient education**

The number of academic and private physicians practicing regenerative medicine as well as the number of patients and chronic conditions being treated with cell-based therapies has grown exponentially in the past decade [132–136]. Although clinical trials throughout the world have been or are being conducted and reported through [clinicaltrials.gov](http://clinicaltrials.gov), and governmental regulatory bodies provide some oversight [134, 137, 138], there is a growing concern

that physicians without prior or proper training in cell-based therapeutics are treating patients with stem cells from various sources with little or no evidence of safety or efficacy [132, 133, 136, 139]. To add to these concerns, there is a growing demand to deregulate the use of these therapies [140–144]. The rules of the FDA as well as the European Medicines Agency define stem cells modified outside the body as medicines and therefore under their regulatory oversight [134, 137, 138]. However, commercial promotion of unsupported therapeutic uses of stem cells has become a world-wide problem that has proven resistant to regulatory efforts and has created unsafe situations that have resulted in harm to patients, both physically and psychologically (i.e., false promises of cure), and avoidable, punitive conflicts between governmental regulatory agencies and physicians [132–135, 137–140, 143, 145, 146]. One approach that has been proposed to promote compliance and uniformity in this growing field is the implementation of physician training programs at academic institutions [135].

It is imperative that the global biomedical research community be the leaders in developing educational programs to not only train physicians, but also inform patients, the general public, and governmental agencies on the appropriate development, investigation, and clinical use of cell-based therapies [37, 132, 147, 148]. It was with this goal in mind that the National Institutes of Health-sponsored cardiovascular cell therapy network (CCTRn) supports physician training programs that provide expertise in all aspects of regenerative medicine. The mission of the CCTRn is to “achieve public health advances for the treatment of cardiovascular diseases, through the conduct and dissemination of collaborative research leading to evidence-based treatment options and improved outcome for patients with heart disease” (<https://sph.uth.edu/research/centers/ccct/cctrn/about-us.htm>) [37, 147, 148].

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