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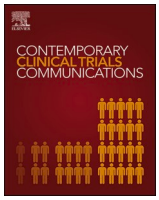

A prospective randomized controlled study of multi-intravenous infusion of umbilical cord mesenchymal stem cells in patients with heart failure and reduced ejection fraction (PRIME-HFrEF) trial: Rationale and design

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ABSTRACT

Background and objective: The use of mesenchymal stem cells for heart failure treatment has gained increasing interest. However, most studies have relied on a single injection approach, with no research yet confirming the effects of multiple administrations. The present trial aims to investigate the safety and efficacy of multi-intravenous infusion of umbilical cord-mesenchymal stem cells (UC-MSCs) in patients with heart failure and reduced ejection fraction (HFrEF).

Methods: The PRIME-HFrEF trial is a single-center, prospective, randomized, triple-blinded, placebo-controlled trial of multi-intravenous infusion of UC-MSCs in HFrEF patients. A total of 40 patients meeting the inclusion criteria for HFrEF were enrolled and randomized 1:1 to the MSC group or the placebo group. Patients enrolled will receive intravenous injections of either UC-MSCs or placebo every 6 weeks for three times. Both groups will be followed up for 12 months. The primary safety endpoint is the incidence of serious adverse events. The primary efficacy endpoint is a change in left ventricular ejection fraction (LVEF) measured by left ventricular opacification (LVO) with contrast echocardiography and magnetic resonance imaging (MRI) at 12 months. The secondary endpoints include a composite of the incidence of death and re-hospitalization caused by heart failure at the 12th month, serum NT-proBNP, growth stimulation expressed gene 2 (ST_2), and a change of right ventricular structure and function.

Conclusions: The PRIME-HFrEF study is designed to shed new light on multiple UC-MSC administration regimens for heart failure treatment.

1. Introduction

Worldwide, approximately 23 million individuals suffer from heart failure (HF), with nearly half of these cases defined by heart failure with reduced ejection fraction (HFrEF) [1]. Even with considerable progress in both drug-based and surgical treatments, the mortality and hospitalization statistics for HF are distressingly elevated, severely affecting patients' quality of life [2]. Innovations in regenerative medicine have opened new therapeutic possibilities for combating HF, and mesenchymal stem cells (MSCs) stand out as a notably promising option [3].

A variety of MSCs exist today, including those sourced from bone marrow, adipose tissue, and the umbilical cord (UC). Of these, UC-MSCs have attracted particular interest due to their plentiful availability and minimal immunogenicity [4]. In the prior RIMECARD clinical trial, UC-MSCs notably enhanced the left ventricular ejection fraction (LVEF) and improved the New York Heart Association (NYHA) classification,

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Minnesota Living with Heart Failure Questionnaire (MLHFQ) score, and Kansas City Cardiomyopathy Questionnaire (KCCQ) score for heart failure patients relative to their baseline [5]. Furthermore, acute myocardial infarction patients who underwent coronary injections of UC-MSCs exhibited significant LVEF augmentation and reductions in left ventricular end-diastolic volume (LVEDV) and left ventricular end-systolic volume (LVESV) over an 18-month observation period [6]. Beyond cardiovascular applications, UC-MSCs have demonstrated promising results in treating other diseases [7–9].

MSCs are known for their pronounced paracrine function, releasing various growth factors and intercellular mediators that maintain bodily homeostasis [10]. UC-MSCs, derived from relatively young tissues, exhibit reduced senescence and enhanced paracrine action and proliferation compared to MSCs from other sources [4]. They also demonstrate anti-inflammatory and immunoregulatory characteristics, suggesting therapeutic benefits in slowing the pathological progression of HF [11]. Considering their potential mechanism of action and existing clinical studies, we posit that intravenous injection, being less invasive and safer, merits further exploration [5,12].

When used as allogeneic cells, MSCs are rapidly cleared from a patient's system upon transplantation. This brief presence is consistent across various cell types [13–16]. Thus, the therapeutic advantages of stem cell infusion may be short-lived. Consequently, some researchers suggest that multiple injections of MSCs might amplify their clinical impact and extend their duration of effect [3]. While a handful of clinical trials have assessed repeated cell therapies using bone marrow mononuclear cells, peripheral blood stem cells, and $CD34^+$ cells [17–21], no study has yet explored a multi-dose regimen of MSC administration for HF patients.

The PRIME-HFrEF trial is a prospective, triple-blinded, randomized, placebo-controlled clinical trial designed to evaluate the safety and efficacy of multi-intravenous UC-MSC treatments for HFrEF patients.

2. Methods

2.1. Study design and setting

The objective of the prospective PRIME-HFrEF trial is to evaluate the safety and efficacy of multi-intravenous injections of allogeneic UC-MSCs in HFrEF patients in a single-center, triple-blind, randomized, placebo-controlled setting. The study protocol complies with the Declaration of Helsinki and the Recommendations for Interventional Trials (SPIRIT) 2013 statement [22]. The study is registered at clinicalTrials.gov (registration no. NCT04992832). The study design is shown in Fig. 1.

Patients were enrolled at Shanghai East Hospital, School of Medicine, Tongji University from July 2021 to June 2022. Written informed consent must be obtained from each participant. All the participants were fully informed before they signed consent to protect their legitimate rights and interests. A total of 40 patients with HFrEF were enrolled and randomized 1:1 to the UC-MSCs group or placebo group. The patients received three intravenous injections of either UC-MSCs or placebo, and clinical evaluations were followed up for 1 year.

2.2. Patient population

Patients between the ages of 18–80 with HFrEF (LVEF ≤40 %), NYHA Classification II-IV, who have received maximally tolerated guideline-directed medical therapy (GDMT) for at least 3 months were included. Patients must not have received an implantable cardioverter-defibrillator (ICD), or cardiac resynchronization therapy (CRT) within the previous 3 months or have been treated via percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG). The baseline parameters including age, sex, blood pressure, body mass index, NYHA class, serum N-terminal pro b-type natriuretic peptide (NT-proBNP), biochemical indicators, underlying disease, left-heart function, tricuspid annular plane systolic excursion (TAPSE), 6-min walking test (6MWT), and medications are shown in Table 1.

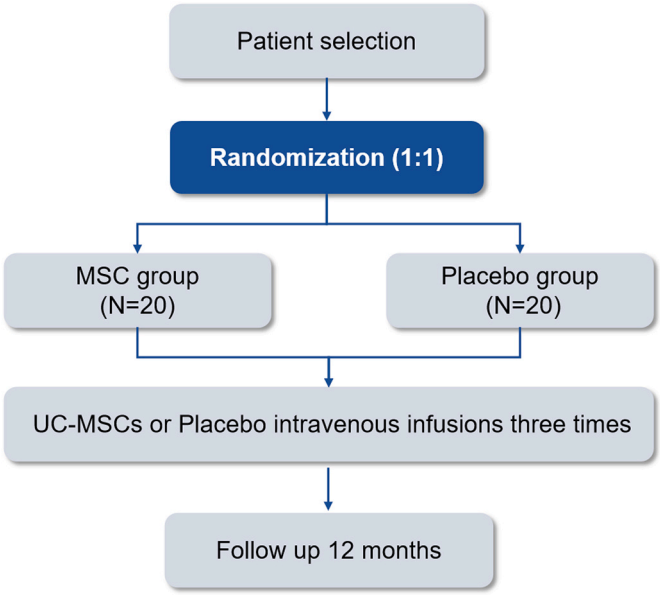

Fig. 1. Flow chart of PRIME-HFrEF

2.3. Randomization and blinding

Patients are randomized in a 1:1 ratio to either the UC-MSCs group or the placebo group in blocks of 10. The randomization list is generated with SAS 9.1.3 software by the Department of Preventive Medicine, Medical College, Tongji University. All patients, study sponsors, nurses, data collectors, and statisticians were blinded to the treatment allocation. Treatments were manufactured by the laboratory of the National Stem Cell Translational Resource Center, and all manufacturing information was withheld from the clinical team. The packaging and appearance of the experimental (UC-MSCs) and control (Placebo) products were indistinguishable. Besides, human serum albumin was used as controls as the UC-MSCs products were stored in human serum albumin.

2.4. Interventions

Based on the results of the RIMECARD Trial, the patients in the UC-MSC group received intravenous injections of either UC-MSCs ($1*10^6$/kg) [5] plus 1 % human serum albumin (UC-MSC group) or 1 % human serum albumin only (Control group) every 6 weeks for three times. Methylprednisolone (40 mg) was administered prior to UC-MSC or Placebo treatment to prevent allergic or nonhemolytic reactions.

Patient blood pressure, heart rate, and temperature were non-invasively monitored throughout the infusion procedure. To minimize donor variability, each patient received the same dose of UC-MSCs isolated from the same donor at 0, 6, and 12 weeks throughout the treatment. To exclude interference of other medications, the dosages of GDMT medications remained constant after patient enrolment, unless clinical intolerance was present, such as a decrease in blood pressure or deterioration in renal function.

2.5. Outcome measures for safety

The primary safety endpoint of the present study is the incidence of any serious adverse events (SAEs) within 12 months after intravenous MSC infusion. Additional safety endpoints include other adverse events (AEs) and clinical abnormalities, such as infection, cardiovascular

Table 1
Baseline characteristics.

Parameter	Prime study
Age (years)	57.10 ± 12.31
Sex, n (%)	
Men	28(71.79)
Women	11(28.21)
Blood pressure	
Systolic (mmHg)	106.92 ± 15.37
Diastolic (mmHg)	66.28 ± 9.82
Heart rate (bpm)	73.97 ± 12.31
Body mass index (kg/m^2)	24.71 ± 3.65
Smoking, n (%)	19(48.72)
Drinking, n (%)	14(35.90)
Stroke, n (%)	5(12.82)
Diabetes mellitus, n (%)	9(23.08)
Cardiac history, n (%)	
Ischemic heart disease	16(41.03)
Cardiomyopathy	28(71.79)
Hypertension	11(28.21)
Atrial fibrillation	13(33.33)
Previous MI	9(23.08)
Previous PCI	10(25.64)
Previous CABG	2(5.26)
Permanent CRT	2(5.13)
ICD implant	3(7.69)
Baseline endpoints	
LVEF (%)	26.08 ± 6.72
LVEDV (mL)	246.62 ± 98.30
LVESV (mL)	185.51 ± 86.97
TAPSE (mm)	16.21 ± 4.50
NYHA Class II-III, n (%)	38(97.44)
6-min walking test (m)	410.64 ± 45.68
MLHFQ	36.56 ± 22.37
Biochemical profile	
ALT (U/L)	16.59 ± 8.69
AST (U/L)	17.51 ± 6.75
eGFR (mL/min/1.73m^2)	74.62 ± 26.26
Total cholesterol (mmol/L)	3.64 ± 0.81
Triglyceride (mmol/L)	1.24 ± 0.62
NT-proBNP (ng/L)	2969.25 ± 4250.75
ST_2 (ng/mL)	12.86 ± 11.24
Troponin-T (ng/mL)	0.02 ± 0.02
CKMB (ng/mL)	2.21 ± 3.31
C-reactive protein (mg/l)	8.09 ± 17.23
D-Dimer (mg/L)	0.46 ± 0.44
Triiodothyronine (ng/mL)	0.91 ± 0.18
TSH (μIU/mL)	3.46 ± 2.54
Medication, n (%)	
Acetylsalicylic acid	9(23.08)
Clopidogrel	19(48.72)
VKA/NOACs	13(33.33)
ARNI	39(100)
β-Blocker	37(94.87)
MRA	33(84.62)
SGLT-2 inhibitor	34(87.18)
Diuretic agent	21(53.84)
Statins	27(69.23)

Values are presented as mean ± SD and n (%).
ALT = alanine aminotransferase; ARNI = angiotensin II receptor enkephalinase inhibitor; AST = aspartate aminotransferase; CABG = coronary artery bypass graft; CKMB = creatine kinase isoenzyme; CRT = cardiac resynchronization therapy; eGFR = estimated glomerular filtration rate; ICD = implantable cardioverter-defibrillator; LVEDV = left ventricular end-diastolic volume; LVEF = left ventricular ejection fraction; LVESV = left ventricular end-systolic volume; MI = myocardial infarction; MLHFQ = Minnesota Living with Heart Failure Questionnaire; MRA = mineralocorticoid receptor antagonist; NOAC = novel oral anticoagulant; NT-proBNP = N-terminal pro-B-type natriuretic peptide; NYHA = New York Heart Association; PCI = percutaneous coronary intervention; SGLT-2 = sodium-dependent glucose transporter 2; ST_2 = soluble growth stimulating gene 2 protein; TAPSE = tricuspid annular plane systolic excursion; TSH = thyroid-stimulating hormone; VKA = vitamin K antagonist.

abnormalities, gastrointestinal disease, neurological disorders, and endocrine metabolic disease, which were detected via serial troponin measurements, hematology, chemistry, urinalysis, 24-h Holter monitoring, chest computed tomography scanning, ultrasound imaging of the abdomen, and immunological profiling.

2.6. Outcome measures for efficacy

The primary efficacy endpoint is a change in LVEF measured by left ventricular opacification (LVO) with contrast echocardiography and magnetic resonance imaging (MRI) at the 12th month.

Secondary endpoints include a composite of death and rehospitalization for heart failure within 12 months, serum NT-proBNP, and ST_2. LVESV, LVEDV, right ventricular end-systolic volume (RVESV), and right ventricular end-diastolic volume (RVEDV) are detected by cardiac MRI, and TAPSE is detected by echocardiography. Additionally, myocardial glucose uptake is evaluated by positron emission tomography MRI (PET/MRI) and myocardial fibrosis is detected by cardiac MRI. Other secondary endpoints include a 6MWT, serum tumor necrosis factor alpha (TNF-α), interleukin 1 (IL-1), interleukin 6 (IL-6), and D-dimer. The follow-up schedule is presented in Fig. 2 and Table 2.

2.7. Mesenchymal stromal cell isolation and expansion

Clinical-grade umbilical cord mesenchymal stem cells (UC-MSCs) were isolated and expanded using a standardized protocol. Umbilical cords from healthy pregnant women were obtained and cleaned. The Wharton's jelly was separated and cut into small pieces, which were then distributed into culture flasks. These flasks were placed in a 37 °C, 5 % CO2 incubator for 6–8 h. α-MEM medium containing 10 % UltraGRO-Advanced was then added, and primary cells were collected once they had migrated from the tissue blocks and reached approximately 80 % confluency. The cells were washed, digested with TrypLE-Express, and collected for subculture. Subsequent passages were performed upon reaching 80 % confluency. At the third passage, the cells were bulk-frozen in liquid nitrogen for long-term preservation.

For experimental use, the frozen cells were thawed, washed, and seeded in culture flasks. UC-MSCs were deemed pure and homogeneous by the fifth passage, exhibiting high expression of specific markers (CD73, CD90, CD105) and low expression of others (CD11, CD19, CD31, CD34, CD45, HLA-DR). The quality and safety of the UC-MSCs were assessed using short tandem repeat (STR) analysis, comparing 21 gene loci (D19S433, D5S818, D21S11, D18S51, D6S1043, AMEL, D3S1358, D13S317, D7S820, D16S539, CSF1PO, PentaD, D2S441, vWA, D8S1179, TPOX, PentaE, TH01, D12S391, D2S1338, FGA).

Additionally, quality and safety evaluations included karyotype analysis (G-banding), trilineage differentiation (adipogenesis, osteogenesis, and chondrogenesis), tumorigenicity (formation of soft agar colonies), immune response assessment (inhibition of microglial cell proliferation), and telomerase activity. Seed cells that met these criteria could be resuscitated and cultured to the fifth passage for clinical applications after passing tests for cell morphology, viability, mycoplasma/bacteria contamination, aerobic/anaerobic bacteria, endotoxin

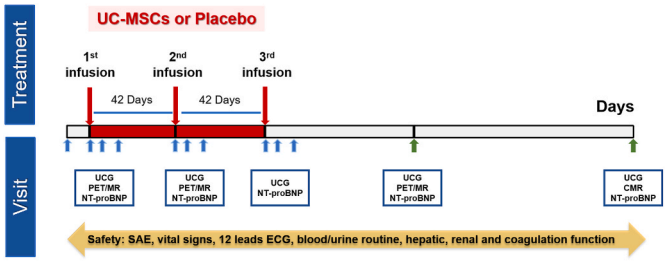

Fig. 2. Outline of PRIME-HFrEF study.

Table 2
Schedule of assessments.

	Screening/Baseline	D1	D3	D7	D43	D45	D49	D85	D87	D91	D180	D360
Informed consent	×											
History/physical	×	×		×	×		×	×		×	×	×
Laboratories	×	×	×	×	×	×	×	×	×	×	×	×
MLHFQ evaluation	×				×			×			×	×
Biomarkers	×	×	×	×	×	×	×	×	×	×	×	×
ECG/MCE	×			×	×		×	×		×	×	×
PET-CMR/SPECT	×				×						×	
CMR												×
Holter monitoring	×			×			×			×	×	×
MSC injection		×			×			×				
Medicine record	×	×		×	×		×	×		×	×	×
AE evaluation		×	×	×	×	×	×	×	×	×	×	×

MLHFQ, Minnesota Living with Heart Failure Questionnaire; ECG, electrocardiogram; MCE, myocardial contrast echocardiography; PET, positron emission tomography; CMR, cardiovascular magnetic resonance; SPECT, single photon emission computed tomography; MSC, mesenchymal stem cell; AE, adverse event.

levels, and human viral detection (e.g., HIV).

2.8. Contrast echocardiography

LVO imaging was performed by using a Philips EPIQ 7C (Philips Health Care, Amsterdam, Netherlands) equipped with an S5–1 (1–5 MHz) probe. The contrast agent (SonoVue; Bracco Diagnostics Inc., Milan, Italy) was infused via the left antecubital vein at a rate of 1 mL/min to a total volume of 1.0–1.5 ml, and the LVO image was obtained with the intermediate mechanical index maintained at approximately 0.3–0.5. A slow flush of 5 mL saline over 10 s was performed to optimize cavity opacification, repeat boluses were administered at 2-min intervals, if necessary, and gain and compression settings were optimized to minimize far-field attenuation. All images containing three heartbeat cycles were digitally captured, stored, and used for measurements.

2.9. Positron emission tomography-cardiac magnetic resonance

Patients underwent hybrid PET-MR imaging at baseline, 6 weeks, and 6 months using an integrated whole-body PET-MR scanner (3.0 T, uPMR790, United Imaging Healthcare Co. Ltd., Shanghai, China). List-mode PET imaging was performed with a median uptake time of 60 min. The data were reconstructed using an ordered subsets expectation maximization (OSEM) iterative reconstruction algorithm with two iterations, 20 subsets, and a matrix size of 150 × 150. Images were then spatially smoothed using 3 mm full width at half-maximum (FWHM) Gaussian kernels. PET emission data were corrected for scatter, random coincidences, dead time, and attenuation. A four-compartment model PET attenuation map was generated using the MR capabilities of the machine, employing fat-only and water-only Dixon-based sequences for automatic PET attenuation correction (AC).

2.10. Data management and statistical analysis

The data management plan is set up by investigators and statisticians according to the International Conference on Harmonization guidelines. Data will be entered by two investigators using the raw data sheet and the CRF. Before database closure, reconciliation will be performed by the data administrator. The data administrator modified and confirmed the data according to the investigators' answers and can propose the questions again if necessary. If the database needs to be modified, track of the changes should be kept. The data administrator will review the completeness and accuracy of the data.

Continuous variables are presented as the mean with standard deviation (SD). Categorical data are expressed as frequencies and proportions. Normally distributed variables are compared by independent *t*-test while the Mann-Whitney *U* test is used for non-normal distribution variables. The Chi-square test is used to compare categorical variables between two groups. Intraindividual comparisons of continuous variables at each follow-up with those at baseline will be performed with paired *t*-test or Wilcoxon rank-sum test depending on normality. Statistical significance was assumed at a value of $P < 0.05$. Analyses will be done by the statistics software SPSS (SPSS Inc, version 22.0, Chicago, IL, USA).

3. Discussion

The PRIME-HFrEF study is the first to investigate the safety and efficacy of multiple intravenous injections of UC-MSCs. More importantly, we adopted a randomized, placebo-controlled, triple-blind study design to mitigate the influence of subjective factors on outcomes, which is critical to advancing cell-based cardiac treatments.

3.1. Rationale for multi-dose regimen

Several clinical studies have revealed that cardiac function recovers in the first and third months after MSC therapy [5]. However, this resurgence is ephemeral. This is consistent with the hypothesis that both the inherent cellular entity and its paracrine effects possess limited longevity in vivo. Research indicates that post-transplant, the surge in c-kit + cardiac progenitor cells (CPCs) stimulated by MSCs peaks around two weeks and returns to baseline levels by approximately day 60 [14,15,23]. Research indicates that post-transplant, the surge in c-kit + cardiac progenitor cells (CPCs) stimulated by MSCs culminates around the fortnight mark and reverts to foundational levels by approximately day 60 [24]. This demonstrates that the benefits from a single stem cell inoculation are transient, underscoring the need for repeated administrations. A preclinical rat model of heart failure showed that three repeat doses of CPCs were more effective than a single large dose in improving cardiac performance, even when the total cell count remained the same, suggesting that multiple doses are preferable in stem cell therapy [25]. Yet, a majority of studies have predicated their outcomes on singular treatments, with a few clinical trials incorporating multiple dosages [18,26,27].

At present, no clinical trials employ successive administrations of UC-MSCs for cardiac patients, encompassing the ongoing research cataloged on ClinicalTrials.gov. In the research concerning the application of stem cells in the treatment of chronic obstructive pulmonary disease, patients received four monthly infusions ($100*10^6$ cells/infusion). During the subsequent two-year follow-up period, no adverse reactions associated with stem cell therapy were observed [28]. Additionally, as noted by Dixon et al., MSCs are detectable in female ovine subjects just 1 h post-injection, but they become undetectable eight weeks post-injection [29]. Consequently, we considered intervals between 2 and 8 weeks. After deliberation, we chose a six-week interval, hypothesizing that overlapping at the peak might increase adverse reactions and that repeated cell therapy every six weeks might stabilize the paracrine action.

3.2. Rationale for choice of administration route

Diverse cellular administration techniques are employed in contemporary clinical research, encompassing intramyocardial, intracoronary, and intravenous modalities. Studies focusing on intracoronary injections have elucidated that MSCs notably ameliorate systolic cardiac dysfunction [30,31]. Likewise, intravenous modalities have demonstrated a marked augmentation in LVEF [5]. In recent years, the benefits of cell therapy for heart failure are thought to come from the release of various biomolecules that induce endogenous repair pathways rather than the integration of transplanted cells within the recipient myocardium [32]. Thus, the effect of intravenous injection should be similar to that of other delivery methods. Meanwhile, the intracoronary or intramyocardial injection has its complications and additional costs while the intravenous injection offers simplicity, particularly for repeated therapy. Therefore, in our study, we chose intravenous injection of MSCs. This investigation endeavors to amass additional empirical insights and validation, fortifying the foundation for the expansive clinical deployment of MSC therapies.

3.3. Safety of multiple UC-MSCs treatments

UC-MSCs are allogeneic stem cells, which may evoke an immune response in patients. Although a single-dose intravenous infusion of UC-MSCs has been demonstrated to be safe for HF patients, it is unknown whether multiple, prolonged infusions will cause adverse clinical problems associated with a severe immune response [5,30,33].

Currently, there is relatively limited research regarding the multiple administrations of UC-MSCs. In a study on hereditary spinocerebellar ataxia, patients underwent intravenous and intrathecal injections on four occasions, with a one-week interval between each injection. Throughout a one-year follow-up, no adverse events associated with UC-MSCs were observed, and laboratory examinations corroborated their safety [8]. Additionally, a patient with multiple sclerosis who underwent multiple injections of BM-MSCs and UC-MSCs from different sources over eleven years did not manifest any discernible side effects [34]. Furthermore, a three-year-old girl suffering from hereditary pulmonary arterial hypertension received five infusions of UC-MSC-conditioned medium via pulmonary artery and central venous catheters within six months. Three years later, the young girl exhibited a remarkable recovery with no apparent adverse reactions [35].

The paramount concern regarding recurrent UC-MSC treatments pertains to the implications for immunological reactions. In vitro, autologous or allogeneic bone marrow stromal cells strongly suppress T-lymphocyte proliferation, including $CD4^+$ and $CD8^+$ T lymphocytes, which are vital for mounting a strong immune response against viral infections [36]. Thus, clinical events associated with immune and immune indicators were evaluated in the present study.

3.4. Selection of efficacy endpoints

Given the ambiguous impact of multiple intravenous infusions of UC-MSCs on HF, PRIME is designed as a phase I, hypothesis-generating study. The primary efficacy endpoint is a change in LVEF. So far, LVEF is the most commonly used and comprehensive parameter for HF diagnosis, characterization, prognosis, monitoring, therapeutic decision-making, and eligibility for HF clinical trials [37]. However, a common criticism is that LVEF is variable and unreliable by 2D echocardiography. Therefore, both LVO measured by contrast echocardiography and MRI are employed to evaluate LVEF.

We have also incorporated composite data on deaths and rehospitalizations due to heart failure within 12 months. Such a composite endpoint offers not only a greater efficacy than singular endpoints but, crucially, permits a formal assessment of treatment efficacy across multiple clinical parameters. Additionally, we will assess alterations in the structure and function of the right ventricle, as intravenously infused UC-MSCs initially enter the right atrium and right ventricle, subsequently proceeding into the pulmonary circulation. NT-proBNP and ST_2, biomarkers of heart failure, were also evaluated. This broad set of endpoints will ensure that the beneficial actions of UC-MSCs are not overlooked and will provide essential information for selecting the most appropriate endpoint and sample size for a subsequent phase II trial.

4. Limitations

Two major limitations of this study could be addressed in future research. First, the study sample was limited to Chinese individuals, the majority of whom had stage NYHA classification II-III. This could be due to our study participants being recruited at a clinic, where a greater proportion of stage II- III disease with more advanced HF diseases would be gathered, and the representativeness of the study findings could not be estimated. Second, because this study is an early phase I exploratory clinical trial, a total of 40 patients that is too small decreases the statistical power of the study. If the safety of repeat therapy can be confirmed, research with a larger sample size could be designed to verify the efficacy outcomes.

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CRediT authorship contribution statement

Xin Gong: Writing – original draft, Methodology. **Yuheng Jiao:** Writing – review & editing, Writing – original draft. **Hao Hu:** Data curation. **Rongzhen Zhang:** Resources. **Wenwen Jia:** Data curation. **Jun Zhao:** Software. **Zhongmin Liu:** Funding acquisition. **Yuanfeng Xin:** Investigation. **Wei Han:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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