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# Epicardial Injection of Allogeneic Human Induced-Pluripotent Stem Cell-derived Cardiomyocytes in Patients with Advanced Ischemic Heart Failure: Protocol for a Phase I Dose-Escalation Clinical Trial

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## ABSTRACT

**Introduction:** Heart failure (HF) is a growing global public health burden. However, due to the very limited regenerative capacity of mature cardiomyocytes in the adult mammalian heart, conventional treatments can only improve the symptoms of HF but fail to restore cardiac function. Heart transplantation is limited by a severe shortage of donors. Cell-based transplantation for the treatment of HF has become a promising strategy. Human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) have been tested in animal models to assess safety and efficacy. This study aims at evaluating the safety and efficacy of epicardial injection of hiPSC-CMs in patients with advanced ischemic heart failure during Coronary Artery Bypass Grafting (CABG) surgery.

**Methods:** This study is a dose-escalation, placebo-controlled, single-blinded, single-centre phase I clinical trial. Dose escalation will be guided by a modified 3+3 design for 3 doses ($1\times10^8$, $2\times10^8$ and $4\times10^8$ cells, sequentially). Patients with advanced ischemic heart failure will be enrolled and randomly allocated to receive epicardial injection of hiPSC-CMs during CABG surgery or CABG surgery alone, followed by a 12-month follow-up investigation. The primary endpoint is to assess the safety of hiPSC-CMs injection, including sustained ventricular arrhythmias, sudden unexpected death and newly formed tumors during 6 months post-operatively. The secondary endpoint is to evaluate the efficacy of epicardial injection of hiPSC-CMs and CABG surgery combination by comparison with the CABG surgery alone.

**Ethics and dissemination:** The study protocol has been approved by the Institutional Ethical Committee of Nanjing Drum Tower Hospital (No.SC202000102). Findings will be disseminated to the academic community through peer-reviewed publications and presentation at national and international meetings.

**Trial registration number:** NCT03763136


**Keywords:** Clinical Trial, Heart Failure, human induced Pluripotent Stem Cell derived cardiomyocytes, Coronary Artery Bypass Grafting surgery

**Word Count: 2780**

## Strengths and limitations of this study

- This study is the first dose-escalation and placebo-controlled trial for the patients with advanced ischemic heart failure treated with epicardial injection of hiPSC-CMs during CABG surgery.
- The results are expected to assess the safety of hiPSC-CMs by dose-escalation, as well as the efficacy of epicardial injection of hiPSC-CMs and CABG surgery combination by comparison with the CABG surgery alone.
- As a phase I trial, the sample size is small, which limited the power of observation.

## INTRODUCTION

Heart failure (HF) is a growing global public health concern with an estimated prevalence of over 37 million individuals worldwide [1]. HF is caused by several causes of cardiovascular diseases (CVD), resulting in poor quality of life, high morbidity and mortality [1, 2]. Ischemic heart disease (IHD) is a major cause of heart failure [2, 3] and represents the number one killer worldwide, causing over 8.9 million or 16% deaths in the year of 2019 globally [4]. Although the treatments for HF, including medications, interventional procedures and surgery have continuously improved in recent decades, they can only improve the symptoms of HF but fail to restore cardiac function by addressing the root cause of the disease, which is the loss of a huge number of contractile cardiomyocytes [5~7]. Restoring cardiac function in HF patients remains a long way to go.

The key pathogenesis of IHD is that loss of these cardiomyocytes results in an irreversible impairment of cardiac function. Unfortunately, the adult mammalian heart has a very limited capability to regenerate after cardiac injury [8~10]. Therefore, transplantation of cardiomyocytes is reasonable and promising to improve cardiac function by remuscularization [11~13]. Human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) can differentiate into cardiomyocytes of high purity [14~16]. However, the clinical application of hESCs faces problems such as limited supply and ethical controversy [17]. In contrast, hiPSCs are derived from adult somatic cells (peripheral blood mononuclear cells, skin fibroblasts, etc.) through reprogramming, which overcomes the supply limit barrier and avoids the ethical issues [18, 19]. Therefore, hiPSCs could be an ideal source for in vitro differentiated cardiomyocytes as the next generation cell therapy for HF.

The First-In-Human (FIH) study involving the transplantation of hESCs-derived cardiac progenitor cells was completed in 2015 by Menasché *et al* in patients suffering from severe ischemic left ventricular dysfunction [20]. A subsequent clinical report from the same team further suggested that transplantation of these cells was safe and potentially promoted some functional recovery in the transplanted myocardial areas [21]. Because of the mentioned limitations of hESCs, hiPSCs-derived cardiomyocytes

have been investigated in rat [22], pig [23] and non-human primate [24] models and shown to restore cardiac function. In addition, based on the FIH clinical trial of epicardial injection of hiPSC-CMs during CABG surgery, "Treating Heart Failure With hPSC-CMs (HEAL-CHF)" (NCT03763136), no serious adverse event, such as mortality or tumorigenicity, related to the epicardial injection of $1\times10^8$ hiPSC-CMs during CABG surgery was reported during the 24-month follow-up investigation. Therefore, we designed this clinical trial to further evaluate the safety and efficacy of epicardial injection of allogeneic hiPSC-CMs in patients with advanced ischemic HF during CABG surgery by comparison with CABG surgery alone.

## METHODS AND ANALYSIS

### Study design

This study is a dose-escalation, placebo-controlled, single-blinded, single-centre phase I clinical trial. An overview of the modified 3+3 dose-escalation trial is presented in Figure 1. The primary endpoint is to assess the safety of the epicardial injection of allogeneic hiPSC-CMs in the treatment of patients with advanced IHF during CABG surgery. The secondary endpoint is to evaluate the efficacy of epicardial injection of hiPSC-CMs and CABG surgery combination by comparison with the CABG surgery alone.

### Study population

Patients with advanced chronic HF secondary to ischemic heart disease fulfilling all inclusion / exclusion criteria will be enrolled at Nanjing Drum Tower Hospital, the affiliated hospital of Nanjing University Medical School, China. The study will be conducted in compliance with the requirements of governmental regulatory bodies and ethics committees.

#### *Inclusion criteria*

1. Patients aged 35-75 years
2. Have signed the Informed Consent Form (ICF).
3. Patients have chronic left ventricular dysfunction.
4. Patients have New York Heart Association (NYHA) Functional Classification III-

IV despite optimal standard of care.

5. Patients have indications for CABG surgery.
6. LVEF ≤ 35% as determined by echocardiogram (data collected up to 6 months prior to inclusion evaluation are valid, excluding the measured values within 1 month of myocardial infarction).
7. Weakening or absence of segmental regional wall motion as determined by standard imaging.

***Exclusion criteria***

1. Patient received implantable cardioverter-defibrillator (ICD), cardiac resynchronization therapy (CRT), left ventricular assist device surgery or similar treatment.
2. Patients with nonischemic cardiomyopathy, viral myocarditis, left ventricular aneurysm / thrombus, untreated congenital heart disease, primary significant organic valvular heart disease (with specified dimensions), pericardial disorders / pericarditis, cerebrovascular disease and/or peripheral vascular disease.
3. In process of being evaluated for heart transplant.
4. Patients screened less than 1 month after the onset of myocardial infarction or PCI.
5. Patients having previously suffered from sustained ventricular tachycardia, atrial fibrillation, conduction abnormalities (including bundle branch block), or sudden cardiac death.
6. Panel Reactive antibody (PRA)≥20% or Donor-specific Antibody (DSA) positive. Autoimmune disorders related the higher risk of immune rejection.
7. Baseline glomerular filtration rate <30ml/min/1.73m$^2$.
8. Liver dysfunction, as evidenced by enzymes (AST and ALT) greater than three times the upper limit of normal (ULN).
9. Hematological abnormality: A hematocrit <25% as determined by HCT, white blood cell<2500/μl or platelet values<100000/μl. Coagulopathy (INR **>** 1.3) not

due to a reversible cause (e.g., warfarin and/or Factor Xa inhibitors).

10. Serious radiographic contrast allergy, penicillin allergy, streptomycin allergy.
11. Contra-indication to performance of a magnetic resonance imaging (MRI) scan.
12. Recipients of organ transplant.
13. Clinical history of malignancy within 5 years (patients with prior malignancy must be disease-free for 5 years).
14. Non-cardiac condition that limits lifespan <1 year.
15. On chronic therapy with immunosuppressant medication, such as glucocorticoid and TNFα antagonist.
16. Contra-indication to take immunosuppressant medication
17. Serum positive for Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), or Treponema Pallidum (TP).
18. Currently enrolled in another investigational therapeutic or device study.
19. Patients who are pregnant or breast feeding.
20. Patients suffering from Orthopedic or Spinal/Neurological Disorders who may have limited ability to participate in post CABG rehabilitation programs or perform efficacy assessments (such as 6 Minute Walk Test)
21. Patients with amyloidosis
22. Other conditions that researchers consider not suitable to participate in this study.

**Randomization and Groups**

Six patients will be enrolled and randomly allocated to CABG +$1\times10^8$ cells group or CABG group. Randomization will be similarly applied for the $2\times10^8$ cells and $4\times10^8$ cells patient groups (Figure 1).

**Intervention**

***1. Screening and Baseline Phase***

See Table 1 for the schedule and assessments to be performed during this phase I clinical trial. Subjects fulfilling all inclusion / exclusion criteria and who have signed the Informed Consent Form will be enrolled. Baseline information and data required

should be collected from all enrolled subjects within 4 weeks before the operation. Key information and data to be collected include subject demographics, vital signs, lab tests, cardiac function evaluation and immunological evaluation (HLA typing, determination of PRA and DSA).

***2. preparation of hiPSC-CMs***

The allogeneic hiPSC-CMs were manufactured at Help Therapeutics under current good manufacturing practice (cGMP) condition and cryopreserved after quality control analysis [22]. The hiPSC-CMs will be thawed in a 37°C water bath (~2 minutes) and resuspended in 5% human serum albumin solution before epicardial injection.

***3. Dose and treatment method***

Six patients will be enrolled and randomly allocated to CABG +$1\times10^8$ group or CABG group (n=3 for each arm). For patients allocated to the CABG +$1\times10^8$ group, hiPSC-CMs will be injected at approximately 10 sites (0.25~0.30 ml of cell suspension at each site). Details regarding injection site location and volume of cell suspension injected at each site will be carefully recorded. patients in CABG groups will receive standard CABG surgery alone. All patients will be transferred to the intensive care unit ( ICU ) after surgery. See Table 1 for the schedule and assessments during this phase.

If no grade 2 or above adverse event occurs within 1 month post-operatively in the CABG + $1\times10^8$ group, dose escalation will proceed to $2\times10^8$ cells. If one grade 2 or above adverse event occurs within 1 month post-operatively, three more patients will be enrolled and injected with $1\times10^8$ cells during CABG surgery. If no grade 2 or above adverse event occurs in the second three-patient cohort, dose escalation will then proceed to $2\times10^8$ cells. Otherwise, the trial will be stopped. The dose escalation design is depicted in Figure 1.

***4. Prohibited drugs***

Subjects in the cell treatment groups will receive immunosuppressive treatment as described below:

1) 2.5g of immunoglobulin will be injected intravenously 1 day pre-operatively, on the day of surgery and 3 days post-operatively, respectively.

2) 500mg of methylprednisolone will be injected intravenously 1 day pre-operatively.
3) 20mg of Simulect® (Basiliximab for Injection) will be injected intravenously on the day of surgery and 4 days post-operatively, respectively.
4) 1g of mycophenolate mofetil (oral) will be given 1 day pre-operatively and subsequently at the dose of 1.5g for 28 days post-operatively.
5) 20mg of Prednisone (oral) will be given daily for 28 days post-operatively.
6) Tacrolimus (oral) will be given from 3 days pre-operatively to 28 days post-operatively, and the dose will be adjusted according to the drug concentration in subject's blood, with a target blood concentration of 3-5ng/ml.

***5. Day 1~21 post-operatively***

See Table 1 for the schedule and assessments to be performed during this phase. Key assessments to be performed include vital sign evaluation, ECG-based evaluation of arrhythmia and laboratory tests including blood routine and biochemistry, cardiac injury markers (NT-Pro-BNP, cardiac troponin, cardiac enzymes, etc.), cytokines (IFNγ, TNFα, IL-2, IL-6 and IL-10), PRA and DSA.

***6. Month 1~12 Visit***

See Table 1 for the schedule and assessments to be performed during this period. Outpatient visits should be completed as close to the scheduled visit dates as possible. The visit window is ± 7 days from the intended date of the visit (1, 3, 6 and 12 months post-operatively). Key assessments to be performed include vital sign evaluation, ECG-based evaluation of arrhythmia, echocardiogram-based and MRI-based cardiac function evaluation, NYHA Classification, 6 min walk test, chest and abdominal PET scan, and laboratory tests including blood routine and biochemistry, cardiac injury markers (NT-Pro-BNP, cardiac troponin, cardiac enzymes, etc.), cytokines (IFNγ, TNFα, IL-2, IL-6 and IL-10), PRA, DSA and tumor markers. Subjects will also fill the Minnesota Living with Heart Failure Questionnaire (MLHFQ).

**Endpoints**

*1)* ***Safety***

(1) procedural complications; vital signs; changes in heart failure medications; sustained ventricular arrhythmias, defined as ventricular arrhythmias lasting longer than 30 seconds as recorded by Holter monitoring; newly formed tumor of allogeneic origin (chest and abdominal CT at 1, 3, 6 months post-operatively, PET scan and 6 and 12 months post-operatively, histopathological analysis of any newly formed tumor tissue);

(2) laboratory tests (including complete blood counts, comprehensive chemistry panels with liver function tests, troponin I, creatinine kinase; PRA and DSA at 1, 3 and 6 months post-operatively); electrocardiogram; all-cause mortality, all-cause hospital admission and need for heart failure co-intervention (Table 1).

***2) Preliminary efficacy***

(1) MRI-based evaluation of left ventricular function

At 1, 3, 6 and 12 months post-operatively, the proportion of infarcted myocardium, left ventricular wall thickness at diastole, interventricular septum thickness, left ventricular ejection fraction, left ventricular end-systolic and end-diastolic volumes, stroke volume, cardiac output, myocardium density and left ventricular mass at diastole will be evaluated.

(2) Echocardiogram-based evaluation of left ventricular function

At 1, 3, 6 and 12 months post-operatively, interventricular septum thickness at diastole, left ventricular end-systolic and end-diastolic diameters, left ventricular posterior wall thickness at diastole, left atrial diameter, left ventricular ejection fraction, mitral flow pattern (E/A) will be evaluated and compared to baseline values.

(3) PET/CT based evaluation of myocardial perfusion at baseline, 6 months and 12 months post-operatively.

(4) 6 Minute Walk Test (baseline, 1, 3, 6 and 12 months post-operatively).

(5) NYHA Classification (baseline, 1, 3, 6 and 12 months post-operatively).

(6) Minnesota Living with Heart Failure Questionnaire (MLHFQ) (baseline, 1, 3, 6 and 12 months post-operatively).

**Statistical Considerations**

This is a phase I dose-escalation clinical trial. The sample size is estimated based on a modified 3+3 design to achieve the primary endpoint. Sample size will be ranged from 6 to 27.

Descriptive statistical analysis will be used for the primary and secondary endpoints. The 95% confidence intervals of the frequency of developing ventricular tachycardia sustained for >30 seconds and tumorigenesis due to allogeneic hiPSC-CMs will be determined with the use of Miettinen's method.

Descriptive statistical analysis will be used for secondary endpoint. Depending on the variables, different statistical methods will be used to compare the outcomes. For measurement data, mean and standard deviation, median, maximum, minimum and range will be calculated and presented. For enumeration data and rating data, frequency (composition ratio), rate, and confidence interval will be calculated and presented. Student's t-Test will be employed to determine the 95% confidence intervals of enumeration data and rating data, while Miettinen's method will be employed to determine the 95% confidence intervals of measurement data. Where appropriate, differences between low dose and high dose groups will be calculated and significance tests will be performed. A bilateral P value less than or equal to 0.05 is considered significant.

**Data collection, management and monitoring**

The schedule of data collection is shown in Table 1. An electronic data capture (EDC) system will be established for this study. A database manager (DM) will be appointed, who will be responsible for the design of the EDC system. Data will be collected from medical notes and hospital records in Nanjing Drum Tower Hospital. Before freezing the database, the DM will compose the data validation report based on the study plan, data validation standards and database contents. The Sponsor, Principal Investigator, Statistician and DM should engage in a meeting to validate the data and come to a resolution regarding database freezing. Once approved, the DM will be responsible for the freezing of the database and the Statistician will conduct statistical analysis afterwards.

Data monitoring and validation will be regularly conducted throughout the study. The frequency of monitoring will be once a year by the Medical Ethics Committee of Affiliated Nanjing Drum Tower Hospital, Nanjing University Medical College, starting from the beginning of this study.

**Quality control**

The clinical trial investigators will implement a quality assurance and quality control system based on the standard operating procedures prescribed by the investigators. Implementation of clinical trial, data creation, recording, monitoring and reporting will be conducted in compliance with "Administrative Measures for Clinical Studies of Stem Cell-based Therapeutics". The study will be monitored by a third-party Data and Safety Monitoring Board.

**Patient and public involvement**

Neither patients nor the public were involved in the development of the research question, choice of outcome measures, design of the trial, recruitment of participants or conduct of the trial. Results of the trial will be disseminated to study participants through direct consultation with a trial clinician at completion of the trial, as well as through the publication of results.

## DISCUSSION

Loss of cardiomyocytes in the myocardium contributes to severe impairment of cardiac function and may lead to heart failure. The implantation of cardiomyocytes presents an alternative treatment to heart transplantation [11~13]. After a roll-in experience as part of the "Treating Heart Failure With hPSC-CMs (HEAL-CHF)" study (NCT03763136), we now initiate a dose-escalation trial to evaluate the safety and efficacy of epicardial injection of hiPSC-CMs during CABG surgery in patients with advanced chronic heart failure. This study will be undertaken with sufficient safety considerations and based on the implementation plan and relevant laws. This clinical trial will shed the light on the hiPSC-CMs cell therapy for the unmet clinical needs for advanced heart failure

patients.

## ETHICS AND DISSEMINATION

The study protocol has been approved by the Medical Ethics Committee of Affiliated Nanjing Drum Tower Hospital, Nanjing University Medical College (No.SC202000102) in May 2020. Participants and their guardians (where applicable) have the right to withdraw at any time and if they do withdraw, will be treated according to hospital standard procedures. Participants who choose to withdraw from the trial will be asked if we can continue to use any data already collected and whether they are willing to participate in the trial follow-up. We will present the trial findings at international meetings and in peer-reviewed publications. We will inform the public through patient organizations and a newsletter to participants.

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## Author contributions

D.W. and J.W. designed the whole protocol, reviewed and approved the paper. H. Z & Z. L wrote the paper and prepared the Figure and Table. T.P, X.Z, X.T, C.X, Y.X, F.F, H.C, B.Z, J.P & Q.Z reviewed the paper and provided valuable suggestion.

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## Competing interests

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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**Figure Legend**

Figure 1. The modified 3+3 dose escalation study design. (SAE, Serious Adverse Event; CABG, Coronary Artery Bypass Graft; MTD, Maximum Tolerated Dose.)

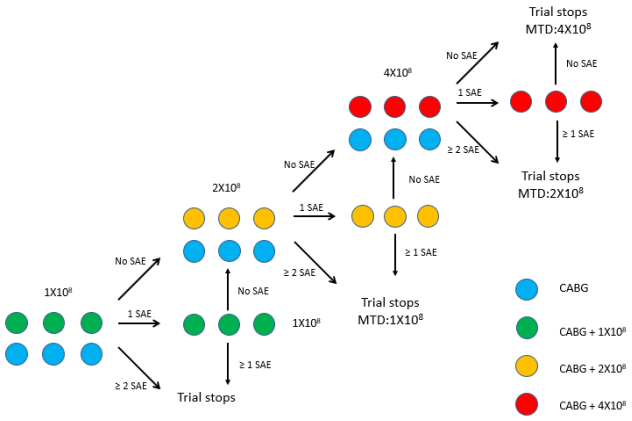

**Table 1. Schedule of Events and Assessments**

| Visit time / Assessments | Baseline Screening | In Patient Visit | | | | Out-patient Monitoring Visits | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Day 0 | Day1---Day 7 | Day14 | Day21 | Month 1±7d | Month 3±7d | Month 6±7d | Month 12±7d |
| Informed Consent Form | X | | | | | | | | |
| Medical History | X | | | | | X | X | X | X |
| Physical Examination | X | X | X | X | X | X | X | X | X |
| 12-lead ECG | X | | X | X | X | X | X | X | X |
| Concomitant medications | X | X | X | X | X | X | X | X | X |
| CAG（SYNTAX score） | X | | | | | | | | |
| iPSC-CM Administration | | X | | | | | | | |
| Echocardiography | X | | | | | X | X | X | X |
| Cardiac MRI | X | | | | | X | X | X | X |
| PET/CT | X | | | | | | | X | X |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **CT ( Brain/Chest/Pelvic )** | X | | | | | X | X | X | X |
| **6 Minute Walk Test (m)** | X | | | | | X | X | X | X |
| **NYHA classification** | X | | | | | X | X | X | X |
| **MLHFQ** | X | | | | | X | X | X | X |
| **24H Holter** | X | | X | X | X | X | X | X | X |
| **Cardiac Enzymes and Troponins** | X | | X | X | X | X | X | X | X |
| **NTproBNP** | X | | X | X | X | X | X | X | X |
| **Blood routine and PCT** | X | | X | X | X | X | X | X | X |
| **Blood Type** | X | | | | | | | | |
| **Biochemistry** | X | | X | X | X | X | X | X | X |
| **Routine urine and stool test** | X | | | | | X | X | X | X |
| **Thyroid function test** | X | | | | | X | X | X | X |

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|---|---|---|---|---|---|---|---|---|---|
| **Tumor marker** | X | | | | | X | X | X | X |
| **Immunoassay（C3、C4、IgA、IgG、IgM）** | X | | | X | | X | X | X | X |
| **Infectious test** | X | | | | | X | X | X | X |
| **Coagulation function** | X | | | X | X | X | X | X | X |
| **HLA typing** | X | | | | | | | | |
| **Plasma Renin Activity** | X | | | X | | X | X | X | X |
| **Donor Specific Antibody** | X | | | X | | X | X | X | X |
| **Cytokines（IFNγ、TNFα、IL-2、IL-4、IL-6、IL-10）** | X | | Day1/3/7 | X | | | | | |
| **Adverse Events** | | X | X | X | X | X | X | X | X |

Note: ECG, electrocardiogram; HLA, human lymphocyte antigen; iPSC-CM, induced Pluripotent Stem Cell derived CardioMyocyte; MLHFQ, The Minnesota Living with Heart Failure Questionnaire ; MRI, Magnetic Resonance Imaging; NTproBNP, N-terminal (NT)-pro hormone BNP; NYHA, New York Heart Association; PCT, procalcitonin; PET/CT, Positron Emission Tomography / Computed Tomography.

# CONSORT 2010 checklist of information to include when reporting a randomised trial*

| Section/Topic | Item No | Checklist item | Reported on page No |
|---|---|---|---|
| **Title and abstract** | | | |
| | 1a | Identification as a randomised trial in the title | 1 |
| | 1b | Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts) | 3 |
| **Introduction** | | | |
| Background and objectives | 2a | Scientific background and explanation of rationale | 5-6 |
| | 2b | Specific objectives or hypotheses | 5 |
| **Methods** | | | |
| Trial design | 3a | Description of trial design (such as parallel, factorial) including allocation ratio | 6 |
| | 3b | Important changes to methods after trial commencement (such as eligibility criteria), with reasons | 6-7 |
| Participants | 4a | Eligibility criteria for participants | 6-7 |
| | 4b | Settings and locations where the data were collected | 6 |
| Interventions | 5 | The interventions for each group with sufficient details to allow replication, including how and when they were actually administered | 8-10 |
| Outcomes | 6a | Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed | 10-11 |
| | 6b | Any changes to trial outcomes after the trial commenced, with reasons | NA |
| Sample size | 7a | How sample size was determined | 11 |
| | 7b | When applicable, explanation of any interim analyses and stopping guidelines | 12-13 |
| Randomisation: | | | |
| Sequence generation | 8a | Method used to generate the random allocation sequence | 6 |
| | 8b | Type of randomisation; details of any restriction (such as blocking and block size) | 6 |
| Allocation concealment mechanism | 9 | Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned | NA |
| Implementation | 10 | Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions | NA |
| Blinding | 11a | If done, who was blinded after assignment to interventions (for example, participants, care providers, those | NA |

| | | | |
|---|---|---|---|
| | | assessing outcomes) and how | |
| | 11b | If relevant, description of the similarity of interventions | NA |
| Statistical methods | 12a | Statistical methods used to compare groups for primary and secondary outcomes | 11-12 |
| | 12b | Methods for additional analyses, such as subgroup analyses and adjusted analyses | NA |
| **Results** | | | |
| Participant flow (a diagram is strongly recommended) | 13a | For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome | 10-12 |
| | 13b | For each group, losses and exclusions after randomisation, together with reasons | NA |
| Recruitment | 14a | Dates defining the periods of recruitment and follow-up | NA |
| | 14b | Why the trial ended or was stopped | 12 |
| Baseline data | 15 | A table showing baseline demographic and clinical characteristics for each group | NA |
| Numbers analysed | 16 | For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups | 12 |
| Outcomes and estimation | 17a | For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval) | 12 |
| | 17b | For binary outcomes, presentation of both absolute and relative effect sizes is recommended | 12 |
| Ancillary analyses | 18 | Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory | NA |
| Harms | 19 | All important harms or unintended effects in each group (for specific guidance see CONSORT for harms) | NA |
| **Discussion** | | | |
| Limitations | 20 | Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses | 13 |
| Generalisability | 21 | Generalisability (external validity, applicability) of the trial findings | 13 |
| Interpretation | 22 | Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence | NA |
| **Other information** | | | |
| Registration | 23 | Registration number and name of trial registry | 3 |
| Protocol | 24 | Where the full trial protocol can be accessed, if available | 3 |
| Funding | 25 | Sources of funding and other support (such as supply of drugs), role of funders | 14 |

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

BMJ Open

# Epicardial Injection of Allogeneic Human Induced-Pluripotent Stem Cell-derived Cardiomyocytes in Patients with Advanced Ischemic Heart Failure: Protocol for a Phase I Dose-Escalation Clinical Trial



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| | |

# Epicardial Injection of Allogeneic Human Induced-Pluripotent Stem Cell-derived Cardiomyocytes in Patients with Advanced Ischemic Heart Failure: Protocol for a Phase I Dose-Escalation Clinical Trial

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## ABSTRACT

**Introduction:** Heart failure (HF) is a growing global public health burden. However, due to the very limited regenerative capacity of mature cardiomyocytes in the adult mammalian heart, conventional treatments can only improve the symptoms of HF but fail to restore cardiac function. Heart transplantation is limited by a severe shortage of donors. Cell-based transplantation for the treatment of HF has become a promising strategy. Human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) have been tested in animal models to assess safety and efficacy. This study aims at evaluating the safety and efficacy of epicardial injection of hiPSC-CMs in patients with advanced ischemic heart failure during Coronary Artery Bypass Grafting (CABG) surgery.

**Methods:** This study is a dose-escalation, randomized control , single-blinded, single-centre phase I clinical trial. Dose escalation will be guided by a modified 3+3 design for 3 doses ($1\times10^8$, $2\times10^8$ and $4\times10^8$ cells, sequentially). Patients with advanced ischemic heart failure will be enrolled and randomly allocated to receive epicardial injection of hiPSC-CMs during CABG surgery or CABG surgery alone, followed by a 12-month follow-up investigation. The primary endpoint is to assess the safety of hiPSC-CMs injection, including sustained ventricular arrhythmias, sudden unexpected death and newly formed tumors during 6 months post-operatively. The secondary endpoint is to evaluate the efficacy of epicardial injection of hiPSC-CMs and CABG surgery combination by comparison with the CABG surgery alone.

**Ethics and dissemination:** The study protocol has been approved by the Institutional Ethical Committee of Nanjing Drum Tower Hospital (No.SC202000102). Findings will be disseminated to the academic community through peer-reviewed publications and presentation at national and international meetings.

**Trial registration number:** NCT03763136


**Keywords:** Clinical Trial, Heart Failure, human induced Pluripotent Stem Cell derived cardiomyocytes, Coronary Artery Bypass Grafting surgery

**Word Count: 2922**

## Strengths and limitations of this study

- This study is the first dose-escalation and randomized control trial for the patients with advanced ischemic heart failure treated with epicardial injection of hiPSC-CMs during CABG surgery.
- The results are expected to assess the safety of hiPSC-CMs by dose-escalation, as well as the efficacy of epicardial injection of hiPSC-CMs and CABG surgery combination by comparison with the CABG surgery alone.
- As a phase I trial, the sample size is small, which limited the power of observation.

## INTRODUCTION

Heart failure (HF) is a growing global public health concern with an estimated prevalence of over 37 million individuals worldwide [1]. HF is caused by several causes of cardiovascular diseases (CVD), resulting in poor quality of life, high morbidity and mortality [1, 2]. Ischemic heart disease (IHD) is a major cause of heart failure [2, 3] and represents the number one killer worldwide, causing over 8.9 million or 16% deaths in the year of 2019 globally [4]. Although the treatments for HF, including medications, interventional procedures and surgery have continuously improved in recent decades, they can only improve the symptoms of HF but fail to restore cardiac function by addressing the root cause of the disease, which is the loss of a huge number of contractile cardiomyocytes [5~7]. Restoring cardiac function in HF patients remains a long way to go.

The key pathogenesis of IHD is that loss of these cardiomyocytes results in an irreversible impairment of cardiac function. Unfortunately, the adult mammalian heart has a very limited capability to regenerate after cardiac injury [8~10]. Therefore, transplantation of cardiomyocytes is reasonable and promising to improve cardiac function by remuscularization [11~13]. Human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) can differentiate into cardiomyocytes of high purity [14~16]. However, the clinical application of hESCs faces problems such as limited supply and ethical controversy [17]. In contrast, hiPSCs are derived from adult somatic cells (peripheral blood mononuclear cells, skin fibroblasts, etc.) through reprogramming, which overcomes the supply limit barrier and avoids the ethical issues [18, 19]. Therefore, hiPSCs could be an ideal source for in vitro differentiated cardiomyocytes as the next generation cell therapy for HF.

The First-In-Human (FIH) study involving the transplantation of hESCs-derived cardiac progenitor cells was completed in 2015 by Menasché *et al* in patients suffering from severe ischemic left ventricular dysfunction [20]. A subsequent clinical report from the same team further suggested that transplantation of these cells was safe and potentially promoted some functional recovery in the transplanted myocardial areas [21]. Because of the mentioned limitations of hESCs, hiPSCs-derived cardiomyocytes

have been investigated in rat [22], pig [23] and non-human primate [24] models and shown to restore cardiac function. In addition, based on our first in human study, two patients with advanced ischemic heart disease received epicardial injection of hiPSC-CMs during CABG surgery, we observed ventricular tachycardia, supraventricular tachycardia and atrial fibrillation in first two weeks. No serious adverse events, such as mortality, malignant arrhythmia or tumorigenicity related to the epicardial injection of $1*10^8$ hiPSC-CMs during 24 months follow-up after CABG surgery. Therefore, we designed this clinical trial to further evaluate the safety and efficacy of epicardial injection of allogeneic hiPSC-CMs in patients with advanced ischemic HF during CABG surgery by comparison with CABG surgery alone.

## METHODS AND ANALYSIS

### Study design

This study is a dose-escalation, randomized control, single-blinded, single-centre phase I clinical trial. An overview of the modified 3+3 dose-escalation trial is presented in Figure 1. The primary endpoint is to assess the safety of the epicardial injection of allogeneic hiPSC-CMs in the treatment of patients with advanced IHF during CABG surgery. The secondary endpoint is to evaluate the efficacy of epicardial injection of hiPSC-CMs and CABG surgery combination by comparison with the CABG surgery alone.

### Study population

Patients with advanced chronic HF secondary to ischemic heart disease fulfilling all inclusion / exclusion criteria will be enrolled at Nanjing Drum Tower Hospital, the affiliated hospital of Nanjing University Medical School, China. The study will be conducted in compliance with the requirements of governmental regulatory bodies and ethics committees.

#### *Inclusion criteria*

1. Patients aged 35-75 years
2. Have signed the Informed Consent Form (ICF).
3. Patients have chronic left ventricular dysfunction.

4. Patients have New York Heart Association (NYHA) Functional Classification III-IV despite optimal standard of care.
5. Patients have indications for CABG surgery.
6. LVEF ≤ 35% as determined by echocardiogram (data collected up to 6 months prior to inclusion evaluation are valid, excluding the measured values within 1 month of myocardial infarction).
7. Weakening or absence of segmental regional wall motion as determined by standard imaging.

***Exclusion criteria***

1. Patient received implantable cardioverter-defibrillator (ICD), cardiac resynchronization therapy (CRT), left ventricular assist device surgery or similar treatment.
2. Patients with nonischemic cardiomyopathy, viral myocarditis, left ventricular aneurysm / thrombus, untreated congenital heart disease, primary significant organic valvular heart disease (with specified dimensions), pericardial disorders / pericarditis, cerebrovascular disease and/or peripheral vascular disease.
3. In process of being evaluated for heart transplant.
4. Patients screened less than 1 month after the onset of myocardial infarction or PCI.
5. Patients having previously suffered from sustained ventricular tachycardia, atrial fibrillation, conduction abnormalities (including bundle branch block), or sudden cardiac death.
6. Panel Reactive antibody (PRA)≥20% or Donor-specific Antibody (DSA) positive. Autoimmune disorders related the higher risk of immune rejection.
7. Baseline glomerular filtration rate <30ml/min/1.73m$^2$.
8. Liver dysfunction, as evidenced by enzymes (AST and ALT) greater than three times the upper limit of normal (ULN).
9. Hematological abnormality: A hematocrit <25% as determined by HCT, white

blood cell<2500/μl or platelet values<100000/μl. Coagulopathy (INR **>** 1.3) not due to a reversible cause (e.g., warfarin and/or Factor Xa inhibitors).

10. Serious radiographic contrast allergy, penicillin allergy, streptomycin allergy.
11. Contra-indication to performance of a magnetic resonance imaging (MRI) scan.
12. Recipients of organ transplant.
13. Clinical history of malignancy within 5 years (patients with prior malignancy must be disease-free for 5 years).
14. Non-cardiac condition that limits lifespan <1 year.
15. On chronic therapy with immunosuppressant medication, such as glucocorticoid and TNFα antagonist.
16. Contra-indication to take immunosuppressant medication
17. Serum positive for Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), or Treponema Pallidum (TP).
18. Currently enrolled in another investigational therapeutic or device study.
19. Patients who are pregnant or breast feeding.
20. Patients suffering from Orthopedic or Spinal/Neurological Disorders who may have limited ability to participate in post CABG rehabilitation programs or perform efficacy assessments (such as 6 Minute Walk Test)
21. Patients with amyloidosis
22. Other conditions that researchers consider not suitable to participate in this study.

**Randomization and Groups**

Six patients will be enrolled and randomly allocated to CABG +$1\times10^8$ cells group or CABG group. Randomization will be similarly applied for the $2\times10^8$ cells and $4\times10^8$ cells patient groups (Figure 1).

**Intervention**

***1. Screening and Baseline Phase***

See Table 1 for the schedule and assessments to be performed during this phase I clinical trial. Subjects fulfilling all inclusion / exclusion criteria and who have signed

the Informed Consent Form will be enrolled. Baseline information and data required should be collected from all enrolled subjects within 4 weeks before the operation. Key information and data to be collected include subject demographics, vital signs, lab tests, cardiac function evaluation and immunological evaluation (HLA typing, determination of PRA and DSA).

***2. preparation of hiPSC-CMs***

The allogeneic hiPSC-CMs were manufactured at Help Therapeutics under current good manufacturing practice (cGMP) condition and cryopreserved after quality control analysis [22]. The hiPSC-CMs will be thawed in a 37°C water bath (~2 minutes) and resuspended in 5% human serum albumin solution before epicardial injection.

***3. Dose and treatment method***

Six patients will be enrolled and randomly allocated to CABG +$1\times10^8$ group or CABG group (n=3 for each arm). For patients allocated to the CABG +$1\times10^8$ group, hiPSC-CMs will be injected at approximately 10 sites (0.25~0.30 ml of cell suspension at each site). Injection sites will be determined by surgeon based on patients' preoperative CMR and wall motion abnormalities during CABG surgery. Details regarding injection site location and volume of cell suspension injected at each site will be carefully recorded. patients in CABG groups will receive standard CABG surgery alone. All patients will be transferred to the intensive care unit ( ICU ) after surgery. See Table 1 for the schedule and assessments during this phase.

If no grade 2 or above adverse event occurs within 1 month post-operatively in the CABG + $1\times10^8$ group, dose escalation will proceed to $2\times10^8$ cells. If one grade 2 or above adverse event occurs within 1 month post-operatively, three more patients will be enrolled and injected with $1\times10^8$ cells during CABG surgery. If no grade 2 or above adverse event occurs in the second three-patient cohort, dose escalation will then proceed to $2\times10^8$ cells. Otherwise, the trial will be stopped. The dose escalation design is depicted in Figure 1.

***4. Prohibited drugs***

Subjects in the cell treatment groups will receive immunosuppressive treatment as

described below:

1) 2.5g of immunoglobulin will be injected intravenously 1 day pre-operatively, on the day of surgery and 3 days post-operatively, respectively.
2) 500mg of methylprednisolone will be injected intravenously 1 day pre-operatively.
3) 20mg of Simulect® (Basiliximab for Injection) will be injected intravenously on the day of surgery and 4 days post-operatively, respectively.
4) 1g of mycophenolate mofetil (oral) will be given 1 day pre-operatively and subsequently at the dose of 1.5g for 28 days post-operatively.
5) 20mg of Prednisone (oral) will be given daily for 28 days post-operatively.
6) Tacrolimus (oral) will be given from 3 days pre-operatively to 28 days post-operatively, and the dose will be adjusted according to the drug concentration in subject's blood, with a target blood concentration of 3-5ng/ml.

***5. Day 1~21 post-operatively***

See Table 1 for the schedule and assessments to be performed during this phase. Key assessments to be performed include vital sign evaluation, ECG-based evaluation of arrhythmia and laboratory tests including blood routine and biochemistry, cardiac injury markers (NT-Pro-BNP, cardiac troponin, cardiac enzymes, etc.), cytokines (IFNγ, TNFα, IL-2, IL-6 and IL-10), PRA and DSA.

***6. Month 1~12 Visit***

See Table 1 for the schedule and assessments to be performed during this period. Outpatient visits should be completed as close to the scheduled visit dates as possible. The visit window is ± 7 days from the intended date of the visit (1, 3, 6 and 12 months post-operatively). Key assessments to be performed include vital sign evaluation, ECG-based evaluation of arrhythmia, echocardiogram-based and MRI-based cardiac function evaluation, NYHA Classification, 6 min walk test, chest and abdominal PET scan, and laboratory tests including blood routine and biochemistry, cardiac injury markers (NT-Pro-BNP, cardiac troponin, cardiac enzymes, etc.), cytokines (IFNγ, TNFα, IL-2, IL-6 and IL-10), PRA, DSA and tumor markers. Subjects will also fill the

Minnesota Living with Heart Failure Questionnaire (MLHFQ).

**Endpoints**

*1)* ***Safety***

(1) procedural complications; vital signs; changes in heart failure medications; sustained ventricular arrhythmias, defined as ventricular arrhythmias lasting longer than 30 seconds as recorded by Holter monitoring; newly formed tumor of allogeneic origin (chest and abdominal CT at 1, 3, 6 months post-operatively, PET scan and 6 and 12 months post-operatively, histopathological analysis of any newly formed tumor tissue);

(2) laboratory tests (including complete blood counts, comprehensive chemistry panels with liver function tests, troponin I, creatinine kinase; PRA and DSA at 1, 3 and 6 months post-operatively); electrocardiogram; all-cause mortality, all-cause hospital admission and need for heart failure co-intervention (Table 1).

(1) ***Exploratory efficacy data***MRI-based evaluation of left ventricular function

At 1, 3, 6 and 12 months post-operatively, the proportion of infarcted myocardium, left ventricular wall thickness at diastole, interventricular septum thickness, left ventricular ejection fraction, left ventricular end-systolic and end-diastolic volumes, stroke volume, cardiac output, myocardium density and left ventricular mass at diastole will be evaluated.

(2) Echocardiogram-based evaluation of left ventricular function

At 1, 3, 6 and 12 months post-operatively, interventricular septum thickness at diastole, left ventricular end-systolic and end-diastolic diameters, left ventricular posterior wall thickness at diastole, left atrial diameter, left ventricular ejection fraction, mitral flow pattern (E/A) will be evaluated and compared to baseline values.

(3) PET/CT based evaluation of myocardial perfusion at baseline, 6 months and 12 months post-operatively.

(4) 6 Minute Walk Test (baseline, 1, 3, 6 and 12 months post-operatively).

(5) NYHA Classification (baseline, 1, 3, 6 and 12 months post-operatively).

(6) Minnesota Living with Heart Failure Questionnaire (MLHFQ) (baseline, 1, 3, 6

and 12 months post-operatively).

**Statistical Considerations**

This is a phase I dose-escalation clinical trial. The sample size is estimated based on a modified 3+3 design to achieve the primary endpoint. Sample size will be ranged from 6 to 27.

Descriptive statistical analysis will be used for the primary and secondary endpoints. The 95% confidence intervals of the frequency of developing ventricular tachycardia sustained for >30 seconds and tumorigenesis due to allogeneic hiPSC-CMs will be determined with the use of Miettinen's method.

Descriptive statistical analysis will be used for secondary endpoint. Depending on the variables, different statistical methods will be used to compare the outcomes. For measurement data, mean and standard deviation, median, maximum, minimum and range will be calculated and presented. For enumeration data and rating data, frequency (composition ratio), rate, and confidence interval will be calculated and presented. Student's t-Test will be employed to determine the 95% confidence intervals of enumeration data and rating data, while Miettinen's method will be employed to determine the 95% confidence intervals of measurement data. Where appropriate, differences between low dose and high dose groups will be calculated and significance tests will be performed. A bilateral P value less than or equal to 0.05 is considered significant.

**Data collection, management and monitoring**

The schedule of data collection is shown in Table 1. An electronic data capture (EDC) system will be established for this study. A database manager (DM) will be appointed, who will be responsible for the design of the EDC system. Data will be collected from medical notes and hospital records in Nanjing Drum Tower Hospital. Before freezing the database, the DM will compose the data validation report based on the study plan, data validation standards and database contents. The Sponsor, Principal Investigator, Statistician and DM should engage in a meeting to validate the data and come to a

resolution regarding database freezing. Once approved, the DM will be responsible for the freezing of the database and the Statistician will conduct statistical analysis afterwards.

Data monitoring and validation will be regularly conducted throughout the study. The frequency of monitoring will be once a year by the Medical Ethics Committee of Affiliated Nanjing Drum Tower Hospital, Nanjing University Medical College, starting from the beginning of this study.

**Quality control**

The clinical trial investigators will implement a quality assurance and quality control system based on the standard operating procedures prescribed by the investigators. Implementation of clinical trial, data creation, recording, monitoring and reporting will be conducted in compliance with "Administrative Measures for Clinical Studies of Stem Cell-based Therapeutics". The study will be monitored by a third-party Data and Safety Monitoring Board.

**Patient and public involvement**

Neither patients nor the public were involved in the development of the research question, choice of outcome measures, design of the trial, recruitment of participants or conduct of the trial. Results of the trial will be disseminated to study participants through direct consultation with a trial clinician at completion of the trial, as well as through the publication of results.

## DISCUSSION

Loss of cardiomyocytes in the myocardium contributes to severe impairment of cardiac function and may lead to heart failure. The implantation of cardiomyocytes presents an alternative treatment to heart transplantation [11~13]. After a roll-in experience as part of the "Treating Heart Failure With hPSC-CMs (HEAL-CHF)" study (NCT03763136), we now initiate a dose-escalation trial to evaluate the safety and efficacy of epicardial injection of hiPSC-CMs during CABG surgery in patients with advanced chronic heart

failure. This study will be undertaken with sufficient safety considerations and based on the implementation plan and relevant laws. This clinical trial will shed the light on the hiPSC-CMs cell therapy for the unmet clinical needs for advanced heart failure patients.

The allogeneic HiPSC-CMs were selected in this trial for following reasons: firstly, the cost of autologous hIPSC-CMs is much higher than the allogeneic hIPSC-CMs. Especially, it is not realistic for cardiac repair in a large patient populations; Secondly, It is very time-consuming about 1 year for autologous HiPSC-CMs production, which limited the clinical application for severe heart failure patients; Thirdly, the ideal therapeutic HiPSC-CMs should be screened as healthy status. A potential genetic susceptibility of heart failure is hard to excluded for every patient. So universal allogeneic HiPSC-CMs were preferred in our trial. At last, the allogeneic HiPSC-CMs derived from heathy donor with critical criteria was selected for clinical trial.

## ETHICS AND DISSEMINATION

The study protocol has been approved by the Medical Ethics Committee of Affiliated Nanjing Drum Tower Hospital, Nanjing University Medical College (No.SC202000102) in May 2020. Participants and their guardians (where applicable) have the right to withdraw at any time and if they do withdraw, will be treated according to hospital standard procedures. Participants who choose to withdraw from the trial will be asked if we can continue to use any data already collected and whether they are willing to participate in the trial follow-up. We will present the trial findings at international meetings and in peer-reviewed publications. We will inform the public through patient organizations and a newsletter to participants.

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## Author contributions

D.W. and J.W. designed the whole protocol, reviewed and approved the paper. H. Z &

Z. L wrote the paper and prepared the Figure and Table. T.P, X.Z, X.T, C.X, Y.X, F.F, H.C, B.Z, J.P & Q.Z reviewed the paper and provided valuable suggestion.

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**Competing interests**

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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**Figure Legend**

Figure 1. The modified 3+3 dose escalation study design. (SAE, Serious Adverse Event; CABG, Coronary Artery Bypass Graft; MTD, Maximum Tolerated Dose.)

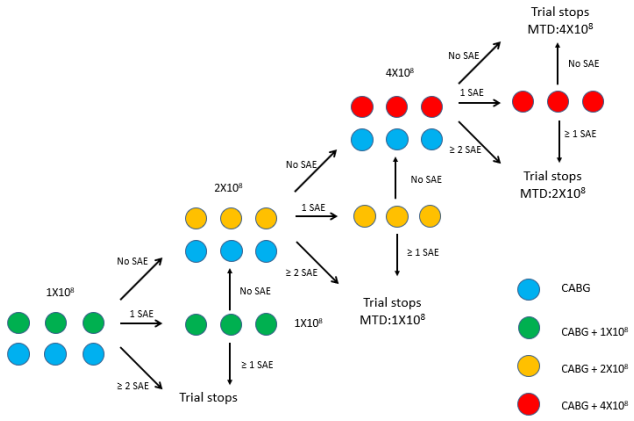
**Table 1. Schedule of Events and Assessments**

| Visit time / Assessments | Baseline Screening | In Patient Visit | | | | Out-patient Monitoring Visits | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Day 0 | Day1---Day 7 | Day14 | Day21 | Month 1±7d | Month 3±7d | Month 6±7d | Month 12±7d |
| Informed Consent Form | X | | | | | | | | |
| Medical History | X | | | | | X | X | X | X |
| Physical Examination | X | X | X | X | X | X | X | X | X |
| 12-lead ECG | X | | X | X | X | X | X | X | X |
| Concomitant medications | X | X | X | X | X | X | X | X | X |
| CAG（SYNTAX score） | X | | | | | | | | |
| iPSC-CM Administration | | X | | | | | | | |
| Echocardiography | X | | | | | X | X | X | X |
| Cardiac MRI | X | | | | | X | X | X | X |
| PET/CT | X | | | | | | | X | X |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **CT ( Brain/Chest/Pelvic )** | X | | | | | X | X | X | X |
| **6 Minute Walk Test (m)** | X | | | | | X | X | X | X |
| **NYHA classification** | X | | | | | X | X | X | X |
| **MLHFQ** | X | | | | | X | X | X | X |
| **24H Holter** | X | | X | X | X | X | X | X | X |
| **Cardiac Enzymes and Troponins** | X | | X | X | X | X | X | X | X |
| **NTproBNP** | X | | X | X | X | X | X | X | X |
| **Blood routine and PCT** | X | | X | X | X | X | X | X | X |
| **Blood Type** | X | | | | | | | | |
| **Biochemistry** | X | | X | X | X | X | X | X | X |
| **Routine urine and stool test** | X | | | | | X | X | X | X |
| **Thyroid function test** | X | | | | | X | X | X | X |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Tumor marker** | X | | | | | X | X | X | X |
| **Immunoassay（C3、C4、IgA、IgG、IgM）** | X | | | X | | X | X | X | X |
| **Infectious test** | X | | | | | X | X | X | X |
| **Coagulation function** | X | | | X | X | X | X | X | X |
| **HLA typing** | X | | | | | | | | |
| **Plasma Renin Activity** | X | | | X | | X | X | X | X |
| **Donor Specific Antibody** | X | | | X | | X | X | X | X |
| **Cytokines（IFNγ、TNFα、IL-2、IL-4、IL-6、IL-10）** | X | | Day1/3/7 | X | | | | | |
| **Adverse Events** | | X | X | X | X | X | X | X | X |

Note: ECG, electrocardiogram; HLA, human lymphocyte antigen; iPSC-CM, induced Pluripotent Stem Cell derived CardioMyocyte; MLHFQ, The Minnesota Living with Heart Failure Questionnaire ; MRI, Magnetic Resonance Imaging; NTproBNP, N-terminal (NT)-pro hormone BNP; NYHA, New York Heart Association; PCT, procalcitonin; PET/CT, Positron Emission Tomography / Computed Tomography.



# Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

## Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

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| | | Reporting Item | Page Number |
|---|---|---|---|
| **Administrative information** | | | |
| Title | #1 | Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym | 1 |
| Trial registration | #2a | Trial identifier and registry name. If not yet registered, | 4 |

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|---|---|---|---|
| | | name of intended registry | |
| Trial registration: data set | #2b | All items from the World Health Organization Trial Registration Data Set | 4 |
| Protocol version | #3 | Date and version identifier | 4 |
| Funding | #4 | Sources and types of financial, material, and other support | 17 |
| Roles and responsibilities: contributorship | #5a | Names, affiliations, and roles of protocol contributors | 2 |
| Roles and responsibilities: sponsor contact information | #5b | Name and contact information for the trial sponsor | 2 |
| Roles and responsibilities: sponsor and funder | #5c | Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities | 17-18 |
| Roles and responsibilities: committees | #5d | Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) | 14 |

## Introduction

| | | | |
|---|---|---|---|
| Background and rationale | #6a | Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention | 5-6 |
| Background and rationale: choice of comparators | #6b | Explanation for choice of comparators | 6 |
| Objectives | #7 | Specific objectives or hypotheses | 6 |
| Trial design | #8 | Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory) | 6 |
| **Methods: Participants, interventions, and outcomes** | | | |
| Study setting | #9 | Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained | 4 |
| Eligibility criteria | #10 | Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, | 7-8 |

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|---|---|---|---|
| | | surgeons, psychotherapists) | |
| Interventions: description | #11a | Interventions for each group with sufficient detail to allow replication, including how and when they will be administered | 9 |
| Interventions: modifications | #11b | Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease) | 11 |
| Interventions: adherance | #11c | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests) | N/A |
| Interventions: concomitant care | #11d | Relevant concomitant care and interventions that are permitted or prohibited during the trial | N/A |
| Outcomes | #12 | Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended | 10-11 |
| Participant timeline | #13 | Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) | 21 |

| | | | |
|---|---|---|---|
| Sample size | #14 | Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations | 12 |
| Recruitment | #15 | Strategies for achieving adequate participant enrolment to reach target sample size | 12 |
| **Methods: Assignment of interventions (for controlled trials)** | | | |
| Allocation: sequence generation | #16a | Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions | 12 |
| Allocation concealment mechanism | #16b | Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned | 8 |
| Allocation: implementation | #16c | Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions | 8 |

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| Blinding (masking) | #17a | Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how | 8 |
| Blinding (masking): emergency unblinding | #17b | If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial | 8 |
| **Methods: Data collection, management, and analysis** | | | |
| Data collection plan | #18a | Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol | 12-13 |
| Data collection plan: retention | #18b | Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols | N/A |
| Data management | #19 | Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). | N/A |

| | | | |
|---|---|---|---|
| | | Reference to where details of data management procedures can be found, if not in the protocol | |
| Statistics: outcomes | #20a | Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol | 13 |
| Statistics: additional analyses | #20b | Methods for any additional analyses (eg, subgroup and adjusted analyses) | N/A |
| Statistics: analysis population and missing data | #20c | Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) | 12 |
| **Methods: Monitoring** | | | |
| Data monitoring: formal committee | #21a | Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed | 12-13 |
| Data monitoring: interim analysis | #21b | Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial | 14 |
| Harms | #22 | Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial | 11-12 |

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|---|---|---|---|
| | | conduct | |
| Auditing | #23 | Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor | 13 |
| **Ethics and dissemination** | | | |
| Research ethics approval | #24 | Plans for seeking research ethics committee / institutional review board (REC / IRB) approval | 13 |
| Protocol amendments | #25 | Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators) | N/A |
| Consent or assent | #26a | Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) | 9 |
| Consent or assent: ancillary studies | #26b | Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable | N/A |
| Confidentiality | #27 | How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial | 15 |
| Declaration of | #28 | Financial and other competing interests for principal | 17 |

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| interests | | investigators for the overall trial and each study site | |
| Data access | #29 | Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators | 18 |
| Ancillary and post trial care | #30 | Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation | N/A |
| Dissemination policy: trial results | #31a | Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions | 15 |
| Dissemination policy: authorship | #31b | Authorship eligibility guidelines and any intended use of professional writers | N/A |
| Dissemination policy: reproducible research | #31c | Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code | N/A |
| **Appendices** | | | |
| Informed consent materials | #32 | Model consent form and other related documentation given to participants and authorised surrogates | N/A |
| Biological specimens | #33 | Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable | N/A |

BMJ Open

# Epicardial Injection of Allogeneic Human Induced-Pluripotent Stem Cell-derived Cardiomyocytes in Patients with Advanced Ischemic Heart Failure: Protocol for a Phase I/IIa Dose-Escalation Clinical Trial



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|---|---|
| Keywords: | Heart failure < CARDIOLOGY, Coronary heart disease < CARDIOLOGY, SURGERY |
| | |

# Epicardial Injection of Allogeneic Human Induced-Pluripotent Stem Cell-derived Cardiomyocytes in Patients with Advanced Heart Failure: Protocol for a Phase I/IIa Dose-Escalation Clinical Trial

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## ABSTRACT

**Introduction:** Heart failure (HF) is a growing global public health burden. However, due to the very limited regenerative capacity of mature cardiomyocytes in the adult mammalian heart, conventional treatments can only improve the symptoms of HF but fail to restore cardiac function. Heart transplantation is limited by a severe shortage of donors. Cell-based transplantation for the treatment of HF has become a promising strategy. Human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) have been tested in animal models to assess safety and efficacy. This study aims at evaluating the safety and efficacy of epicardial injection of hiPSC-CMs in patients with advanced heart failure during Coronary Artery Bypass Grafting (CABG) surgery.

**Methods:** This study is a dose-escalation, placebo-controlled, single-center phase I/IIa clinical trial. Dose escalation will be guided by a modified 3+3 design for 3 doses ($1\times10^8$, $2\times10^8$ and $4\times10^8$ cells, sequentially). Patients with advanced heart failure will be enrolled and randomly allocated to receive epicardial injection of hiPSC-CMs during CABG surgery or CABG surgery alone, followed by a 12-month follow-up investigation. The primary endpoint is to assess the safety of hiPSC-CMs transplantation, including hemodynamic compromised sustained ventricular arrhythmias and newly formed tumors during 6 months post-operatively. The secondary endpoint is to evaluate the efficacy of epicardial injection of hiPSC-CMs and CABG surgery combination by comparison with CABG surgery alone.

**Ethics and dissemination:** The study protocol has been approved by the Institutional Ethical Committee of Nanjing Drum Tower Hospital (No.SC202000102) and approved by National Health Commission of the PRC (MR-32-21-014649). Findings will be disseminated to the academic community through peer-reviewed publications and presentation at national and international meetings.

**Trial registration number:** NCT03763136


**Keywords:** Clinical Trial, Heart Failure, human induced Pluripotent Stem Cell derived cardiomyocytes, Coronary Artery Bypass Grafting surgery

**Word Count: 2882**

## Strengths and limitations of this study

- This study is the first dose-finding and placebo-controlled trial of induced pluripotent stem cell based cardiac regenerative therapy in patients with advanced heart failure;
- This dose-finding study will assess both the safety and efficacy of epicardial injection of hiPSC-CMs during CABG surgery for treating advanced heart failure;
- This study will be limited to a Chinese population with a target sample size.

## INTRODUCTION

Heart failure (HF) is a growing global public health concern with an estimated prevalence of over 37 million individuals worldwide [1]. HF is caused by several causes of cardiovascular diseases (CVD), resulting in poor quality of life, high morbidity and mortality [1 2]. Ischemic heart disease (IHD) is a major cause of heart failure [2 3], causing over 8.9 million or 16% deaths in the year of 2019 globally [4]. Although the treatments for HF, including medications and interventional devices have continuously improved in the past few decades, currently there is no treatment to restore cardiac function by addressing the underlying mechanism of IHD, loss of massive contractile cardiomyocytes [5-7]. Adult mammalian heart has limited capability to regenerate after cardiac injury [8-10]. Hence, it is reasonable to hypothesize that transplantation of exogenous cardiomyocytes as a promising therapeutic to repair cardiac function by remuscularization of the otherwise irreversibly impaired human myocardium [11-13].

Human pluripotent stem cells, including embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs), can differentiate into cardiomyocytes with high purity in vitro[14-16], providing an ideal source to regenerate the impaired cardiac function. Ethical controversy has long been a key concern of clinical application of hESCs [17]. In contrast, hiPSCs are derived from adult somatic cells (peripheral blood mononuclear cells, skin fibroblasts, etc.) through reprogramming, which overcomes supply limits and avoids ethical issues[18 19]. In 2015, the First-In-Human (FIH) study involving transplantation of hESCs-derived cardiac progenitor cells was completed by Dr. Menasché *et al* in patients with severe ischemic left ventricular dysfunction[20]. A subsequent clinical report from the same team further suggested that transplantation of these cells was safe and potentially promoted some functional recovery in the transplanted myocardial areas [21]. Because of the mentioned limitations of hESCs, hiPSCs-derived cardiomyocytes have been investigated in both small and large animals, including non-human primates [22-24], demonstrating promising results to remuscularize and restore cardiac function.

Previously, our group has performed a FIH clinical study of hiPSC-CMs transplantation during open-chest surgery[25], “Treating Heart Failure with hPSC-CMs

(HEAL-CHF)" (NCT03763136), and observed no serious adverse event, such as mortality or tumorigenicity, which related to the epicardial injection exogenous hiPSC-CMs during a 24-month follow-up. Here, we design a dose-escalation, placebo-controlled, Phase I/IIa clinical trial to evaluate the safety and efficacy of epicardial injection of allogeneic hiPSC-CMs in patients with advanced HF during CABG surgery by comparison with CABG surgery alone.

## METHODS AND ANALYSIS

### Study design

This study is a dose-escalation, placebo-controlled, single-center phase I/IIa clinical trial. An overview of the modified 3+3 dose-escalation trial is presented in Figure 1. This study protocol follows the Standard Protocol Items: Recommendations for Interventional Trials guidelines, developed to provide a standardised guidance for recommended items to be included in a clinical trial protocol[26]. The primary endpoint is to assess the safety of the epicardial injection of allogeneic hiPSC-CMs in the treatment of patients with advanced IHF during CABG surgery. The secondary endpoint is to evaluate the efficacy of epicardial injection of hiPSC-CMs and CABG surgery combination by comparison with the CABG surgery alone.

### Study population

Patients with advanced chronic HF secondary to ischemic heart disease fulfilling all inclusion / exclusion criteria will be enrolled at Nanjing Drum Tower Hospital, the affiliated hospital of Nanjing University Medical School, China. The study will be conducted in compliance with the requirements of governmental regulatory bodies and ethics committees.

#### *Inclusion criteria*

1. Patients aged 35-75 years (including 35 and 75).
2. Have signed the Informed Consent Form (ICF).
3. Patients have chronic left ventricular dysfunction.

4. Patients have New York Heart Association (NYHA) Functional Classification III-IV despite receiving guideline-directed medical therapy.
5. Patients have indications for Coronary Artery Bypass Grafting.
6. 20% ≤ LVEF ≤ 45% as determined by echocardiographic assessment (data collected up to 6 months prior to inclusion evaluation are valid; data collected within 1 month since a myocardial infarction are invalid).
7. Weakening or absence of segmental regional wall motion as determined by standard imaging.

***Exclusion criteria***

1. Panel Reactive Antibody (PRA) ≥ 20% or Donor Specific Antibody (DSA) positive.
2. Patients with valvular heart disease or received heart valvular disease.
3. Patients with acute myocardial infarction or percutaneous transluminal coronary intervention (PCI) treatment within 1 month.
4. Patients requiring atrial fibrillation radiofrequency ablation.
5. Patients having previously suffered from sustained ventricular tachycardia.
6. Baseline glomerular filtration rate <30ml/min/1.73m$^2$.
7. Liver dysfunction, as evidenced by enzymes (AST and ALT) greater than three times the upper limit of normal (ULN).
8. Hematological abnormality: A hematocrit <25% as determined by HCT, white blood cell<2500/μl or platelet values<100000/μl.
9. Serious radiographic contrast allergy, penicillin allergy, streptomycin allergy.
10. Coagulopathy (INR **>** 1.3) not due to a reversible cause.
11. Contra-indication to performance of magnetic resonance imaging (MRI) scan and positron emission tomography/ emission computed tomography (PET/ECT) scan.
12. Recipients of organ transplant.
13. Clinical history of malignancy within 5 years (patients with prior malignancy must be disease-free for 5 years).
14. Non-cardiac condition that limits lifespan <1 year.

15. On chronic therapy with immunosuppressant medication, such as glucocorticoid and TNFα antagonist.
16. Contra-indication to take immunosuppressant medication.
17. Serum positive for Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), or Treponema Pallidum (TP).
18. Currently enrolled in another investigational therapeutic or device study.
19. Patients who are pregnant or breast feeding.
20. Other conditions that researchers consider not suitable to participate in this study.

**Randomization and Groups**

Six patients will be enrolled and randomly allocated to CABG +$1\times10^8$ cells group or CABG group. Randomization will be similarly applied for the $2\times10^8$ cells and $4\times10^8$ cells patient groups (Figure 1).

**Intervention**

***1. Screening and Baseline Phase***

See Table 1 for the schedule and assessments to be performed during this phase I/IIa clinical trial. Subjects fulfilling all inclusion / exclusion criteria and who have signed the Informed Consent Form will be enrolled. Baseline information and data required should be collected from all enrolled subjects within 4 weeks before the operation. Key information and data to be collected include subject demographics, vital signs, lab tests, cardiac function evaluation and immunological evaluation (HLA typing, determination of PRA and DSA).

***2. Preparation of hiPSC-CMs***

Donors were screened and tested for relevant communicable disease agents and diseases, including HIV-1 (antigen and nucleic acid), HIV-2, hepatitis B virus (HBV, nucleic acid and surface and core antigen), hepatitis C virus (HCV, antigen and nucleic acid) and Treponema pallidum (syphilis), according to "Guidance for Industry: Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-

Based Products (HCT/Ps)" by FDA. In order to prevent promotion of delayed carcinogenesis, donors were also screened and tested by exome sequencing for target genes presumably responsible for primary somatic cell mutation in cancer, according to COSMIC, an existing cancer genome mutation database. A health, 28 years old, Chinese female, who met the criteria of donor eligibility tests was selected. Her peripheral mononucleate cells were collected and reprogrammed to induced pluripotent stem cells under current good manufacturing practice (cGMP) condition.

The allogeneic hiPSC-CMs were manufactured at Help Therapeutics under cGMP condition and cryopreserved after quality control analysis [22]. The hiPSC-CMs will be thawed in a 37°C water bath and resuspended in 5% human serum albumin solution before epicardial injection.

### *3. Dose and treatment method*

Six patients will be enrolled and randomly allocated to CABG +$1\times10^8$ group or CABG group (n=3 for each arm). For patients allocated to the CABG +$1\times10^8$ group, hiPSC-CMs will be injected at 10 sites (0.25~0.30 ml of cell suspension at each site). Details regarding injection site location and volume of cell suspension injected at each site will be carefully recorded. Patients in CABG groups will receive standard CABG surgery alone. All patients will be transferred to the intensive care unit (ICU) for 1 week after surgery. If no grade 4 or above cell implant related adverse event occurs within 1 month post-operatively in the CABG + $1\times10^8$ group, dose escalation will proceed to $2\times10^8$ cells. If one grade 4 or above cell implant related adverse event occurs within 1 month post-operatively, three more patients will be enrolled and injected with $1\times10^8$ cells during CABG surgery. If no grade 4 or above cell implant related adverse event occurs in the second three-patient cohort, dose escalation will then proceed to $2\times10^8$ cells. Otherwise, the trial will be stopped. The dose escalation design is depicted in Figure 1.

### *4. Concomitant Medications*

#### *4.1 Immunosuppressive drugs*

Subjects in the cell treatment groups will receive immunosuppressive treatment as described below:

1) 2.5g of immunoglobulin will be injected intravenously 1 day pre-operatively, on the day of surgery and 3 days post-operatively, respectively
2) 20mg of Simulect® (Basiliximab for Injection) will be injected intravenously on the day of surgery and 4 days post-operatively, respectively.
3) 1g of mycophenolate mofetil (oral) will be given 1 day pre-operatively and subsequently at the dose of 1.5g for 28 days post-operatively.
4) Tacrolimus tablets will be given from 3 days pre-operatively to 28 days post-operatively, dose will be adjusted according to a target blood concentration of 3-5ng/ml.

***4.2 Anti-arrhythmic drugs***

The following anti-arrhythmic medications will be provided for subjects who developed accelerated idioventricular rhythm over 100 bpm:

1) 450 mg amiodarone hydrochloride, intravenous injection.
2) 200 mg Amiodarone tablet, TID.
3) 5mg Ivabradine tablet, BID.

***5. Day 1~21 post-operatively***

See Table 1 for different timepoints of assessments to be performed during this period. Key assessments to be performed include vital sign evaluation, ECG-based heart rhythm monitoring, and laboratory tests including biochemistry, cardiac injury markers (NT-Pro-BNP, cardiac troponin, cardiac enzymes, etc.), cytokines (IFNγ, TNFα, IL-2, IL-6 and IL-10), PRA and DSA.

***6. Month 1~12 Visit***

See Table 1 for the schedule and assessments to be performed during this period. Outpatient visits should be completed as close to the scheduled visit dates as possible. The visit window is ± 7 days from the intended date of the visit (1, 3, 6 and 12 months post-operatively). Key assessments to be performed include vital sign evaluation, ECG, echocardiogram-based and MRI-based cardiac function evaluation, NYHA Classification, 6-minute walk test, chest and abdominal PET scan, and laboratory tests including biochemistry, cardiac injury markers (NT-Pro-BNP, cardiac troponin, cardiac

enzymes, etc.), cytokines (IFNγ, TNFα, IL-2, IL-6 and IL-10), PRA, DSA and tumor markers. Subjects will also fill the Minnesota Living with Heart Failure Questionnaire (MLHFQ).

**Endpoints**

**1. *Primary Endpoints***

1.1. Dose Limiting Toxicity (DLT), the adverse event occurs within 30 days post CABG surgery and is considered to be related to hiPSC-CMs transplantation, including:

(1) Grade 4 cardiac arrhythmia based on Common Terminology Criteria for Adverse Events (CTCAE) V5.0.;

(2) Graft versus host disease (GvHD) disregard the continuous prophylaxis immunosuppressant treatment

(3) Death;

1.2. Incidence of newly formed tumor, chest and abdominal CT at 1, 3, 6 months and PET/CT at 6 months post-operatively;

1.3. Hemodynamic compromised sustained ventricular tachycardia, from 1-6 months post-operatively.

***2. Secondary Endpoints***

(1) Changes in left ventricle function evaluation by cardiac MRI-based evaluation of left ventricular function at baseline, 1, 3, 6 and 12 months post-operatively, including:

a. Infarct size;

b. Left ventricular ejection fraction (LVEF, %);

c. Left ventricular fractional shortening (LVFS, %);

d. Left ventricular end-diastolic volume (LVEDV, mL);

e. Left ventricular end-systolic volume (LVESV, mL);

f. Left ventricular thickness at sites of injection;

(2) Changes in left ventricle function evaluation by Echocardiogram-based evaluation of left ventricular function at baseline, 1, 3, 6 and 12 months post-operatively.

(3) PET/ECT based evaluation of myocardial perfusion at baseline, 6 months and 12 months post-operatively.

(4) Functional status by 6 Minute Walk Test at baseline, 1, 3, 6 and 12 months post-operatively.

(5) Functional status by NYHA Classification at baseline, 1, 3, 6 and 12 months post-operatively.

(6) Functional status by Minnesota Living with Heart Failure Questionnaire (MLHFQ) at baseline, 1, 3, 6 and 12 months post-operatively.

(7) Incidence of Major Adverse Cardiac Events (MACE) during Month 1-12 visit post-operatively, including death, non-lethal myocardial infraction and hospitalization for worsening HF.

(8) Changes in penal reactive antibodies (PRA), donor specific antibodies (DSA) and NT-pro BNP at baseline, 1, 3, 6 and 12 months post-operatively.

**Statistical Considerations**

This is a phase I/IIa dose-escalation clinical trial. The sample size is estimated based on a modified 3+3 design to achieve the primary endpoint. Sample size will be ranged from 6 to 27.

Descriptive statistical analysis will be used for the primary and secondary endpoints. The 95% confidence intervals of the frequency of developing ventricular tachycardia sustained for >30 seconds and tumorigenesis due to allogeneic hiPSC-CMs will be determined with the use of Miettinen's method.

Descriptive statistical analysis will be used for secondary endpoint. Depending on the variables, different statistical methods will be used to compare the outcomes. For measurement data, mean and standard deviation, median, maximum, minimum and range will be calculated and presented. For enumeration data and rating data, frequency (composition ratio), rate, and confidence interval will be calculated and presented. Student's t-Test will be employed to determine the 95% confidence intervals of

enumeration data and rating data, while Miettinen's method will be employed to determine the 95% confidence intervals of measurement data. Where appropriate, differences between low dose and high dose groups will be calculated and significance tests will be performed. A bilateral P value less than or equal to 0.05 is considered significant.

**Data collection, management and monitoring**

The schedule of data collection is shown in Table 1. An electronic data capture (EDC) system will be established for this study. A database manager (DM) will be appointed, who will be responsible for the design of the EDC system. Data will be collected from medical notes and hospital records in Nanjing Drum Tower Hospital. Before freezing the database, the DM will compose the data validation report based on the study plan, data validation standards and database contents. The Sponsor, Principal Investigator, Statistician and DM should engage in a meeting to validate the data and come to a resolution regarding database freezing. Once approved, the DM will be responsible for the freezing of the database and the Statistician will conduct statistical analysis afterwards.

Data monitoring and validation will be regularly conducted throughout the study. The frequency of monitoring will be once a year by the Medical Ethics Committee of Affiliated Nanjing Drum Tower Hospital, Nanjing University Medical College, starting from the beginning of this study.

**Quality control**

The clinical trial investigators will implement a quality assurance and quality control system based on the standard operating procedures prescribed by the investigators. Implementation of clinical trial, data creation, recording, monitoring and reporting will be conducted in compliance with "Administrative Measures for Clinical Studies of Stem Cell-based Therapeutics". The study will be monitored by a third-party Data and Safety Monitoring Board.

## Patient and public involvement

Neither patients nor the public were involved in the development of the research question, choice of outcome measures, design of the trial, recruitment of participants or conduct of the trial. Results of the trial will be disseminated to study participants through direct consultation with a trial clinician at completion of the trial, as well as through the publication of results.

## DISCUSSION

Loss of cardiomyocytes in the myocardium contributes to severe impairment of cardiac function and may lead to heart failure. The implantation of cardiomyocytes presents an alternative treatment to heart transplantation [11-13]. After a roll-in experience as part of the "Treating Heart Failure With hPSC-CMs (HEAL-CHF)" study (NCT03763136), we now initiate a dose-escalation Phase I/IIa trial to evaluate the safety and efficacy of epicardial injection of hiPSC-CMs during CABG surgery in patients with advanced heart failure. This study will be undertaken with sufficient safety considerations and based on the implementation plan and relevant laws. The allogenic approach can bring down the cost for iPSC-based cell therapy compared to the autologous approach and will also obviate the need for approval of individual patient-derived products by regulatory authorities[27]. This clinical trial will shed the light on the hiPSC-CMs cell therapy for the unmet clinical needs for advanced heart failure patients.

## ETHICS AND DISSEMINATION

The study protocol has been approved by the Medical Ethics Committee of Affiliated Nanjing Drum Tower Hospital, Nanjing University Medical College (No.SC202000102) in May 2020. This study has then been registered and approved by National Health Commission of People's Republic of China (MR-32-21-014649). Participants and their guardians (where applicable) have the right to withdraw at any time and if they do withdraw, will be treated according to hospital standard procedures. Participants who choose to withdraw from the trial will be asked if we can continue to use any data already collected and whether they are willing to participate in the trial follow-up. We will present the trial findings at international meetings and in peer-

reviewed publications. We will inform the public through patient organizations and a newsletter to participants.

**Acknowledgement**

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**Author contributions**

D.W. and J.W. designed the whole protocol, reviewed and approved the paper. H. Z & Y.X wrote the paper and prepared the Figure and Table. T.P, X.Z, H.C, C.X, F.F, H.C, B.Z, J.P, Q.Z & G.Y reviewed the paper and provided valuable suggestions.

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**Competing interests**

J.W. is a full-time employee of HELP Therapeutics. All other authors declare no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Figure Legend**

Figure 1. The modified 3+3 dose escalation study design. (SAE, Serious Adverse Event, grade 4 or above cell implant related adverse event; CABG, Coronary Artery Bypass Graft; MTD, Maximum Tolerated Dose.)

529

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**Table 1. Schedule of Events and Assessments**

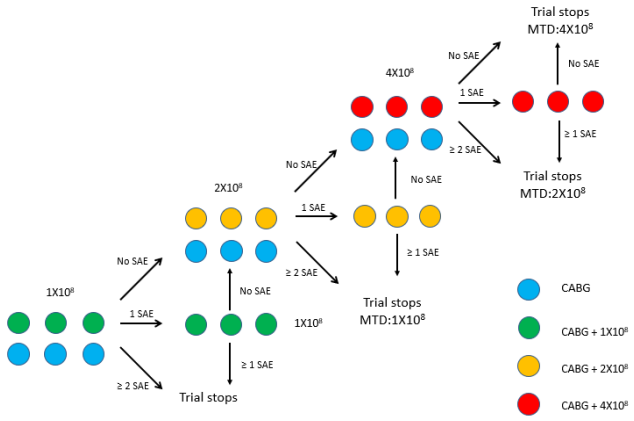
| Visit time / Assessments | Baseline Screening | In Patient Visit | | | | Out-patient Monitoring Visits | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Day 0 | Day1---Day 7 | Day14 | Day21 | Month 1±7d | Month 3±7d | Month 6±7d | Month 12±7d |
| Informed Consent Form | X | | | | | | | | |
| Medical History | X | | | | | X | X | X | X |
| Physical Examination | X | X | X | X | X | X | X | X | X |
| 12-lead ECG | X | | X | X | X | X | X | X | X |
| Concomitant medications | X | X | X | X | X | X | X | X | X |
| CAG ( SYNTAX score ) | X | | | | | | | | |
| iPSC-CM Administration | | X | | | | | | | |
| Echocardiography | X | | | | | X | X | X | X |
| Cardiac MRI | X | | | | | X | X | X | X |
| PET/CT | X | | | | | | | X | X |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **CT ( Brain/Chest/Pelvic )** | X | | | | | X | X | X | X |
| **6 Minute Walk Test (m)** | X | | | | | X | X | X | X |
| **NYHA classification** | X | | | | | X | X | X | X |
| **MLHFQ** | X | | | | | X | X | X | X |
| **24H Holter** | X | | X | X | X | X | X | X | X |
| **Cardiac Enzymes and Troponins** | X | | X | X | X | X | X | X | X |
| **NTproBNP** | X | | X | X | X | X | X | X | X |
| **Blood routine and PCT** | X | | X | X | X | X | X | X | X |
| **Blood Type** | X | | | | | | | | |
| **Biochemistry** | X | | X | X | X | X | X | X | X |
| **Routine urine and stool test** | X | | | | | X | X | X | X |
| **Thyroid function test** | X | | | | | X | X | X | X |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Tumor marker** | X | | | | | X | X | X | X |
| **Immunoassay（C3、C4、IgA、IgG、IgM）** | X | | | X | | X | X | X | X |
| **Infectious test** | X | | | | | X | X | X | X |
| **Coagulation function** | X | | | X | X | X | X | X | X |
| **HLA typing** | X | | | | | | | | |
| **Plasma Renin Activity** | X | | | X | | X | X | X | X |
| **Donor Specific Antibody** | X | | | X | | X | X | X | X |
| **Cytokines（IFNγ、TNFα、IL-2、IL-4、IL-6、IL-10）** | X | | Day1/3/7 | X | | | | | |
| **Adverse Events** | | X | X | X | X | X | X | X | X |

Note: ECG, electrocardiogram; HLA, human lymphocyte antigen; iPSC-CM, induced Pluripotent Stem Cell derived CardioMyocyte; MLHFQ, The Minnesota Living with Heart Failure Questionnaire ; MRI, Magnetic Resonance Imaging; NTproBNP, N-terminal (NT)-pro hormone BNP; NYHA, New York Heart Association; PCT, procalcitonin; PET/CT, Positron Emission Tomography / Computed Tomography.

535



# Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

## Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

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| | | Reporting Item | Page Number |
|---|---|---|---|
| **Administrative information** | | | |
| Title | #1 | Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym | 1 |
| Trial registration | #2a | Trial identifier and registry name. If not yet registered, | 4 |

| | | | |
|---|---|---|---|
| | | name of intended registry | |
| Trial registration: data set | #2b | All items from the World Health Organization Trial Registration Data Set | 4 |
| Protocol version | #3 | Date and version identifier | 4 |
| Funding | #4 | Sources and types of financial, material, and other support | 17 |
| Roles and responsibilities: contributorship | #5a | Names, affiliations, and roles of protocol contributors | 2 |
| Roles and responsibilities: sponsor contact information | #5b | Name and contact information for the trial sponsor | 2 |
| Roles and responsibilities: sponsor and funder | #5c | Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities | 17-18 |
| Roles and responsibilities: committees | #5d | Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) | 14 |

## Introduction

| | | | |
|---|---|---|---|
| Background and rationale | #6a | Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention | 5-6 |
| Background and rationale: choice of comparators | #6b | Explanation for choice of comparators | 6 |
| Objectives | #7 | Specific objectives or hypotheses | 6 |
| Trial design | #8 | Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory) | 6 |
| **Methods: Participants, interventions, and outcomes** | | | |
| Study setting | #9 | Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained | 4 |
| Eligibility criteria | #10 | Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, | 7-8 |

| | | | |
|---|---|---|---|
| | | surgeons, psychotherapists) | |
| Interventions: description | #11a | Interventions for each group with sufficient detail to allow replication, including how and when they will be administered | 9 |
| Interventions: modifications | #11b | Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease) | 11 |
| Interventions: adherance | #11c | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests) | N/A |
| Interventions: concomitant care | #11d | Relevant concomitant care and interventions that are permitted or prohibited during the trial | N/A |
| Outcomes | #12 | Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended | 10-11 |
| Participant timeline | #13 | Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) | 21 |

| | | | |
|---|---|---|---|
| Sample size | #14 | Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations | 12 |
| Recruitment | #15 | Strategies for achieving adequate participant enrolment to reach target sample size | 12 |
| **Methods: Assignment of interventions (for controlled trials)** | | | |
| Allocation: sequence generation | #16a | Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions | 12 |
| Allocation concealment mechanism | #16b | Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned | 8 |
| Allocation: implementation | #16c | Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions | 8 |

| | | | |
|---|---|---|---|
| Blinding (masking) | #17a | Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how | 8 |
| Blinding (masking): emergency unblinding | #17b | If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial | 8 |
| **Methods: Data collection, management, and analysis** | | | |
| Data collection plan | #18a | Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol | 12-13 |
| Data collection plan: retention | #18b | Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols | N/A |
| Data management | #19 | Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). | N/A |

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|---|---|---|---|
| | | Reference to where details of data management procedures can be found, if not in the protocol | |
| Statistics: outcomes | #20a | Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol | 13 |
| Statistics: additional analyses | #20b | Methods for any additional analyses (eg, subgroup and adjusted analyses) | N/A |
| Statistics: analysis population and missing data | #20c | Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) | 12 |
| **Methods: Monitoring** | | | |
| Data monitoring: formal committee | #21a | Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed | 12-13 |
| Data monitoring: interim analysis | #21b | Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial | 14 |
| Harms | #22 | Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial | 11-12 |

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|---|---|---|---|
| | | conduct | |
| Auditing | #23 | Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor | 13 |
| **Ethics and dissemination** | | | |
| Research ethics approval | #24 | Plans for seeking research ethics committee / institutional review board (REC / IRB) approval | 13 |
| Protocol amendments | #25 | Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators) | N/A |
| Consent or assent | #26a | Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) | 9 |
| Consent or assent: ancillary studies | #26b | Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable | N/A |
| Confidentiality | #27 | How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial | 15 |
| Declaration of | #28 | Financial and other competing interests for principal | 17 |

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|---|---|---|---|
| interests | | investigators for the overall trial and each study site | |
| Data access | #29 | Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators | 18 |
| Ancillary and post trial care | #30 | Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation | N/A |
| Dissemination policy: trial results | #31a | Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions | 15 |
| Dissemination policy: authorship | #31b | Authorship eligibility guidelines and any intended use of professional writers | N/A |
| Dissemination policy: reproducible research | #31c | Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code | N/A |
| **Appendices** | | | |
| Informed consent materials | #32 | Model consent form and other related documentation given to participants and authorised surrogates | N/A |
| Biological specimens | #33 | Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable | N/A |