

BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email info.bmjopen@bmj.com

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

| | |
|-------------------------------|---|
| Journal: | <i>BMJ Open</i> |
| Manuscript ID | bmjopen-2021-056264 |
| Article Type: | Protocol |
| Date Submitted by the Author: | 12-Aug-2021 |
| Complete List of Authors: | Zhang, He; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery; Chinese Academy of Medical Sciences & Peking Union Medical College, Graduate School of Peking Union Medical College Li, Zhaomin; HELP Therapeutics Pan, Tuo; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery; Chinese Academy of Medical Sciences & Peking Union Medical College, Graduate School of Peking Union Medical College Zhu, xiyu; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery Tang, Xinlong; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery Xu, Can; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery Xue, Yunxing; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery Fan, Fudong; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery Cao, Hailong; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery Zhang, bomin; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery Pan, Jun; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery Zhou, Qing; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery Wang, Jiaxian; HELP Therapeutics Wang, Dong-Jin ; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery; Chinese Academy of Medical Sciences & Peking Union Medical College, Graduate School of Peking Union Medical College |
| Keywords: | Heart failure < CARDIOLOGY, Coronary heart disease < CARDIOLOGY, SURGERY |
| | |

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



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

4
5 He Zhang^{1,3#}, Zhaomin Li^{2#}, Tuo Pan^{1,3}, Xiyu Zhu¹, Xinlong Tang¹, Can Xu¹, Yunxing
6 Xue¹, Fudong Fan¹, Hailong Cao¹, Bomin Zhang¹, Jun Pan¹, Qing Zhou¹, Jiaxian
7 Wang^{2*}, and Dongjin Wang^{1,3*}

8
9 **Affiliations:**

- 10 1. Department of Cardio-Thoracic Surgery, Nanjing Drum Tower Hospital, The
11 Affiliated Hospital of Nanjing University Medical School, Nanjing, Jiangsu, 210008,
12 China.
13 2. HELP Therapeutics, Nanjing, Jiangsu, 211166, China.
14 3. Chinese Academy of Medical Sciences& Peking Union Medical College, Graduate
15 School of Peking Union Medical College, Beijing, 100010, China.

16
17 **E-mail address:**

- 18 He Zhang: pumczhanghe@163.com
19 Zhaomin Li: lizm@helptx.com.cn
20 Tuo Pan: pan_tuo@126.com
21 Xiyu Zhu: zhuxy_nju@163.com
22 Xinlong Tang: jstangxinlong@126.com
23 Can Xu: skytiankong1023@smail.nju.edu.cn
24 Yunxing Xue: albert_xue@163.com
25 Fudong Fan: ffd19610169@126.com
26 Hailong Cao: 13675186233@163.com

1
2
3
4 27 Bomin Zhang: zhangbomin_gl@163.com
5

6
7 28 Jun Pan: pj791028@163.com
8

9 29 Qing Zhou: zhouqing_penn@163.com
10

11 30 Jiaxian Wang: wangjx@helptx.com.cn
12

13
14 31 Dongjin Wang: dongjin_wang@126.com
15

16 32
17

18 33
19

20 34 #, these authors contributed equally to this work.
21

22 35 *, Correspondence to Dong-Jin Wang, Department of Cardio-thoracic Surgery, Nanjing
23

24 36 Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School,
25

26 37 Nanjing, Jiangsu, 210008, China. E-mail: dongjin_wang@126.com; Jiaxian Wang,
27

28 38 HELP Therapeutics, Nanjing, Jiangsu, 211166, China. E-mail: wangjx@helptx.com.cn
29

30 39
31

32 40
33

34 41
35

36 42
37

38 43
39

40 44
41

42 45
43

44 46
45

46 47
47

48 48
49

50 49
51

52 50
53

54
55
56
57
58
59
60

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 (1×10^8 , 2×10^8 and 4×10^8 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

1
2
3 82 **Strengths and limitations of this study**
4
5

- 6 83 ● This study is the first dose-escalation and placebo-controlled trial for the patients
7
8 84 with advanced ischemic heart failure treated with epicardial injection of hiPSC-
9
10 85 CMs during CABG surgery.
11
12 86 ● The results are expected to assess the safety of hiPSC-CMs by dose-escalation, as
13
14 87 well as the efficacy of epicardial injection of hiPSC-CMs and CABG surgery
15
16 88 combination by comparison with the CABG surgery alone.
17
18 89 ● As a phase I trial, the sample size is small, which limited the power of observation.
19
20

21 90

22
23 91

24
25 92

26
27 93

28
29 94

30
31 95

32
33 96

34
35 97

36
37 98

38
39 99

40 100

41
42 101

43
44 102

45
46 103

47
48 104

49
50 105

51
52 106

53
54 107

55
56 108

57
58 109

59
60 110

111 INTRODUCTION

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

123 The key pathogenesis of IHD is that loss of these cardiomyocytes results in an
124 irreversible impairment of cardiac function. Unfortunately, the adult mammalian heart
125 has a very limited capability to regenerate after cardiac injury [8~10]. Therefore,
126 transplantation of cardiomyocytes is reasonable and promising to improve cardiac
127 function by remuscularization [11~13]. Human embryonic stem cells (hESCs) and
128 induced pluripotent stem cells (hiPSCs) can differentiate into cardiomyocytes of high
129 purity [14~16]. However, the clinical application of hESCs faces problems such as
130 limited supply and ethical controversy [17]. In contrast, hiPSCs are derived from adult
131 somatic cells (peripheral blood mononuclear cells, skin fibroblasts, etc.) through
132 reprogramming, which overcomes the supply limit barrier and avoids the ethical issues
133 [18, 19]. Therefore, hiPSCs could be an ideal source for in vitro differentiated
134 cardiomyocytes as the next generation cell therapy for HF.

135 The First-In-Human (FIH) study involving the transplantation of hESCs-derived
136 cardiac progenitor cells was completed in 2015 by Menasché *et al* in patients suffering
137 from severe ischemic left ventricular dysfunction [20]. A subsequent clinical report
138 from the same team further suggested that transplantation of these cells was safe and
139 potentially promoted some functional recovery in the transplanted myocardial areas
140 [21]. Because of the mentioned limitations of hESCs, hiPSCs-derived cardiomyocytes

1
2
3
4 141 have been investigated in rat [22], pig [23] and non-human primate [24] models and
5
6 142 shown to restore cardiac function. In addition, based on the FIH clinical trial of
7
8 143 epicardial injection of hiPSC-CMs during CABG surgery, “Treating Heart Failure With
9
10 144 hiPSC-CMs (HEAL-CHF)” (NCT03763136), no serious adverse event, such as
11
12 145 mortality or tumorigenicity, related to the epicardial injection of 1×10^8 hiPSC-CMs
13
14 146 during CABG surgery was reported during the 24-month follow-up investigation.
15
16 147 Therefore, we designed this clinical trial to further evaluate the safety and efficacy of
17
18 148 epicardial injection of allogeneic hiPSC-CMs in patients with advanced ischemic HF
19
20 149 during CABG surgery by comparison with CABG surgery alone.
21
22 150

23 151 **METHODS AND ANALYSIS**

24 152 **Study design**

25
26
27 153 This study is a dose-escalation, placebo-controlled, single-blinded, single-centre phase
28
29 154 I clinical trial. An overview of the modified 3+3 dose-escalation trial is presented in
30
31 155 Figure 1. The primary endpoint is to assess the safety of the epicardial injection of
32
33 156 allogeneic hiPSC-CMs in the treatment of patients with advanced IHF during CABG
34
35 157 surgery. The secondary endpoint is to evaluate the efficacy of epicardial injection of
36
37 158 hiPSC-CMs and CABG surgery combination by comparison with the CABG surgery
38
39 159 alone.

40 160 **Study population**

41
42 161 Patients with advanced chronic HF secondary to ischemic heart disease fulfilling all
43
44 162 inclusion / exclusion criteria will be enrolled at Nanjing Drum Tower Hospital, the
45
46 163 affiliated hospital of Nanjing University Medical School, China. The study will be
47
48 164 conducted in compliance with the requirements of governmental regulatory bodies and
49
50 165 ethics committees.

51 166 **Inclusion criteria**

- 52 167 1. Patients aged 35-75 years
- 53 168 2. Have signed the Informed Consent Form (ICF).
- 54 169 3. Patients have chronic left ventricular dysfunction.
- 55 170 4. Patients have New York Heart Association (NYHA) Functional Classification III-

- 1
2
3
4 171 IV despite optimal standard of care.
5
6 172 5. Patients have indications for CABG surgery.
7
8 173 6. LVEF $\leq 35\%$ as determined by echocardiogram (data collected up to 6 months
9
10 174 prior to inclusion evaluation are valid, excluding the measured values within 1
11
12 175 month of myocardial infarction).
13
14 176 7. Weakening or absence of segmental regional wall motion as determined by
15
16 177 standard imaging.
17
18 178

19 179 ***Exclusion criteria***

- 20
21 180 1. Patient received implantable cardioverter-defibrillator (ICD), cardiac
22
23 181 resynchronization therapy (CRT), left ventricular assist device surgery or similar
24
25 182 treatment.
26
27 183 2. Patients with nonischemic cardiomyopathy, viral myocarditis, left ventricular
28
29 184 aneurysm / thrombus, untreated congenital heart disease, primary significant
30
31 185 organic valvular heart disease (with specified dimensions), pericardial disorders /
32
33 186 pericarditis, cerebrovascular disease and/or peripheral vascular disease.
34
35 187 3. In process of being evaluated for heart transplant.
36
37 188 4. Patients screened less than 1 month after the onset of myocardial infarction or
38
39 189 PCI.
40
41 190 5. Patients having previously suffered from sustained ventricular tachycardia, atrial
42
43 191 fibrillation, conduction abnormalities (including bundle branch block), or sudden
44
45 192 cardiac death.
46
47 193 6. Panel Reactive antibody (PRA) $\geq 20\%$ or Donor-specific Antibody (DSA)
48
49 194 positive. Autoimmune disorders related the higher risk of immune rejection.
50
51 195 7. Baseline glomerular filtration rate $< 30\text{ml}/\text{min}/1.73\text{m}^2$.
52
53 196 8. Liver dysfunction, as evidenced by enzymes (AST and ALT) greater than three
54
55 197 times the upper limit of normal (ULN).
56
57 198 9. Hematological abnormality: A hematocrit $< 25\%$ as determined by HCT, white
58
59 199 blood cell $< 2500/\mu\text{l}$ or platelet values $< 100000/\mu\text{l}$. Coagulopathy (INR > 1.3) not
60

- 1
2
3
4 200 due to a reversible cause (e.g., warfarin and/or Factor Xa inhibitors).
5
6 201 10. Serious radiographic contrast allergy, penicillin allergy, streptomycin allergy.
7
8 202 11. Contra-indication to performance of a magnetic resonance imaging (MRI) scan.
9
10 203 12. Recipients of organ transplant.
11
12 204 13. Clinical history of malignancy within 5 years (patients with prior malignancy
13 must be disease-free for 5 years).
14
15 206 14. Non-cardiac condition that limits lifespan <1 year.
16
17 207 15. On chronic therapy with immunosuppressant medication, such as glucocorticoid
18 and TNF α antagonist.
19
20 209 16. Contra-indication to take immunosuppressant medication
21
22 210 17. Serum positive for Human Immunodeficiency Virus (HIV), Hepatitis B Virus
23 (HBV), Hepatitis C Virus (HCV), or Treponema Pallidum (TP).
24
25 211
26 212 18. Currently enrolled in another investigational therapeutic or device study.
27
28 213 19. Patients who are pregnant or breast feeding.
29
30 214 20. Patients suffering from Orthopedic or Spinal/Neurological Disorders who may
31 have limited ability to participate in post CABG rehabilitation programs or
32 perform efficacy assessments (such as 6 Minute Walk Test)
33
34 216
35 217 21. Patients with amyloidosis
36
37
38 218 22. Other conditions that researchers consider not suitable to participate in this study.
39
40
41

42 220 **Randomization and Groups**

43
44 221 Six patients will be enrolled and randomly allocated to CABG +1 \times 10⁸ cells group or
45 CABG group. Randomization will be similarly applied for the 2 \times 10⁸ cells and 4 \times 10⁸
46 222 cells patient groups (Figure 1).
47
48 223
49
50 224

51 225 **Intervention**

52 226 **1. Screening and Baseline Phase**

53
54 227 See Table 1 for the schedule and assessments to be performed during this phase I
55 clinical trial. Subjects fulfilling all inclusion / exclusion criteria and who have signed
56 228 the Informed Consent Form will be enrolled. Baseline information and data required
57
58
59 229
60

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

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

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

239 **3. Dose and treatment method**

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

247 If no grade 2 or above adverse event occurs within 1 month post-operatively in the
248 CABG + 1×10^8 group, dose escalation will proceed to 2×10^8 cells. If one grade 2 or
249 above adverse event occurs within 1 month post-operatively, three more patients will
250 be enrolled and injected with 1×10^8 cells during CABG surgery. If no grade 2 or above
251 adverse event occurs in the second three-patient cohort, dose escalation will then
252 proceed to 2×10^8 cells. Otherwise, the trial will be stopped. The dose escalation design
253 is depicted in Figure 1.

254 **4. Prohibited drugs**

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

- 257 1) 2.5g of immunoglobulin will be injected intravenously 1 day pre-operatively, on
258 the day of surgery and 3 days post-operatively, respectively.

- 1
2
3
4 259 2) 500mg of methylprednisolone will be injected intravenously 1 day pre-
5
6 260 operatively.
7
8 261 3) 20mg of Simulect® (Basiliximab for Injection) will be injected intravenously on
9
10 262 the day of surgery and 4 days post-operatively, respectively.
11
12 263 4) 1g of mycophenolate mofetil (oral) will be given 1 day pre-operatively and
13
14 264 subsequently at the dose of 1.5g for 28 days post-operatively.
15
16 265 5) 20mg of Prednisone (oral) will be given daily for 28 days post-operatively.
17
18 266 6) Tacrolimus (oral) will be given from 3 days pre-operatively to 28 days post-
19
20 267 operatively, and the dose will be adjusted according to the drug concentration in
21
22 268 subject's blood, with a target blood concentration of 3-5ng/ml.

23
24 269 **5. Day 1~21 post-operatively**

25
26 270 See Table 1 for the schedule and assessments to be performed during this phase. Key
27
28 271 assessments to be performed include vital sign evaluation, ECG-based evaluation of
29
30 272 arrhythmia and laboratory tests including blood routine and biochemistry, cardiac
31
32 273 injury markers (NT-Pro-BNP, cardiac troponin, cardiac enzymes, etc.), cytokines (IFN γ ,
33
34 274 TNF α , IL-2, IL-6 and IL-10), PRA and DSA.

35
36 275 **6. Month 1~12 Visit**

37
38 276 See Table 1 for the schedule and assessments to be performed during this period.
39
40 277 Outpatient visits should be completed as close to the scheduled visit dates as possible.
41
42 278 The visit window is \pm 7 days from the intended date of the visit (1, 3, 6 and 12 months
43
44 279 post-operatively). Key assessments to be performed include vital sign evaluation, ECG-
45
46 280 based evaluation of arrhythmia, echocardiogram-based and MRI-based cardiac
47
48 281 function evaluation, NYHA Classification, 6 min walk test, chest and abdominal PET
49
50 282 scan, and laboratory tests including blood routine and biochemistry, cardiac injury
51
52 283 markers (NT-Pro-BNP, cardiac troponin, cardiac enzymes, etc.), cytokines (IFN γ ,
53
54 284 TNF α , IL-2, IL-6 and IL-10), PRA, DSA and tumor markers. Subjects will also fill the
55
56 285 Minnesota Living with Heart Failure Questionnaire (MLHFQ).

57 286

58 287 **Endpoints**

59 288 1) **Safety**
60

1
2
3
4 289 (1) procedural complications; vital signs; changes in heart failure medications;
5
6 290 sustained ventricular arrhythmias, defined as ventricular arrhythmias lasting longer
7
8 291 than 30 seconds as recorded by Holter monitoring; newly formed tumor of allogeneic
9
10 292 origin (chest and abdominal CT at 1, 3, 6 months post-operatively, PET scan and 6
11
12 293 and 12 months post-operatively, histopathological analysis of any newly formed
13
14 294 tumor tissue);
15
16 295 (2) laboratory tests (including complete blood counts, comprehensive chemistry panels
17
18 296 with liver function tests, troponin I, creatinine kinase; PRA and DSA at 1, 3 and 6
19
20 297 months post-operatively); electrocardiogram; all-cause mortality, all-cause hospital
21
22 298 admission and need for heart failure co-intervention (Table 1).

23 299 **2) Preliminary efficacy**

24 300 (1) MRI-based evaluation of left ventricular function

25
26 301 At 1, 3, 6 and 12 months post-operatively, the proportion of infarcted myocardium, left
27
28 302 ventricular wall thickness at diastole, interventricular septum thickness, left ventricular
29
30 303 ejection fraction, left ventricular end-systolic and end-diastolic volumes, stroke volume,
31
32 304 cardiac output, myocardium density and left ventricular mass at diastole will be
33
34 305 evaluated.

35 306 (2) Echocardiogram-based evaluation of left ventricular function

36
37 307 At 1, 3, 6 and 12 months post-operatively, interventricular septum thickness at diastole,
38
39 308 left ventricular end-systolic and end-diastolic diameters, left ventricular posterior wall
40
41 309 thickness at diastole, left atrial diameter, left ventricular ejection fraction, mitral flow
42
43 310 pattern (E/A) will be evaluated and compared to baseline values.

44
45 311 (3) PET/CT based evaluation of myocardial perfusion at baseline, 6 months and 12
46
47 312 months post-operatively.

48
49 313 (4) 6 Minute Walk Test (baseline, 1, 3, 6 and 12 months post-operatively).

50
51 314 (5) NYHA Classification (baseline, 1, 3, 6 and 12 months post-operatively).

52
53 315 (6) Minnesota Living with Heart Failure Questionnaire (MLHFQ) (baseline, 1, 3, 6
54
55 316 and 12 months post-operatively).

56
57 317

58 318 **Statistical Considerations**

1
2
3
4 319 This is a phase I dose-escalation clinical trial. The sample size is estimated based on a
5
6 320 modified 3+3 design to achieve the primary endpoint. Sample size will be ranged from
7
8 321 6 to 27.

9 322 Descriptive statistical analysis will be used for the primary and secondary endpoints.
10
11 323 The 95% confidence intervals of the frequency of developing ventricular tachycardia
12
13 324 sustained for >30 seconds and tumorigenesis due to allogeneic hiPSC-CMs will be
14
15 325 determined with the use of Miettinen's method.

16
17 326 Descriptive statistical analysis will be used for secondary endpoint. Depending on the
18
19 327 variables, different statistical methods will be used to compare the outcomes. For
20
21 328 measurement data, mean and standard deviation, median, maximum, minimum and
22
23 329 range will be calculated and presented. For enumeration data and rating data, frequency
24
25 330 (composition ratio), rate, and confidence interval will be calculated and presented.
26
27 331 Student's t-Test will be employed to determine the 95% confidence intervals of
28
29 332 enumeration data and rating data, while Miettinen's method will be employed to
30
31 333 determine the 95% confidence intervals of measurement data. Where appropriate,
32
33 334 differences between low dose and high dose groups will be calculated and significance
34
35 335 tests will be performed. A bilateral P value less than or equal to 0.05 is considered
36
37 336 significant.

38
39 337

40 338 **Data collection, management and monitoring**

41
42 339 The schedule of data collection is shown in Table 1. An electronic data capture (EDC)
43
44 340 system will be established for this study. A database manager (DM) will be appointed,
45
46 341 who will be responsible for the design of the EDC system. Data will be collected from
47
48 342 medical notes and hospital records in Nanjing Drum Tower Hospital. Before freezing
49
50 343 the database, the DM will compose the data validation report based on the study plan,
51
52 344 data validation standards and database contents. The Sponsor, Principal Investigator,
53
54 345 Statistician and DM should engage in a meeting to validate the data and come to a
55
56 346 resolution regarding database freezing. Once approved, the DM will be responsible for
57
58 347 the freezing of the database and the Statistician will conduct statistical analysis
59
60 348 afterwards.

1
2
3
4 349 Data monitoring and validation will be regularly conducted throughout the study. The
5
6 350 frequency of monitoring will be once a year by the Medical Ethics Committee of
7
8 351 Affiliated Nanjing Drum Tower Hospital, Nanjing University Medical College, starting
9
10 352 from the beginning of this study.

11 353

13 354 **Quality control**

15 355 The clinical trial investigators will implement a quality assurance and quality control
16
17 356 system based on the standard operating procedures prescribed by the investigators.
18
19 357 Implementation of clinical trial, data creation, recording, monitoring and reporting will
20
21 358 be conducted in compliance with “Administrative Measures for Clinical Studies of
22
23 359 Stem Cell-based Therapeutics”. The study will be monitored by a third-party Data and
24
25 360 Safety Monitoring Board.

26
27 361

29 362 **Patient and public involvement**

31 363 Neither patients nor the public were involved in the development of the research
32
33 364 question, choice of outcome measures, design of the trial, recruitment of participants or
34
35 365 conduct of the trial. Results of the trial will be disseminated to study participants
36
37 366 through direct consultation with a trial clinician at completion of the trial, as well as
38
39 367 through the publication of results.

40 368

42 369 **DISCUSSION**

43 370 Loss of cardiomyocytes in the myocardium contributes to severe impairment of cardiac
44
45 371 function and may lead to heart failure. The implantation of cardiomyocytes presents an
46
47 372 alternative treatment to heart transplantation [11~13]. After a roll-in experience as part
48
49 373 of the “Treating Heart Failure With hPSC-CMs (HEAL-CHF)” study (NCT03763136),
50
51 374 we now initiate a dose-escalation trial to evaluate the safety and efficacy of epicardial
52
53 375 injection of hiPSC-CMs during CABG surgery in patients with advanced chronic heart
54
55 376 failure. This study will be undertaken with sufficient safety considerations and based
56
57 377 on the implementation plan and relevant laws. This clinical trial will shed the light on
58
59 378 the hiPSC-CMs cell therapy for the unmet clinical needs for advanced heart failure
60

1
2
3
4 379 patients.

5 380

6
7 381 **ETHICS AND DISSEMINATION**

8
9 382 The study protocol has been approved by the Medical Ethics Committee of Affiliated
10
11 383 Nanjing Drum Tower Hospital, Nanjing University Medical College
12
13 384 (No.SC202000102) in May 2020. Participants and their guardians (where applicable)
14
15 385 have the right to withdraw at any time and if they do withdraw, will be treated according
16
17 386 to hospital standard procedures. Participants who choose to withdraw from the trial will
18
19 387 be asked if we can continue to use any data already collected and whether they are
20
21 388 willing to participate in the trial follow-up. We will present the trial findings at
22
23 389 international meetings and in peer-reviewed publications. We will inform the public
24
25 390 through patient organizations and a newsletter to participants.

26 391

27
28 392 **Acknowledgement**

29
30 393 The authors would like to thank Dr Ph Menasché for his help during preparation of this
31
32 394 manuscript.

33 395

34
35 396 **Author contributions**

36
37 397 D.W. and J.W. designed the whole protocol, reviewed and approved the paper. H. Z &
38
39 398 Z. L wrote the paper and prepared the Figure and Table. T.P, X.Z, X.T, C.X, Y.X, F.F,
40
41 399 H.C, B.Z, J.P & Q.Z reviewed the paper and provided valuable suggestion.

42
43 400 **Funding**

44 401 The dose escalation study is fully sponsored by Help Therapeutics.

45 402

46
47 403 **Competing interests**

48
49 404 The authors declare that they have no known competing financial interests or personal
50
51 405 relationships that could have appeared to influence the work reported in this paper.

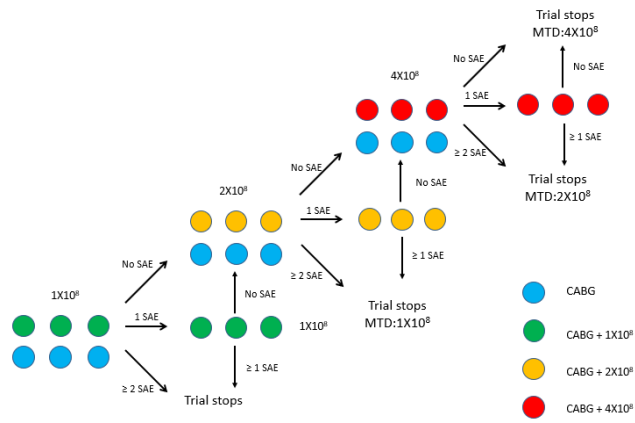
52
53 406

54
55 407 **REFERENCES**

- 56
57 408 1. Ziaeeian B, Fonarow G. Epidemiology and aetiology of heart failure. *Nature reviews*
58
59 409 *Cardiology* 2016;13(6):368-78.
60
410 2. Peng H, Abdel-Latif A. Cellular Therapy for Ischemic Heart Disease: An Update.

- 411 Advances in experimental medicine and biology 2019;1201:195-213.
- 412 3. Elgendy I, Mahtta D, Pepine C. Medical Therapy for Heart Failure Caused by
413 Ischemic Heart Disease. *Circulation research* 2019;124(11):1520-35.
- 414 4. Rana J, Khan S, Lloyd-Jones D, et al. Changes in Mortality in Top 10 Causes of
415 Death from 2011 to 2018. *Journal of general internal medicine* 2020
- 416 5. Rossignol P, Hernandez A, Solomon S, et al. Heart failure drug treatment. *Lancet*
417 (London, England) 2019;393(10175):1034-44.
- 418 6. Normand C, Kaye D, Povsic T, et al. Beyond pharmacological treatment: an insight
419 into therapies that target specific aspects of heart failure pathophysiology. *Lancet*
420 (London, England) 2019;393(10175):1045-55.
- 421 7. Willerson J. The Medical and Device-Related Treatment of Heart Failure.
422 *Circulation research* 2019;124(11):1519.
- 423 8. González A, Schelbert E, Díez J, et al. Myocardial Interstitial Fibrosis in
424 Heart Failure: Biological and Translational Perspectives. *Journal of the American*
425 *College of Cardiology* 2018;71(15):1696-706.
- 426 9. Uygur A, Lee R. Mechanisms of Cardiac Regeneration. *Developmental cell*
427 2016;36(4):362-74.
- 428 10. Ponnusamy M, Liu F, Zhang Y, et al. Long Noncoding RNA CPR
429 (Cardiomyocyte Proliferation Regulator) Regulates Cardiomyocyte Proliferation and
430 Cardiac Repair. *Circulation* 2019;139(23):2668-84.
- 431 11. Bertero A, Murry C. Hallmarks of cardiac regeneration. *Nature reviews*
432 *Cardiology* 2018;15(10):579-80.
- 433 12. Nakamura K, Murry C. Function Follows Form - A Review of Cardiac Cell
434 Therapy. *Circulation journal: official journal of the Japanese Circulation Society*
435 2019;83(12):2399-412.
- 436 13. Murry C, MacLellan W. Stem cells and the heart-the road ahead. *Science (New*
437 *York, NY)* 2020;367(6480):854-55.
- 438 14. Burridge P, Matsa E, Shukla P, et al. Chemically defined generation of human
439 cardiomyocytes. *Nature methods* 2014;11(8):855-60.
- 440 15. Lian X, Bao X, Zilberter M, et al. Chemically defined, albumin-free human

- 1
2
3
4 441 cardiomyocyte generation. *Nature methods* 2015;12(7):595-6.
5
6 442 16. Liu Y, Chen B, Yang X, et al. Human embryonic stem cell-derived
7
8 443 cardiomyocytes restore function in infarcted hearts of non-human primates. *Nature*
9
10 444 *biotechnology* 2018;36(7):597-605.
11
12 445 17. Ilic D, Ogilvie C. Concise Review: Human Embryonic Stem Cells-What Have We
13
14 446 Done? What Are We Doing? Where Are We Going? *Stem cells* (Dayton, Ohio)
15
16 447 2017;35(1):17-25.
17
18 448 18. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from
19
20 449 adult human fibroblasts by defined factors. *Cell* 2007;131(5):861-72.
21
22 450 19. Yu J, Vodyanik M, Smuga-Otto K, et al. Induced pluripotent stem cell lines
23
24 451 derived from human somatic cells. *Science* 2007; 318(5858): 1917-20.
25
26 452 20. Menasché P, Vanneaux V, Hagege A, et al. Human embryonic stem cell-derived
27
28 453 cardiac progenitors for severe heart failure treatment: first clinical case report.
29
30 454 *European heart journal* 2015;36(30):2011-7.
31
32 455 21. Menasché P, Vanneaux V, Hagege A, et al. Transplantation of Human Embryonic
33
34 456 Stem Cell-Derived Cardiovascular Progenitors for Severe Ischemic Left Ventricular
35
36 457 Dysfunction. *Journal of the American College of Cardiology* 2018;71(4):429-38.
37
38 458 22. Guan X, Xu W, Zhang H, et al. Transplantation of human induced pluripotent
39
40 459 stem cell-derived cardiomyocytes improves myocardial function and reverses
41
42 460 ventricular remodeling in infarcted rat hearts. *Stem cell research & therapy*
43
44 461 2020;11(1):73.
45
46 462 23. Kawamura M, Miyagawa S, Miki K, et al. Feasibility, safety, and therapeutic
47
48 463 efficacy of human induced pluripotent stem cell-derived cardiomyocyte sheets in a
49
50 464 porcine ischemic cardiomyopathy model. *Circulation* 2012;126:S29-37.
51
52 465 24. Shiba Y, Gomibuchi T, Seto T, et al. Allogeneic transplantation of iPS cell-
53
54 466 derived cardiomyocytes regenerates primate hearts. *Nature* 2016; 538(7625): 388-91.
55
56 467
57 468 **Figure Legend**
58 469 Figure 1. The modified 3+3 dose escalation study design. (SAE, Serious Adverse
59
60 470 Event; CABG, Coronary Artery Bypass Graft; MTD, Maximum Tolerated Dose.)



For peer review only

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.



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 |

| | | | |
|----|--------------------------|---|-------|
| 1 | | assessing outcomes) and how | |
| 2 | | 11b If relevant, description of the similarity of interventions | NA |
| 3 | Statistical methods | 12a Statistical methods used to compare groups for primary and secondary outcomes | 11-12 |
| 4 | | 12b Methods for additional analyses, such as subgroup analyses and adjusted analyses | NA |
| 5 | | | |
| 6 | Results | | |
| 7 | Participant flow (a | 13a For each group, the numbers of participants who were randomly assigned, received intended treatment, and | 10-12 |
| 8 | diagram is strongly | were analysed for the primary outcome | |
| 9 | recommended) | 13b For each group, losses and exclusions after randomisation, together with reasons | NA |
| 10 | Recruitment | 14a Dates defining the periods of recruitment and follow-up | NA |
| 11 | | 14b Why the trial ended or was stopped | 12 |
| 12 | Baseline data | 15 A table showing baseline demographic and clinical characteristics for each group | NA |
| 13 | Numbers analysed | 16 For each group, number of participants (denominator) included in each analysis and whether the analysis was | |
| 14 | | by original assigned groups | 12 |
| 15 | Outcomes and | 17a For each primary and secondary outcome, results for each group, and the estimated effect size and its | |
| 16 | estimation | precision (such as 95% confidence interval) | 12 |
| 17 | | 17b For binary outcomes, presentation of both absolute and relative effect sizes is recommended | 12 |
| 18 | Ancillary analyses | 18 Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing | |
| 19 | | pre-specified from exploratory | NA |
| 20 | Harms | 19 All important harms or unintended effects in each group (for specific guidance see CONSORT for harms) | NA |
| 21 | | | |
| 22 | Discussion | | |
| 23 | Limitations | 20 Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses | 13 |
| 24 | Generalisability | 21 Generalisability (external validity, applicability) of the trial findings | 13 |
| 25 | Interpretation | 22 Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence | NA |
| 26 | | | |
| 27 | Other information | | |
| 28 | Registration | 23 Registration number and name of trial registry | 3 |
| 29 | Protocol | 24 Where the full trial protocol can be accessed, if available | 3 |
| 30 | Funding | 25 Sources of funding and other support (such as supply of drugs), role of funders | 14 |

36
37 *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
38 recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials.
39 Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.
40
41
42

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

| | |
|---------------------------------|---|
| Journal: | <i>BMJ Open</i> |
| Manuscript ID | bmjopen-2021-056264.R1 |
| Article Type: | Protocol |
| Date Submitted by the Author: | 04-Jan-2022 |
| Complete List of Authors: | Zhang, He; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery; Chinese Academy of Medical Sciences & Peking Union Medical College, Graduate School of Peking Union Medical College Li, Zhaomin; HELP Therapeutics Pan, Tuo; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery; Chinese Academy of Medical Sciences & Peking Union Medical College, Graduate School of Peking Union Medical College Zhu, xiyu; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery Tang, Xinlong; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery Xu, Can; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery Xue, Yunxing; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery Fan, Fudong; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery Cao, Hailong; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery Zhang, bomin; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery Pan, Jun; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery Zhou, Qing; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery Wang, Jiaxian; HELP Therapeutics Wang, Dong-Jin ; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery; Chinese Academy of Medical Sciences & Peking Union Medical College, Graduate School of Peking Union Medical College |
| Primary Subject Heading: | Cardiovascular medicine |
| Secondary Subject Heading: | Cardiovascular medicine, Surgery |

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

SCHOLARONE™
Manuscripts

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

4
5 He Zhang^{1,3#}, Zhaomin Li^{2#}, Tuo Pan^{1,3}, Xiyu Zhu¹, Xinlong Tang¹, Can Xu¹, Yunxing
6 Xue¹, Fudong Fan¹, Hailong Cao¹, Bomin Zhang¹, Jun Pan¹, Qing Zhou¹, Jiaxian
7 Wang^{2*}, and Dongjin Wang^{1,3*}

8
9 **Affiliations:**

- 10 1. Department of Cardio-Thoracic Surgery, Nanjing Drum Tower Hospital, The
11 Affiliated Hospital of Nanjing University Medical School, Nanjing, Jiangsu, 210008,
12 China.
13 2. HELP Therapeutics, Nanjing, Jiangsu, 211166, China.
14 3. Chinese Academy of Medical Sciences& Peking Union Medical College, Graduate
15 School of Peking Union Medical College, Beijing, 100010, China.

16
17 **E-mail address:**

- 18 He Zhang: pumczhanghe@163.com
19 Zhaomin Li: lizm@helptx.com.cn
20 Tuo Pan: pan_tuo@126.com
21 Xiyu Zhu: zhuxy_nju@163.com
22 Xinlong Tang: jstangxinlong@126.com
23 Can Xu: skytiankong1023@smail.nju.edu.cn
24 Yunxing Xue: albert_xue@163.com
25 Fudong Fan: ffd19610169@126.com
26 Hailong Cao: 13675186233@163.com

1
2
3
4 27 Bomin Zhang: zhangbomin_gl@163.com
5

6
7 28 Jun Pan: pj791028@163.com
8

9 29 Qing Zhou: zhouqing_penn@163.com
10

11 30 Jiaxian Wang: wangjx@helptx.com.cn
12

13
14 31 Dongjin Wang: dongjin_wang@126.com
15

16 32
17

18 33
19

20 34 #, these authors contributed equally to this work.
21

22 35 *, Correspondence to Dong-Jin Wang, Department of Cardio-thoracic Surgery, Nanjing
23

24 36 Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School,
25

26 37 Nanjing, Jiangsu, 210008, China. E-mail: dongjin_wang@126.com; Jiaxian Wang,
27

28 38 HELP Therapeutics, Nanjing, Jiangsu, 211166, China. E-mail: wangjx@helptx.com.cn
29

30 39
31

32 40
33

34 41
35

36 42
37

38 43
39

40 44
41

42 45
43

44 46
45

46 47
47

48 48
49

50 49
51

52 50
53

54
55
56
57
58
59
60

1
2
3
4 51 **ABSTRACT**
5

6 52 **Introduction:** Heart failure (HF) is a growing global public health burden. However,
7
8 53 due to the very limited regenerative capacity of mature cardiomyocytes in the adult
9
10 54 mammalian heart, conventional treatments can only improve the symptoms of HF but
11
12 55 fail to restore cardiac function. Heart transplantation is limited by a severe shortage of
13
14 56 donors. Cell-based transplantation for the treatment of HF has become a promising
15
16 57 strategy. Human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs)
17
18 58 have been tested in animal models to assess safety and efficacy. This study aims at
19
20 59 evaluating the safety and efficacy of epicardial injection of hiPSC-CMs in patients with
21
22 60 advanced ischemic heart failure during Coronary Artery Bypass Grafting (CABG)
23
24 61 surgery.

25
26 62 **Methods:** This study is a dose-escalation, randomized control, single-blinded, single-
27
28 63 centre phase I clinical trial. Dose escalation will be guided by a modified 3+3 design
29
30 64 for 3 doses (1×10^8 , 2×10^8 and 4×10^8 cells, sequentially). Patients with advanced
31
32 65 ischemic heart failure will be enrolled and randomly allocated to receive epicardial
33
34 66 injection of hiPSC-CMs during CABG surgery or CABG surgery alone, followed by a
35
36 67 12-month follow-up investigation. The primary endpoint is to assess the safety of
37
38 68 hiPSC-CMs injection, including sustained ventricular arrhythmias, sudden unexpected
39
40 69 death and newly formed tumors during 6 months post-operatively. The secondary
41
42 70 endpoint is to evaluate the efficacy of epicardial injection of hiPSC-CMs and CABG
43
44 71 surgery combination by comparison with the CABG surgery alone.

45
46 72 **Ethics and dissemination:** The study protocol has been approved by the Institutional
47
48 73 Ethical Committee of Nanjing Drum Tower Hospital (No.SC202000102). Findings will
49
50 74 be disseminated to the academic community through peer-reviewed publications and
51
52 75 presentation at national and international meetings.

53
54 76 **Trial registration number:** NCT03763136

55
56 77 **Keywords:** Clinical Trial, Heart Failure, human induced Pluripotent Stem Cell derived
57
58 78 cardiomyocytes, Coronary Artery Bypass Grafting surgery

59
60 79 **Word Count: 2922**
80
81

1
2
3 82 **Strengths and limitations of this study**
4
5

- 6 83 ● This study is the first dose-escalation and randomized control trial for the patients
7
8 84 with advanced ischemic heart failure treated with epicardial injection of hiPSC-
9
10 85 CMs during CABG surgery.
11
12 86 ● The results are expected to assess the safety of hiPSC-CMs by dose-escalation, as
13
14 87 well as the efficacy of epicardial injection of hiPSC-CMs and CABG surgery
15
16 88 combination by comparison with the CABG surgery alone.
17
18 89 ● As a phase I trial, the sample size is small, which limited the power of observation.
19
20

21 90

22
23 91

24
25 92

26
27 93

28
29 94

30
31 95

32
33 96

34
35 97

36
37 98

38
39 99

40 100

41
42 101

43
44 102

45
46 103

47
48 104

49
50 105

51
52 106

53
54 107

55
56 108

57
58 109

59
60 110

111 INTRODUCTION

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

123 The key pathogenesis of IHD is that loss of these cardiomyocytes results in an
124 irreversible impairment of cardiac function. Unfortunately, the adult mammalian heart
125 has a very limited capability to regenerate after cardiac injury [8~10]. Therefore,
126 transplantation of cardiomyocytes is reasonable and promising to improve cardiac
127 function by remuscularization [11~13]. Human embryonic stem cells (hESCs) and
128 induced pluripotent stem cells (hiPSCs) can differentiate into cardiomyocytes of high
129 purity [14~16]. However, the clinical application of hESCs faces problems such as
130 limited supply and ethical controversy [17]. In contrast, hiPSCs are derived from adult
131 somatic cells (peripheral blood mononuclear cells, skin fibroblasts, etc.) through
132 reprogramming, which overcomes the supply limit barrier and avoids the ethical issues
133 [18, 19]. Therefore, hiPSCs could be an ideal source for in vitro differentiated
134 cardiomyocytes as the next generation cell therapy for HF.

135 The First-In-Human (FIH) study involving the transplantation of hESCs-derived
136 cardiac progenitor cells was completed in 2015 by Menasché *et al* in patients suffering
137 from severe ischemic left ventricular dysfunction [20]. A subsequent clinical report
138 from the same team further suggested that transplantation of these cells was safe and
139 potentially promoted some functional recovery in the transplanted myocardial areas
140 [21]. Because of the mentioned limitations of hESCs, hiPSCs-derived cardiomyocytes

1
2
3
4 141 have been investigated in rat [22], pig [23] and non-human primate [24] models and
5
6 142 shown to restore cardiac function. In addition, based on our first in human study, two
7
8 143 patients with advanced ischemic heart disease received epicardial injection of hiPSC-
9
10 144 CMs during CABG surgery, we observed ventricular tachycardia, supraventricular
11
12 145 tachycardia and atrial fibrillation in first two weeks. No serious adverse events, such as
13
14 146 mortality, malignant arrhythmia or tumorigenicity related to the epicardial injection of
15
16 147 1×10^8 hiPSC-CMs during 24 months follow-up after CABG surgery. Therefore, we
17
18 148 designed this clinical trial to further evaluate the safety and efficacy of epicardial
19
20 149 injection of allogeneic hiPSC-CMs in patients with advanced ischemic HF during
21
22 150 CABG surgery by comparison with CABG surgery alone.

23 151

24 152 **METHODS AND ANALYSIS**

25 153 **Study design**

26
27
28
29 154 This study is a dose-escalation, randomized control, single-blinded, single-centre phase
30
31 155 I clinical trial. An overview of the modified 3+3 dose-escalation trial is presented in
32
33 156 Figure 1. The primary endpoint is to assess the safety of the epicardial injection of
34
35 157 allogeneic hiPSC-CMs in the treatment of patients with advanced IHF during CABG
36
37 158 surgery. The secondary endpoint is to evaluate the efficacy of epicardial injection of
38
39 159 hiPSC-CMs and CABG surgery combination by comparison with the CABG surgery
40
41 160 alone.

42 161 **Study population**

43
44 162 Patients with advanced chronic HF secondary to ischemic heart disease fulfilling all
45
46 163 inclusion / exclusion criteria will be enrolled at Nanjing Drum Tower Hospital, the
47
48 164 affiliated hospital of Nanjing University Medical School, China. The study will be
49
50 165 conducted in compliance with the requirements of governmental regulatory bodies and
51
52 166 ethics committees.

53 167 **Inclusion criteria**

- 54
55
56 168 1. Patients aged 35-75 years
57
58 169 2. Have signed the Informed Consent Form (ICF).
59
60 170 3. Patients have chronic left ventricular dysfunction.

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

179

180 Exclusion criteria

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

- 1
2
3
4 200 blood cell < 2500/ μ l or platelet values < 100000/ μ l. Coagulopathy (INR > 1.3) not
5
6 201 due to a reversible cause (e.g., warfarin and/or Factor Xa inhibitors).
7
8 202 10. Serious radiographic contrast allergy, penicillin allergy, streptomycin allergy.
9
10 203 11. Contra-indication to performance of a magnetic resonance imaging (MRI) scan.
11
12 204 12. Recipients of organ transplant.
13
14 205 13. Clinical history of malignancy within 5 years (patients with prior malignancy
15
16 206 must be disease-free for 5 years).
17
18 207 14. Non-cardiac condition that limits lifespan < 1 year.
19
20 208 15. On chronic therapy with immunosuppressant medication, such as glucocorticoid
21
22 209 and TNF α antagonist.
23
24 210 16. Contra-indication to take immunosuppressant medication
25
26 211 17. Serum positive for Human Immunodeficiency Virus (HIV), Hepatitis B Virus
27
28 212 (HBV), Hepatitis C Virus (HCV), or Treponema Pallidum (TP).
29
30 213 18. Currently enrolled in another investigational therapeutic or device study.
31
32 214 19. Patients who are pregnant or breast feeding.
33
34 215 20. Patients suffering from Orthopedic or Spinal/Neurological Disorders who may
35
36 216 have limited ability to participate in post CABG rehabilitation programs or
37
38 217 perform efficacy assessments (such as 6 Minute Walk Test)
39
40 218 21. Patients with amyloidosis
41
42 219 22. Other conditions that researchers consider not suitable to participate in this study.
43
44 220

221 **Randomization and Groups**

222 Six patients will be enrolled and randomly allocated to CABG + 1×10^8 cells group or
223 CABG group. Randomization will be similarly applied for the 2×10^8 cells and 4×10^8
224 cells patient groups (Figure 1).
225

226 **Intervention**

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

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

1
2
3
4 230 the Informed Consent Form will be enrolled. Baseline information and data required
5
6 231 should be collected from all enrolled subjects within 4 weeks before the operation. Key
7
8 232 information and data to be collected include subject demographics, vital signs, lab tests,
9
10 233 cardiac function evaluation and immunological evaluation (HLA typing, determination
11
12 234 of PRA and DSA).

13 235 **2. preparation of hiPSC-CMs**

14
15 236 The allogeneic hiPSC-CMs were manufactured at Help Therapeutics under current
16
17 237 good manufacturing practice (cGMP) condition and cryopreserved after quality control
18
19 238 analysis [22]. The hiPSC-CMs will be thawed in a 37°C water bath (~2 minutes) and
20
21 239 resuspended in 5% human serum albumin solution before epicardial injection.

22 240 **3. Dose and treatment method**

23
24 241 Six patients will be enrolled and randomly allocated to CABG + 1×10^8 group or CABG
25
26 242 group (n=3 for each arm). For patients allocated to the CABG + 1×10^8 group, hiPSC-
27
28 243 CMs will be injected at approximately 10 sites (0.25~0.30 ml of cell suspension at each
29
30 244 site). Injection sites will be determined by surgeon based on patients' preoperative
31
32 245 CMR and wall motion abnormalities during CABG surgery. Details regarding injection
33
34 246 site location and volume of cell suspension injected at each site will be carefully
35
36 247 recorded. patients in CABG groups will receive standard CABG surgery alone. All
37
38 248 patients will be transferred to the intensive care unit (ICU) after surgery. See Table 1
39
40 249 for the schedule and assessments during this phase.

41
42 250 If no grade 2 or above adverse event occurs within 1 month post-operatively in the
43
44 251 CABG + 1×10^8 group, dose escalation will proceed to 2×10^8 cells. If one grade 2 or
45
46 252 above adverse event occurs within 1 month post-operatively, three more patients will
47
48 253 be enrolled and injected with 1×10^8 cells during CABG surgery. If no grade 2 or above
49
50 254 adverse event occurs in the second three-patient cohort, dose escalation will then
51
52 255 proceed to 2×10^8 cells. Otherwise, the trial will be stopped. The dose escalation design
53
54 256 is depicted in Figure 1.

55 257 **4. Prohibited drugs**

56
57 258 Subjects in the cell treatment groups will receive immunosuppressive treatment as
58
59
60

1
2
3
4 259 described below:

5 260 1) 2.5g of immunoglobulin will be injected intravenously 1 day pre-operatively, on
6
7 261 the day of surgery and 3 days post-operatively, respectively.

8
9 262 2) 500mg of methylprednisolone will be injected intravenously 1 day pre-
10
11 263 operatively.

12
13
14 264 3) 20mg of Simulect® (Basiliximab for Injection) will be injected intravenously on
15
16 265 the day of surgery and 4 days post-operatively, respectively.

17
18 266 4) 1g of mycophenolate mofetil (oral) will be given 1 day pre-operatively and
19
20 267 subsequently at the dose of 1.5g for 28 days post-operatively.

21 268 5) 20mg of Prednisone (oral) will be given daily for 28 days post-operatively.

22
23
24 269 6) Tacrolimus (oral) will be given from 3 days pre-operatively to 28 days post-
25
26 270 operatively, and the dose will be adjusted according to the drug concentration in
27
28 271 subject's blood, with a target blood concentration of 3-5ng/ml.

29
30 272 **5. Day 1~21 post-operatively**

31 273 See Table 1 for the schedule and assessments to be performed during this phase. Key
32
33 274 assessments to be performed include vital sign evaluation, ECG-based evaluation of
34
35 275 arrhythmia and laboratory tests including blood routine and biochemistry, cardiac
36
37 276 injury markers (NT-Pro-BNP, cardiac troponin, cardiac enzymes, etc.), cytokines (IFN γ ,
38
39 277 TNF α , IL-2, IL-6 and IL-10), PRA and DSA.

40
41 278 **6. Month 1~12 Visit**

42
43 279 See Table 1 for the schedule and assessments to be performed during this period.

44
45 280 Outpatient visits should be completed as close to the scheduled visit dates as possible.

46
47 281 The visit window is \pm 7 days from the intended date of the visit (1, 3, 6 and 12 months
48
49 282 post-operatively). Key assessments to be performed include vital sign evaluation, ECG-

50
51 283 based evaluation of arrhythmia, echocardiogram-based and MRI-based cardiac

52
53 284 function evaluation, NYHA Classification, 6 min walk test, chest and abdominal PET

54
55 285 scan, and laboratory tests including blood routine and biochemistry, cardiac injury

56
57 286 markers (NT-Pro-BNP, cardiac troponin, cardiac enzymes, etc.), cytokines (IFN γ ,

58
59 287 TNF α , IL-2, IL-6 and IL-10), PRA, DSA and tumor markers. Subjects will also fill the
60

1
2
3
4 288 Minnesota Living with Heart Failure Questionnaire (MLHFQ).

5 289

6 290 **Endpoints**

7
8 291 *1) Safety*

9
10 292 (1) procedural complications; vital signs; changes in heart failure medications;
11
12 293 sustained ventricular arrhythmias, defined as ventricular arrhythmias lasting longer
13
14 294 than 30 seconds as recorded by Holter monitoring; newly formed tumor of allogeneic
15
16 295 origin (chest and abdominal CT at 1, 3, 6 months post-operatively, PET scan and 6
17
18 296 and 12 months post-operatively, histopathological analysis of any newly formed
19
20 297 tumor tissue);

21
22 298 (2) laboratory tests (including complete blood counts, comprehensive chemistry panels
23
24 299 with liver function tests, troponin I, creatinine kinase; PRA and DSA at 1, 3 and 6
25
26 300 months post-operatively); electrocardiogram; all-cause mortality, all-cause hospital
27
28 301 admission and need for heart failure co-intervention (Table 1).

29 302 (1) *Exploratory efficacy data* MRI-based evaluation of left ventricular function

30
31 303 At 1, 3, 6 and 12 months post-operatively, the proportion of infarcted myocardium, left
32
33 304 ventricular wall thickness at diastole, interventricular septum thickness, left ventricular
34
35 305 ejection fraction, left ventricular end-systolic and end-diastolic volumes, stroke volume,
36
37 306 cardiac output, myocardium density and left ventricular mass at diastole will be
38
39 307 evaluated.

40
41 308 (2) Echocardiogram-based evaluation of left ventricular function

42
43 309 At 1, 3, 6 and 12 months post-operatively, interventricular septum thickness at diastole,
44
45 310 left ventricular end-systolic and end-diastolic diameters, left ventricular posterior wall
46
47 311 thickness at diastole, left atrial diameter, left ventricular ejection fraction, mitral flow
48
49 312 pattern (E/A) will be evaluated and compared to baseline values.

50
51 313 (3) PET/CT based evaluation of myocardial perfusion at baseline, 6 months and 12
52
53 314 months post-operatively.

54
55 315 (4) 6 Minute Walk Test (baseline, 1, 3, 6 and 12 months post-operatively).

56
57 316 (5) NYHA Classification (baseline, 1, 3, 6 and 12 months post-operatively).

58
59 317 (6) Minnesota Living with Heart Failure Questionnaire (MLHFQ) (baseline, 1, 3, 6
60

1
2
3
4 318 and 12 months post-operatively).

5 319

6
7 320 **Statistical Considerations**

8
9 321 This is a phase I dose-escalation clinical trial. The sample size is estimated based on a
10 322 modified 3+3 design to achieve the primary endpoint. Sample size will be ranged from
11
12 323 6 to 27.

13
14
15 324 Descriptive statistical analysis will be used for the primary and secondary endpoints.

16 325 The 95% confidence intervals of the frequency of developing ventricular tachycardia
17
18 326 sustained for >30 seconds and tumorigenesis due to allogeneic hiPSC-CMs will be
19
20 327 determined with the use of Miettinen's method.

21
22 328 Descriptive statistical analysis will be used for secondary endpoint. Depending on the
23
24 329 variables, different statistical methods will be used to compare the outcomes. For
25
26 330 measurement data, mean and standard deviation, median, maximum, minimum and
27
28 331 range will be calculated and presented. For enumeration data and rating data, frequency
29
30 332 (composition ratio), rate, and confidence interval will be calculated and presented.

31
32 333 Student's t-Test will be employed to determine the 95% confidence intervals of
33
34 334 enumeration data and rating data, while Miettinen's method will be employed to
35
36 335 determine the 95% confidence intervals of measurement data. Where appropriate,
37
38 336 differences between low dose and high dose groups will be calculated and significance
39
40 337 tests will be performed. A bilateral P value less than or equal to 0.05 is considered
41
42 338 significant.

43
44 339

45
46 340 **Data collection, management and monitoring**

47
48 341 The schedule of data collection is shown in Table 1. An electronic data capture (EDC)
49
50 342 system will be established for this study. A database manager (DM) will be appointed,
51
52 343 who will be responsible for the design of the EDC system. Data will be collected from
53
54 344 medical notes and hospital records in Nanjing Drum Tower Hospital. Before freezing
55
56 345 the database, the DM will compose the data validation report based on the study plan,
57
58 346 data validation standards and database contents. The Sponsor, Principal Investigator,
59
60 347 Statistician and DM should engage in a meeting to validate the data and come to a

1
2
3
4 348 resolution regarding database freezing. Once approved, the DM will be responsible for
5
6 349 the freezing of the database and the Statistician will conduct statistical analysis
7
8 350 afterwards.

9
10 351 Data monitoring and validation will be regularly conducted throughout the study. The
11
12 352 frequency of monitoring will be once a year by the Medical Ethics Committee of
13
14 353 Affiliated Nanjing Drum Tower Hospital, Nanjing University Medical College, starting
15
16 354 from the beginning of this study.

17
18 355

19 356 **Quality control**

20
21 357 The clinical trial investigators will implement a quality assurance and quality control
22
23 358 system based on the standard operating procedures prescribed by the investigators.
24
25 359 Implementation of clinical trial, data creation, recording, monitoring and reporting will
26
27 360 be conducted in compliance with “Administrative Measures for Clinical Studies of
28
29 361 Stem Cell-based Therapeutics”. The study will be monitored by a third-party Data and
30
31 362 Safety Monitoring Board.

32
33 363

34 364 **Patient and public involvement**

35
36 365 Neither patients nor the public were involved in the development of the research
37
38 366 question, choice of outcome measures, design of the trial, recruitment of participants or
39
40 367 conduct of the trial. Results of the trial will be disseminated to study participants
41
42 368 through direct consultation with a trial clinician at completion of the trial, as well as
43
44 369 through the publication of results.

45
46 370

47 371 **DISCUSSION**

48
49 372 Loss of cardiomyocytes in the myocardium contributes to severe impairment of cardiac
50
51 373 function and may lead to heart failure. The implantation of cardiomyocytes presents an
52
53 374 alternative treatment to heart transplantation [11~13]. After a roll-in experience as part
54
55 375 of the “Treating Heart Failure With hPSC-CMs (HEAL-CHF)” study (NCT03763136),
56
57 376 we now initiate a dose-escalation trial to evaluate the safety and efficacy of epicardial
58
59 377 injection of hiPSC-CMs during CABG surgery in patients with advanced chronic heart
60

1
2
3
4 378 failure. This study will be undertaken with sufficient safety considerations and based
5
6 379 on the implementation plan and relevant laws. This clinical trial will shed the light on
7
8 380 the hiPSC-CMs cell therapy for the unmet clinical needs for advanced heart failure
9
10 381 patients.

11 382 The allogeneic HiPSC-CMs were selected in this trial for following reasons: firstly, the
12
13 383 cost of autologous hIPSC-CMs is much higher than the allogeneic hIPSC-CMs.
14
15 384 Especially, it is not realistic for cardiac repair in a large patient populations; Secondly,
16
17 385 It is very time-consuming about 1 year for autologous HiPSC-CMs production, which
18
19 386 limited the clinical application for severe heart failure patients; Thirdly, the ideal
20
21 387 therapeutic HiPSC-CMs should be screened as healthy status. A potential genetic
22
23 388 susceptibility of heart failure is hard to excluded for every patient. So universal
24
25 389 allogeneic HiPSC-CMs were preferred in our trial. At last, the allogeneic HiPSC-CMs
26
27 390 derived from heathy donor with critical criteria was selected for clinical trial.

28
29 391

30 392 **ETHICS AND DISSEMINATION**

31
32 393 The study protocol has been approved by the Medical Ethics Committee of Affiliated
33
34 394 Nanjing Drum Tower Hospital, Nanjing University Medical College
35
36 395 (No.SC202000102) in May 2020. Participants and their guardians (where applicable)
37
38 396 have the right to withdraw at any time and if they do withdraw, will be treated according
39
40 397 to hospital standard procedures. Participants who choose to withdraw from the trial will
41
42 398 be asked if we can continue to use any data already collected and whether they are
43
44 399 willing to participate in the trial follow-up. We will present the trial findings at
45
46 400 international meetings and in peer-reviewed publications. We will inform the public
47
48 401 through patient organizations and a newsletter to participants.

49 402

50 403 **Acknowledgement**

51
52 404 The authors would like to thank Dr Ph Menasché for his help during preparation of this
53
54 405 manuscript.

55 406

56 407 **Author contributions**

57
58 408 D.W. and J.W. designed the whole protocol, reviewed and approved the paper. H. Z &
59
60

1
2
3
4 409 Z. L wrote the paper and prepared the Figure and Table. T.P, X.Z, X.T, C.X, Y.X, F.F,
5
6 410 H.C, B.Z, J.P & Q.Z reviewed the paper and provided valuable suggestion.

7
8 411 **Funding**

9
10 412 The dose escalation study is fully sponsored by Help Therapeutics.

11
12 413

13
14 414 **Competing interests**

15
16 415 The authors declare that they have no known competing financial interests or personal
17
18 416 relationships that could have appeared to influence the work reported in this paper.

19
20 417

21
22 418 **REFERENCES**

23
24 419 1. Ziaieian B, Fonarow G. Epidemiology and aetiology of heart failure. *Nature reviews*
25
26 420 *Cardiology* 2016;13(6):368-78.

27
28 421 2. Peng H, Abdel-Latif A. Cellular Therapy for Ischemic Heart Disease: An Update.

29
30 422 *Advances in experimental medicine and biology* 2019;1201:195-213.

31
32 423 3. Elgendy I, Mahtta D, Pepine C. Medical Therapy for Heart Failure Caused by

33
34 424 Ischemic Heart Disease. *Circulation research* 2019;124(11):1520-35.

35
36 425 4. Rana J, Khan S, Lloyd-Jones D, et al. Changes in Mortality in Top 10 Causes of

37
38 426 Death from 2011 to 2018. *Journal of general internal medicine* 2020

39
40 427 5. Rossignol P, Hernandez A, Solomon S, et al. Heart failure drug treatment. *Lancet*

41
42 428 (London, England) 2019;393(10175):1034-44.

43
44 429 6. Normand C, Kaye D, Povsic T, et al. Beyond pharmacological treatment: an insight

45
46 430 into therapies that target specific aspects of heart failure pathophysiology. *Lancet*

47
48 431 (London, England) 2019;393(10175):1045-55.

49
50 432 7. Willerson J. The Medical and Device-Related Treatment of Heart Failure.

51
52 433 *Circulation research* 2019;124(11):1519.

53
54 434 8. González A, Schelbert E, Díez J, et al. Myocardial Interstitial Fibrosis in

55
56 435 Heart Failure: Biological and Translational Perspectives. *Journal of the American*

57
58 436 *College of Cardiology* 2018;71(15):1696-706.

59
60 437 9. Uygur A, Lee R. Mechanisms of Cardiac Regeneration. *Developmental cell*

2016;36(4):362-74.

- 1
2
3
4 439 10. Ponnusamy M, Liu F, Zhang Y, et al. Long Noncoding RNA CPR
5
6 440 (Cardiomyocyte Proliferation Regulator) Regulates Cardiomyocyte Proliferation and
7
8 441 Cardiac Repair. *Circulation* 2019;139(23):2668-84.
- 9
10 442 11. Bertero A, Murry C. Hallmarks of cardiac regeneration. *Nature reviews*
11
12 443 *Cardiology* 2018;15(10):579-80.
- 13
14 444 12. Nakamura K, Murry C. Function Follows Form - A Review of Cardiac Cell
15
16 445 Therapy. *Circulation journal: official journal of the Japanese Circulation Society*
17
18 446 2019;83(12):2399-412.
- 19
20 447 13. Murry C, MacLellan W. Stem cells and the heart-the road ahead. *Science (New*
21
22 448 *York, NY)* 2020;367(6480):854-55.
- 23
24 449 14. Burrige P, Matsa E, Shukla P, et al. Chemically defined generation of human
25
26 450 cardiomyocytes. *Nature methods* 2014;11(8):855-60.
- 27
28 451 15. Lian X, Bao X, Zilberter M, et al. Chemically defined, albumin-free human
29
30 452 cardiomyocyte generation. *Nature methods* 2015;12(7):595-6.
- 31
32 453 16. Liu Y, Chen B, Yang X, et al. Human embryonic stem cell-derived
33
34 454 cardiomyocytes restore function in infarcted hearts of non-human primates. *Nature*
35
36 455 *biotechnology* 2018;36(7):597-605.
- 37
38 456 17. Ilic D, Ogilvie C. Concise Review: Human Embryonic Stem Cells-What Have We
39
40 457 Done? What Are We Doing? Where Are We Going? *Stem cells (Dayton, Ohio)*
41
42 458 2017;35(1):17-25.
- 43
44 459 18. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from
45
46 460 adult human fibroblasts by defined factors. *Cell* 2007;131(5):861-72.
- 47
48 461 19. Yu J, Vodyanik M, Smuga-Otto K, et al. Induced pluripotent stem cell lines
49
50 462 derived from human somatic cells. *Science* 2007; 318(5858): 1917-20.
- 51
52 463 20. Menasché P, Vanneaux V, Hagege A, et al. Human embryonic stem cell-derived
53
54 464 cardiac progenitors for severe heart failure treatment: first clinical case report.
55
56 465 *European heart journal* 2015;36(30):2011-7.
- 57
58 466 21. Menasché P, Vanneaux V, Hagege A, et al. Transplantation of Human Embryonic
59
60 467 Stem Cell-Derived Cardiovascular Progenitors for Severe Ischemic Left Ventricular
468 468 Dysfunction. *Journal of the American College of Cardiology* 2018;71(4):429-38.

- 1
2
3
4 469 22. Guan X, Xu W, Zhang H, et al. Transplantation of human induced pluripotent
5
6 470 stem cell-derived cardiomyocytes improves myocardial function and reverses
7
8 471 ventricular remodeling in infarcted rat hearts. *Stem cell research & therapy*
9
10 472 2020;11(1):73.
- 11
12 473 23. Kawamura M, Miyagawa S, Miki K, et al. Feasibility, safety, and therapeutic
13
14 474 efficacy of human induced pluripotent stem cell-derived cardiomyocyte sheets in a
15
16 475 porcine ischemic cardiomyopathy model. *Circulation* 2012;126:S29-37.
- 17
18 476 24. Shiba Y, Gomibuchi T, Seto T, et al. Allogeneic transplantation of iPS cell-
19
20 477 derived cardiomyocytes regenerates primate hearts. *Nature* 2016; 538(7625): 388-91.
21
22 478

479 **Figure Legend**

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

482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498

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 |

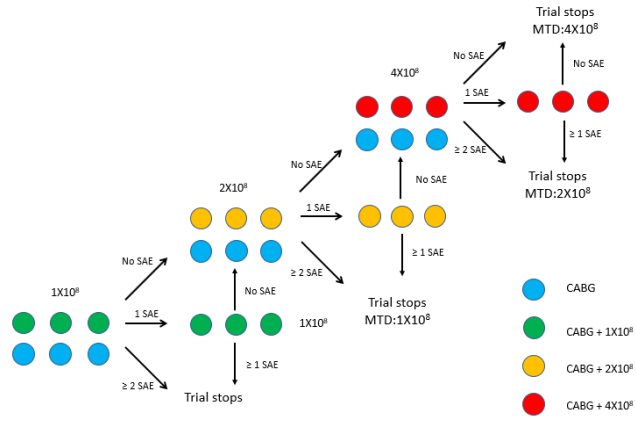
| | | | | | | | | | |
|---|---|---|----------|---|---|---|---|---|---|
| Tumor marker | X | | | | | X | X | X | X |
| Immunoassay (C3, C4, IgA, IgG, IgM) | X | | | X | | X | X | X | X |
| Infectious test | X | | | | | X | X | X | X |
| Coagulation function | X | | | X | X | X | X | X | X |
| HLA typing | 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.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46

For peer review only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



For peer review only

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 SPIRIT reporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. *BMJ*. 2013;346:e7586

| | | | Page |
|-----------------------------------|---------------------|--|--------|
| | | Reporting Item | 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 |

| | | | |
|----|--------------------------|--|-------|
| 1 | | name of intended registry | |
| 2 | | | |
| 3 | | | |
| 4 | Trial registration: data | #2b All items from the World Health Organization Trial | 4 |
| 5 | | | |
| 6 | set | Registration Data Set | |
| 7 | | | |
| 8 | | | |
| 9 | Protocol version | #3 Date and version identifier | 4 |
| 10 | | | |
| 11 | | | |
| 12 | Funding | #4 Sources and types of financial, material, and other support | 17 |
| 13 | | | |
| 14 | | | |
| 15 | Roles and | #5a Names, affiliations, and roles of protocol contributors | 2 |
| 16 | | | |
| 17 | responsibilities: | | |
| 18 | | | |
| 19 | contributorship | | |
| 20 | | | |
| 21 | | | |
| 22 | | | |
| 23 | Roles and | #5b Name and contact information for the trial sponsor | 2 |
| 24 | | | |
| 25 | responsibilities: | | |
| 26 | | | |
| 27 | sponsor contact | | |
| 28 | | | |
| 29 | information | | |
| 30 | | | |
| 31 | | | |
| 32 | | | |
| 33 | Roles and | #5c Role of study sponsor and funders, if any, in study design; | 17-18 |
| 34 | | | |
| 35 | responsibilities: | collection, management, analysis, and interpretation of | |
| 36 | | | |
| 37 | sponsor and funder | data; writing of the report; and the decision to submit the | |
| 38 | | | |
| 39 | | report for publication, including whether they will have | |
| 40 | | | |
| 41 | | ultimate authority over any of these activities | |
| 42 | | | |
| 43 | | | |
| 44 | | | |
| 45 | Roles and | #5d Composition, roles, and responsibilities of the coordinating | 14 |
| 46 | | | |
| 47 | responsibilities: | centre, steering committee, endpoint adjudication | |
| 48 | | | |
| 49 | committees | committee, data management team, and other individuals | |
| 50 | | | |
| 51 | | or groups overseeing the trial, if applicable (see Item 21a | |
| 52 | | | |
| 53 | | for data monitoring committee) | |
| 54 | | | |
| 55 | | | |
| 56 | | | |
| 57 | Introduction | | |
| 58 | | | |
| 59 | | | |
| 60 | | | |

| | | | | |
|----|---------------------------|---------------------|---|-----|
| 1 | Background and | #6a | Description of research question and justification for | 5-6 |
| 2 | | | | |
| 3 | rationale | | undertaking the trial, including summary of relevant studies | |
| 4 | | | (published and unpublished) examining benefits and harms | |
| 5 | | | for each intervention | |
| 6 | | | | |
| 7 | | | | |
| 8 | | | | |
| 9 | | | | |
| 10 | | | | |
| 11 | Background and | #6b | Explanation for choice of comparators | 6 |
| 12 | | | | |
| 13 | rationale: choice of | | | |
| 14 | | | | |
| 15 | comparators | | | |
| 16 | | | | |
| 17 | | | | |
| 18 | Objectives | #7 | Specific objectives or hypotheses | 6 |
| 19 | | | | |
| 20 | | | | |
| 21 | | | | |
| 22 | Trial design | #8 | Description of trial design including type of trial (eg, parallel | 6 |
| 23 | | | group, crossover, factorial, single group), allocation ratio, | |
| 24 | | | and framework (eg, superiority, equivalence, non-inferiority, | |
| 25 | | | exploratory) | |
| 26 | | | | |
| 27 | | | | |
| 28 | | | | |
| 29 | | | | |
| 30 | | | | |
| 31 | Methods: | | | |
| 32 | | | | |
| 33 | Participants, | | | |
| 34 | | | | |
| 35 | interventions, and | | | |
| 36 | | | | |
| 37 | outcomes | | | |
| 38 | | | | |
| 39 | | | | |
| 40 | | | | |
| 41 | Study setting | #9 | Description of study settings (eg, community clinic, | 4 |
| 42 | | | academic hospital) and list of countries where data will be | |
| 43 | | | collected. Reference to where list of study sites can be | |
| 44 | | | obtained | |
| 45 | | | | |
| 46 | | | | |
| 47 | | | | |
| 48 | | | | |
| 49 | | | | |
| 50 | | | | |
| 51 | Eligibility criteria | #10 | Inclusion and exclusion criteria for participants. If | 7-8 |
| 52 | | | applicable, eligibility criteria for study centres and | |
| 53 | | | individuals who will perform the interventions (eg, | |
| 54 | | | | |
| 55 | | | | |
| 56 | | | | |
| 57 | | | | |
| 58 | | | | |
| 59 | | | | |
| 60 | | | | |

| | | | |
|----|----------------------|---|-------|
| 1 | | surgeons, psychotherapists) | |
| 2 | | | |
| 3 | | | |
| 4 | Interventions: | #11a Interventions for each group with sufficient detail to allow | 9 |
| 5 | | | |
| 6 | description | replication, including how and when they will be | |
| 7 | | | |
| 8 | | administered | |
| 9 | | | |
| 10 | | | |
| 11 | Interventions: | #11b Criteria for discontinuing or modifying allocated | 11 |
| 12 | | | |
| 13 | modifications | interventions for a given trial participant (eg, drug dose | |
| 14 | | | |
| 15 | | change in response to harms, participant request, or | |
| 16 | | | |
| 17 | | improving / worsening disease) | |
| 18 | | | |
| 19 | | | |
| 20 | | | |
| 21 | Interventions: | #11c Strategies to improve adherence to intervention protocols, | N/A |
| 22 | | | |
| 23 | adherence | and any procedures for monitoring adherence (eg, drug | |
| 24 | | | |
| 25 | | tablet return; laboratory tests) | |
| 26 | | | |
| 27 | | | |
| 28 | | | |
| 29 | Interventions: | #11d Relevant concomitant care and interventions that are | N/A |
| 30 | | | |
| 31 | concomitant care | permitted or prohibited during the trial | |
| 32 | | | |
| 33 | | | |
| 34 | Outcomes | #12 Primary, secondary, and other outcomes, including the | 10-11 |
| 35 | | | |
| 36 | | specific measurement variable (eg, systolic blood | |
| 37 | | | |
| 38 | | pressure), analysis metric (eg, change from baseline, final | |
| 39 | | | |
| 40 | | value, time to event), method of aggregation (eg, median, | |
| 41 | | | |
| 42 | | proportion), and time point for each outcome. Explanation | |
| 43 | | | |
| 44 | | of the clinical relevance of chosen efficacy and harm | |
| 45 | | | |
| 46 | | outcomes is strongly recommended | |
| 47 | | | |
| 48 | | | |
| 49 | | | |
| 50 | | | |
| 51 | Participant timeline | #13 Time schedule of enrolment, interventions (including any | 21 |
| 52 | | | |
| 53 | | run-ins and washouts), assessments, and visits for | |
| 54 | | | |
| 55 | | participants. A schematic diagram is highly recommended | |
| 56 | | | |
| 57 | | (see Figure) | |
| 58 | | | |
| 59 | | | |
| 60 | | | |

| | | | | |
|----|----------------------------------|----------------------|--|----|
| 1 | 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 |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |
| 6 | | | | |
| 7 | | | | |
| 8 | | | | |
| 9 | | | | |
| 10 | | | | |
| 11 | Recruitment | #15 | Strategies for achieving adequate participant enrolment to reach target sample size | 12 |
| 12 | | | | |
| 13 | | | | |
| 14 | | | | |
| 15 | | | | |
| 16 | Methods: Assignment | | | |
| 17 | of interventions (for | | | |
| 18 | controlled trials) | | | |
| 19 | | | | |
| 20 | | | | |
| 21 | | | | |
| 22 | | | | |
| 23 | | | | |
| 24 | 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 |
| 25 | | | | |
| 26 | | | | |
| 27 | | | | |
| 28 | | | | |
| 29 | | | | |
| 30 | | | | |
| 31 | | | | |
| 32 | | | | |
| 33 | | | | |
| 34 | | | | |
| 35 | | | | |
| 36 | | | | |
| 37 | | | | |
| 38 | | | | |
| 39 | | | | |
| 40 | | | | |
| 41 | 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 |
| 42 | | | | |
| 43 | | | | |
| 44 | | | | |
| 45 | | | | |
| 46 | | | | |
| 47 | | | | |
| 48 | | | | |
| 49 | | | | |
| 50 | | | | |
| 51 | Allocation: implementation | #16c | Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions | 8 |
| 52 | | | | |
| 53 | | | | |
| 54 | | | | |
| 55 | | | | |
| 56 | | | | |
| 57 | | | | |
| 58 | | | | |
| 59 | | | | |
| 60 | | | | |

| | | | | |
|----|------------------------|----------------------|--|-------|
| 1 | Blinding (masking) | #17a | Who will be blinded after assignment to interventions (eg, | 8 |
| 2 | | | trial participants, care providers, outcome assessors, data | |
| 3 | | | analysts), and how | |
| 4 | | | | |
| 5 | | | | |
| 6 | | | | |
| 7 | | | | |
| 8 | Blinding (masking): | #17b | If blinded, circumstances under which unblinding is | 8 |
| 9 | emergency | | permissible, and procedure for revealing a participant's | |
| 10 | | | allocated intervention during the trial | |
| 11 | unblinding | | | |
| 12 | | | | |
| 13 | | | | |
| 14 | | | | |
| 15 | | | | |
| 16 | Methods: Data | | | |
| 17 | collection, | | | |
| 18 | management, and | | | |
| 19 | analysis | | | |
| 20 | | | | |
| 21 | | | | |
| 22 | | | | |
| 23 | | | | |
| 24 | | | | |
| 25 | | | | |
| 26 | Data collection plan | #18a | Plans for assessment and collection of outcome, baseline, | 12-13 |
| 27 | | | and other trial data, including any related processes to | |
| 28 | | | promote data quality (eg, duplicate measurements, training | |
| 29 | | | of assessors) and a description of study instruments (eg, | |
| 30 | | | questionnaires, laboratory tests) along with their reliability | |
| 31 | | | and validity, if known. Reference to where data collection | |
| 32 | | | forms can be found, if not in the protocol | |
| 33 | | | | |
| 34 | | | | |
| 35 | | | | |
| 36 | | | | |
| 37 | | | | |
| 38 | | | | |
| 39 | | | | |
| 40 | | | | |
| 41 | | | | |
| 42 | | | | |
| 43 | Data collection plan: | #18b | Plans to promote participant retention and complete follow- | N/A |
| 44 | retention | | up, including list of any outcome data to be collected for | |
| 45 | | | participants who discontinue or deviate from intervention | |
| 46 | | | protocols | |
| 47 | | | | |
| 48 | | | | |
| 49 | | | | |
| 50 | | | | |
| 51 | | | | |
| 52 | | | | |
| 53 | Data management | #19 | Plans for data entry, coding, security, and storage, | N/A |
| 54 | | | including any related processes to promote data quality | |
| 55 | | | (eg, double data entry; range checks for data values). | |
| 56 | | | | |
| 57 | | | | |
| 58 | | | | |
| 59 | | | | |
| 60 | | | | |

| | | | |
|----|----------------------------|--|-------|
| 1 | | Reference to where details of data management | |
| 2 | | | |
| 3 | | procedures can be found, if not in the protocol | |
| 4 | | | |
| 5 | | | |
| 6 | Statistics: outcomes | #20a Statistical methods for analysing primary and secondary | 13 |
| 7 | | | |
| 8 | | outcomes. Reference to where other details of the | |
| 9 | | | |
| 10 | | statistical analysis plan can be found, if not in the protocol | |
| 11 | | | |
| 12 | | | |
| 13 | Statistics: additional | #20b Methods for any additional analyses (eg, subgroup and | N/A |
| 14 | | | |
| 15 | analyses | adjusted analyses) | |
| 16 | | | |
| 17 | | | |
| 18 | Statistics: analysis | #20c Definition of analysis population relating to protocol non- | 12 |
| 19 | | | |
| 20 | population and | adherence (eg, as randomised analysis), and any statistical | |
| 21 | | | |
| 22 | missing data | methods to handle missing data (eg, multiple imputation) | |
| 23 | | | |
| 24 | | | |
| 25 | | | |
| 26 | Methods: Monitoring | | |
| 27 | | | |
| 28 | | | |
| 29 | Data monitoring: | #21a Composition of data monitoring committee (DMC); | 12-13 |
| 30 | | | |
| 31 | formal committee | summary of its role and reporting structure; statement of | |
| 32 | | | |
| 33 | | whether it is independent from the sponsor and competing | |
| 34 | | | |
| 35 | | interests; and reference to where further details about its | |
| 36 | | | |
| 37 | | charter can be found, if not in the protocol. Alternatively, an | |
| 38 | | | |
| 39 | | explanation of why a DMC is not needed | |
| 40 | | | |
| 41 | | | |
| 42 | | | |
| 43 | | | |
| 44 | Data monitoring: | #21b Description of any interim analyses and stopping | 14 |
| 45 | | | |
| 46 | interim analysis | guidelines, including who will have access to these interim | |
| 47 | | | |
| 48 | | results and make the final decision to terminate the trial | |
| 49 | | | |
| 50 | | | |
| 51 | Harms | #22 Plans for collecting, assessing, reporting, and managing | 11-12 |
| 52 | | | |
| 53 | | solicited and spontaneously reported adverse events and | |
| 54 | | | |
| 55 | | other unintended effects of trial interventions or trial | |
| 56 | | | |
| 57 | | | |
| 58 | | | |
| 59 | | | |
| 60 | | | |

| | | | |
|----|----------------------|--|-----|
| 1 | | conduct | |
| 2 | | | |
| 3 | | | |
| 4 | Auditing | #23 Frequency and procedures for auditing trial conduct, if any, | 13 |
| 5 | | and whether the process will be independent from | |
| 6 | | | |
| 7 | | | |
| 8 | | investigators and the sponsor | |
| 9 | | | |
| 10 | | | |
| 11 | Ethics and | | |
| 12 | | | |
| 13 | dissemination | | |
| 14 | | | |
| 15 | | | |
| 16 | Research ethics | #24 Plans for seeking research ethics committee / institutional | 13 |
| 17 | approval | review board (REC / IRB) approval | |
| 18 | | | |
| 19 | Protocol | #25 Plans for communicating important protocol modifications | N/A |
| 20 | amendments | (eg, changes to eligibility criteria, outcomes, analyses) to | |
| 21 | | relevant parties (eg, investigators, REC / IRBs, trial | |
| 22 | | participants, trial registries, journals, regulators) | |
| 23 | | | |
| 24 | Consent or assent | #26a Who will obtain informed consent or assent from potential | 9 |
| 25 | | trial participants or authorised surrogates, and how (see | |
| 26 | | Item 32) | |
| 27 | | | |
| 28 | | | |
| 29 | Consent or assent: | #26b Additional consent provisions for collection and use of | N/A |
| 30 | ancillary studies | participant data and biological specimens in ancillary | |
| 31 | | studies, if applicable | |
| 32 | | | |
| 33 | | | |
| 34 | Confidentiality | #27 How personal information about potential and enrolled | 15 |
| 35 | | participants will be collected, shared, and maintained in | |
| 36 | | order to protect confidentiality before, during, and after the | |
| 37 | | trial | |
| 38 | | | |
| 39 | Declaration of | #28 Financial and other competing interests for principal | 17 |
| 40 | | | |
| 41 | | | |
| 42 | | | |
| 43 | | | |
| 44 | | | |
| 45 | | | |
| 46 | | | |
| 47 | | | |
| 48 | | | |
| 49 | | | |
| 50 | | | |
| 51 | | | |
| 52 | | | |
| 53 | | | |
| 54 | | | |
| 55 | | | |
| 56 | | | |
| 57 | | | |
| 58 | | | |
| 59 | | | |
| 60 | | | |

| | | | | |
|----|-----------------------|----------------------|---|-----|
| 1 | interests | | investigators for the overall trial and each study site | |
| 2 | | | | |
| 3 | | | | |
| 4 | Data access | #29 | Statement of who will have access to the final trial dataset, | 18 |
| 5 | | | and disclosure of contractual agreements that limit such | |
| 6 | | | access for investigators | |
| 7 | | | | |
| 8 | | | | |
| 9 | | | | |
| 10 | | | | |
| 11 | Ancillary and post | #30 | Provisions, if any, for ancillary and post-trial care, and for | N/A |
| 12 | | | compensation to those who suffer harm from trial | |
| 13 | trial care | | participation | |
| 14 | | | | |
| 15 | | | | |
| 16 | | | | |
| 17 | | | | |
| 18 | | | | |
| 19 | Dissemination policy: | #31a | Plans for investigators and sponsor to communicate trial | 15 |
| 20 | | | results to participants, healthcare professionals, the public, | |
| 21 | trial results | | and other relevant groups (eg, via publication, reporting in | |
| 22 | | | results databases, or other data sharing arrangements), | |
| 23 | | | including any publication restrictions | |
| 24 | | | | |
| 25 | | | | |
| 26 | | | | |
| 27 | | | | |
| 28 | | | | |
| 29 | | | | |
| 30 | | | | |
| 31 | Dissemination policy: | #31b | Authorship eligibility guidelines and any intended use of | N/A |
| 32 | | | professional writers | |
| 33 | authorship | | | |
| 34 | | | | |
| 35 | | | | |
| 36 | Dissemination policy: | #31c | Plans, if any, for granting public access to the full protocol, | N/A |
| 37 | | | participant-level dataset, and statistical code | |
| 38 | reproducible research | | | |
| 39 | | | | |
| 40 | | | | |
| 41 | | | | |
| 42 | Appendices | | | |
| 43 | | | | |
| 44 | | | | |
| 45 | Informed consent | #32 | Model consent form and other related documentation given | N/A |
| 46 | | | to participants and authorised surrogates | |
| 47 | materials | | | |
| 48 | | | | |
| 49 | | | | |
| 50 | Biological specimens | #33 | Plans for collection, laboratory evaluation, and storage of | N/A |
| 51 | | | biological specimens for genetic or molecular analysis in | |
| 52 | | | the current trial and for future use in ancillary studies, if | |
| 53 | | | applicable | |
| 54 | | | | |
| 55 | | | | |
| 56 | | | | |
| 57 | | | | |
| 58 | | | | |
| 59 | | | | |
| 60 | | | | |

1 None The SPIRIT Explanation and Elaboration paper is distributed under the terms of the Creative
2 Commons Attribution License CC-BY-NC. This checklist can be completed online using
3 <https://www.goodreports.org/>, a tool made by the [EQUATOR Network](#) in collaboration with
4 [Penelope.ai](#)
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

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

| | |
|---------------------------------|--|
| Journal: | <i>BMJ Open</i> |
| Manuscript ID | bmjopen-2021-056264.R2 |
| Article Type: | Protocol |
| Date Submitted by the Author: | 24-Mar-2022 |
| Complete List of Authors: | Zhang, He; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery; Chinese Academy of Medical Sciences & Peking Union Medical College, Graduate School of Peking Union Medical College Xue, Yunxing; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery Pan, Tuo; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery; Chinese Academy of Medical Sciences & Peking Union Medical College, Graduate School of Peking Union Medical College Zhu, xiyu; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery Chong, Hoshun; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery Xu, Can; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery Fan, Fudong; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery Cao, Hailong; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery Zhang, bomin; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery Pan, Jun; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery Zhou, Qing; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery Yang, Gang; HELP Therapeutics Wang, Jiaxian; HELP Therapeutics Wang, Dong-Jin ; Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Department of Cardio-Thoracic Surgery; Chinese Academy of Medical Sciences & Peking Union Medical College, Graduate School of Peking Union Medical College |
| Primary Subject Heading: | Cardiovascular medicine |
| Secondary Subject Heading: | Cardiovascular medicine, Surgery |

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

SCHOLARONE™
Manuscripts

1
2
3
4 1 **Epicardial Injection of Allogeneic Human Induced-Pluripotent Stem**
5
6 2 **Cell-derived Cardiomyocytes in Patients with Advanced Heart Failure:**
7
8 3 **Protocol for a Phase I/IIa Dose-Escalation Clinical Trial**
9
10
11 4

13 5 He Zhang^{1,3}, Yunxing Xue¹, Tuo Pan^{1,3}, Xiyu Zhu¹, Hoshun Chong¹, Can Xu¹, Fudong
14 6 Fan¹, Hailong Cao¹, Bomin Zhang¹, Jun Pan¹, Qing Zhou¹, Gang Yang², Jiaxian Wang^{2*},
15 7 and Dongjin Wang^{1,3*}
16
17
18
19
20

21 9 **Affiliations:**

- 23 10 1. Department of Cardio-Thoracic Surgery, Nanjing Drum Tower Hospital, The
24 11 Affiliated Hospital of Nanjing University Medical School, Nanjing, Jiangsu, 210008,
25 12 China.
26 13 2. HELP Therapeutics, Nanjing, Jiangsu, 211166, China.
27 14 3. Chinese Academy of Medical Sciences& Peking Union Medical College, Graduate
28 15 School of Peking Union Medical College, Beijing, 100010, China.
29
30
31
32
33
34
35
36
37

38 17 **E-mail address:**

- 39 18 He Zhang: pumczhanghe@163.com
40
41 19 Yunxing Xue: albert_xue@163.com
42
43 20 Tuo Pan: pan_tuo@126.com
44
45 21 Xiyu Zhu: zhuxy_nju@163.com
46
47 22 Hoshun Chong: hoshunchong@163.com
48
49 23 Can Xu: skytiankong1023@smail.nju.edu.cn
50
51 24 Fudong Fan: ffd19610169@126.com
52
53 25 Hailong Cao: 13675186233@163.com
54
55 26 Bomin Zhang: zhangbomin_gl@163.com
56
57
58
59
60

1
2
3
4 27 Jun Pan: pj791028@163.com
5

6 28 Qing Zhou: zhouqing_penn@163.com
7
8

9 29 Gang Yang: yanggang201301@163.com
10

11 30 Jiaxian Wang: wangjx@helptx.com.cn
12
13

14 31 Dongjin Wang: dongjin_wang@126.com
15
16

17 32

18 33

19
20 34 *, Correspondence to Dong-Jin Wang, Department of Cardio-thoracic Surgery, Nanjing
21

22 35 Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School,
23

24 36 Nanjing, Jiangsu, 210008, China. E-mail: dongjin_wang@126.com; Jiaxian Wang,
25

26 37 HELP Therapeutics, Nanjing, Jiangsu, 211166, China. E-mail: wangjx@helptx.com.cn
27
28

29 38

30 39

31 40

32 41

33 42

34 43

35 44

36 45

37 46

38 47

39 48

40 49

41 50
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4 51 **ABSTRACT**
5

6 52 **Introduction:** Heart failure (HF) is a growing global public health burden. However,
7
8 53 due to the very limited regenerative capacity of mature cardiomyocytes in the adult
9
10 54 mammalian heart, conventional treatments can only improve the symptoms of HF but
11
12 55 fail to restore cardiac function. Heart transplantation is limited by a severe shortage of
13
14 56 donors. Cell-based transplantation for the treatment of HF has become a promising
15
16 57 strategy. Human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs)
17
18 58 have been tested in animal models to assess safety and efficacy. This study aims at
19
20 59 evaluating the safety and efficacy of epicardial injection of hiPSC-CMs in patients with
21
22 60 advanced heart failure during Coronary Artery Bypass Grafting (CABG) surgery.

23
24 61 **Methods:** This study is a dose-escalation, placebo-controlled, single-center phase I/IIa
25
26 62 clinical trial. Dose escalation will be guided by a modified 3+3 design for 3 doses
27
28 63 (1×10^8 , 2×10^8 and 4×10^8 cells, sequentially). Patients with advanced heart failure will
29
30 64 be enrolled and randomly allocated to receive epicardial injection of hiPSC-CMs during
31
32 65 CABG surgery or CABG surgery alone, followed by a 12-month follow-up
33
34 66 investigation. The primary endpoint is to assess the safety of hiPSC-CMs
35
36 67 transplantation, including hemodynamic compromised sustained ventricular
37
38 68 arrhythmias and newly formed tumors during 6 months post-operatively. The
39
40 69 secondary endpoint is to evaluate the efficacy of epicardial injection of hiPSC-CMs and
41
42 70 CABG surgery combination by comparison with CABG surgery alone.

43
44 71 **Ethics and dissemination:** The study protocol has been approved by the Institutional
45
46 72 Ethical Committee of Nanjing Drum Tower Hospital (No.SC202000102) and approved
47
48 73 by National Health Commission of the PRC (MR-32-21-014649). Findings will be
49
50 74 disseminated to the academic community through peer-reviewed publications and
51
52 75 presentation at national and international meetings.

53
54 76 **Trial registration number:** NCT03763136

55
56 77 **Keywords:** Clinical Trial, Heart Failure, human induced Pluripotent Stem Cell derived
57
58 78 cardiomyocytes, Coronary Artery Bypass Grafting surgery

59
60 79 **Word Count: 2882**
80
81

1
2
3 **82 Strengths and limitations of this study**
4
5

- 6 **83** ● This study is the first dose-finding and placebo-controlled trial of induced
7
8 **84** pluripotent stem cell based cardiac regenerative therapy in patients with advanced
9
10 **85** heart failure;
11
12 **86** ● This dose-finding study will assess both the safety and efficacy of epicardial
13
14 **87** injection of hiPSC-CMs during CABG surgery for treating advanced heart failure;
15
16 **88** ● This study will be limited to a Chinese population with a target sample size.
17
18

19 89

20 90

21 91

22 92

23 93

24 94

25 95

26 96

27 97

28 98

29 99

30 100

31 101

32 102

33 103

34 104

35 105

36 106

37 107

38 108

39 109

40 110

111 INTRODUCTION

112 Heart failure (HF) is a growing global public health concern with an estimated
113 prevalence of over 37 million individuals worldwide¹. HF is caused by several causes
114 of cardiovascular diseases (CVD), resulting in poor quality of life, high morbidity and
115 mortality^{1,2}. Ischemic heart disease (IHD) is a major cause of heart failure^{2,3}, causing
116 over 8.9 million or 16% deaths in the year of 2019 globally⁴. Although the treatments
117 for HF, including medications and interventional devices have continuously improved
118 in the past few decades, currently there is no treatment to restore cardiac function by
119 addressing the underlying mechanism of IHD, loss of massive contractile
120 cardiomyocytes⁵⁻⁷. Adult mammalian heart has limited capability to regenerate
121 after cardiac injury⁸⁻¹⁰. Hence, it is reasonable to hypothesize that transplantation of
122 exogenous cardiomyocytes as a promising therapeutic to repair cardiac function by
123 remuscularization of the otherwise irreversibly impaired human myocardium¹¹⁻¹³.

124 Human pluripotent stem cells, including embryonic stem cells (hESCs) and
125 induced pluripotent stem cells (hiPSCs), can differentiate into cardiomyocytes with
126 high purity in vitro¹⁴⁻¹⁶, providing an ideal source to regenerate the impaired cardiac
127 function. Ethical controversy has long been a key concern of clinical application of
128 hESCs¹⁷. In contrast, hiPSCs are derived from adult somatic cells (peripheral blood
129 mononuclear cells, skin fibroblasts, etc.) through reprogramming, which overcomes
130 supply limits and avoids ethical issues^{18,19}. In 2015, the First-In-Human (FIH) study
131 involving transplantation of hESCs-derived cardiac progenitor cells was completed by
132 Dr. Menasché *et al* in patients with severe ischemic left ventricular dysfunction²⁰. A
133 subsequent clinical report from the same team further suggested that transplantation of
134 these cells was safe and potentially promoted some functional recovery in the
135 transplanted myocardial areas²¹. Because of the mentioned limitations of hESCs,
136 hiPSCs-derived cardiomyocytes have been investigated in both small and large animals,
137 including non-human primates²²⁻²⁴, demonstrating promising results to remuscularize
138 and restore cardiac function.

139 Previously, our group has performed a FIH clinical study of hiPSC-CMs
140 transplantation during open-chest surgery²⁵, “Treating Heart Failure with hPSC-CMs

1
2
3
4 141 (HEAL-CHF)” (NCT03763136), and observed no serious adverse event, such as
5
6 142 mortality or tumorigenicity, which related to the epicardial injection exogenous hiPSC-
7
8 143 CMs during a 24-month follow-up. Here, we design a dose-escalation, placebo-
9
10 144 controlled, Phase I/IIa clinical trial to evaluate the safety and efficacy of epicardial
11
12 145 injection of allogeneic hiPSC-CMs in patients with advanced HF during CABG surgery
13
14 146 by comparison with CABG surgery alone.

15
16 147

17 148 **METHODS AND ANALYSIS**

19 149 **Study design**

21 150 This study is a dose-escalation, placebo-controlled, single-center phase I/IIa
22
23 151 clinical trial. An overview of the modified 3+3 dose-escalation trial is presented in
24
25 152 Figure 1. This study protocol follows the Standard Protocol Items: Recommendations
26
27 153 for Interventional Trials guidelines, developed to provide a standardised guidance for
28
29 154 recommended items to be included in a clinical trial protocol²⁶. The primary endpoint
30
31 155 is to assess the safety of the epicardial injection of allogeneic hiPSC-CMs in the
32
33 156 treatment of patients with advanced IHF during CABG surgery. The secondary
34
35 157 endpoint is to evaluate the efficacy of epicardial injection of hiPSC-CMs and CABG
36
37 158 surgery combination by comparison with the CABG surgery alone.

38
39 159

40 160 **Study population**

41 161 Patients with advanced chronic HF secondary to ischemic heart disease fulfilling all
42
43 162 inclusion / exclusion criteria will be enrolled at Nanjing Drum Tower Hospital, the
44
45 163 affiliated hospital of Nanjing University Medical School, China. The study will be
46
47 164 conducted in compliance with the requirements of governmental regulatory bodies and
48
49 165 ethics committees.

50
51 166

53 167 ***Inclusion criteria***

- 55 168 1. Patients aged 35-75 years (including 35 and 75).
56
57 169 2. Have signed the Informed Consent Form (ICF).
58
59 170 3. Patients have chronic left ventricular dysfunction.
60

- 171 4. Patients have New York Heart Association (NYHA) Functional Classification III-
172 IV despite receiving guideline-directed medical therapy.
- 173 5. Patients have indications for Coronary Artery Bypass Grafting.
- 174 6. $20\% \leq \text{LVEF} \leq 45\%$ as determined by echocardiographic assessment (data
175 collected up to 6 months prior to inclusion evaluation are valid; data collected
176 within 1 month since a myocardial infarction are invalid).
- 177 7. Weakening or absence of segmental regional wall motion as determined by
178 standard imaging.

179

180 Exclusion criteria

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

- 1
2
3
4 200 15. On chronic therapy with immunosuppressant medication, such as glucocorticoid
5 201 and TNF α antagonist.
6
7 202 16. Contra-indication to take immunosuppressant medication.
8
9 203 17. Serum positive for Human Immunodeficiency Virus (HIV), Hepatitis B Virus
10 204 (HBV), Hepatitis C Virus (HCV), or Treponema Pallidum (TP).
11
12 205 18. Currently enrolled in another investigational therapeutic or device study.
13
14 206 19. Patients who are pregnant or breast feeding.
15
16 207 20. Other conditions that researchers consider not suitable to participate in this study.
17
18
19
20
21
22

23 210 **Randomization and Groups**

24
25 211 Six patients will be enrolled and randomly allocated to CABG +1 \times 10⁸ cells group
26 212 or CABG group. Randomization will be similarly applied for the 2 \times 10⁸ cells and 4 \times 10⁸
27 213 cells patient groups (Figure 1).
28
29
30
31
32

33 215 **Intervention**

34 216 **1. Screening and Baseline Phase**

35
36 217 See Table 1 for the schedule and assessments to be performed during this phase
37 218 I/IIa clinical trial. Subjects fulfilling all inclusion / exclusion criteria and who have
38 219 signed the Informed Consent Form will be enrolled. Baseline information and data
39 220 required should be collected from all enrolled subjects within 4 weeks before the
40 221 operation. Key information and data to be collected include subject demographics, vital
41 222 signs, lab tests, cardiac function evaluation and immunological evaluation (HLA typing,
42 223 determination of PRA and DSA).
43
44
45
46
47
48
49

50 224 **2. Preparation of hiPSC-CMs**

51
52 225 Donors were screened and tested for relevant communicable disease agents and
53 226 diseases, including HIV-1 (antigen and nucleic acid), HIV-2, hepatitis B virus (HBV,
54 227 nucleic acid and surface and core antigen), hepatitis C virus (HCV, antigen and nucleic
55 228 acid) and Treponema pallidum (syphilis), according to "Guidance for Industry:
56 229 Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-

1
2
3
4 230 Based Products (HCT/Ps)” by FDA. In order to prevent promotion of delayed
5
6 231 carcinogenesis, donors were also screened and tested by exome sequencing for target
7
8 232 genes presumably responsible for primary somatic cell mutation in cancer, according
9
10 233 to COSMIC, an existing cancer genome mutation database. A health, 28 years old,
11
12 234 Chinese female, who met the criteria of donor eligibility tests was selected. Her
13
14 235 peripheral mononucleate cells were collected and reprogrammed to induced pluripotent
15
16 236 stem cells under current good manufacturing practice (cGMP) condition.

17 237 The allogeneic hiPSC-CMs were manufactured at Help Therapeutics under cGMP
18
19 238 condition and cryopreserved after quality control analysis ²². The hiPSC-CMs will be
20
21 239 thawed in a 37°C water bath and resuspended in 5% human serum albumin solution
22
23 240 before epicardial injection.

24 241 **3. Dose and treatment method**

25
26
27 242 Six patients will be enrolled and randomly allocated to CABG +1×10⁸ group or
28
29 243 CABG group (n=3 for each arm). For patients allocated to the CABG +1×10⁸ group,
30
31 244 hiPSC-CMs will be injected at 10 sites (0.25~0.30 ml of cell suspension at each site).
32
33 245 Details regarding injection site location and volume of cell suspension injected at each
34
35 246 site will be carefully recorded. Patients in CABG groups will receive standard CABG
36
37 247 surgery alone. All patients will be transferred to the intensive care unit (ICU) for 1 week
38
39 248 after surgery. If no grade 4 or above cell implant related adverse event occurs within 1
40
41 249 month post-operatively in the CABG + 1×10⁸ group, dose escalation will proceed to
42
43 250 2×10⁸ cells. If one grade 4 or above cell implant related adverse event occurs within 1
44
45 251 month post-operatively, three more patients will be enrolled and injected with 1×10⁸
46
47 252 cells during CABG surgery. If no grade 4 or above cell implant related adverse event
48
49 253 occurs in the second three-patient cohort, dose escalation will then proceed to 2×10⁸
50
51 254 cells. Otherwise, the trial will be stopped. The dose escalation design is depicted in
52
53 255 Figure 1.

54 256 **4. Concomitant Medications**

55 257 **4.1 Immunosuppressive drugs**

56
57
58 258 Subjects in the cell treatment groups will receive immunosuppressive treatment as
59
60 259 described below:

- 1
2
3
4 260 1) 2.5g of immunoglobulin will be injected intravenously 1 day pre-operatively, on
5
6 261 the day of surgery and 3 days post-operatively, respectively
7
8 262 2) 20mg of Simulect® (Basiliximab for Injection) will be injected intravenously on
9
10 263 the day of surgery and 4 days post-operatively, respectively.
11
12 264 3) 1g of mycophenolate mofetil (oral) will be given 1 day pre-operatively and
13
14 265 subsequently at the dose of 1.5g for 28 days post-operatively.
15
16 266 4) Tacrolimus tablets will be given from 3 days pre-operatively to 28 days post-
17
18 267 operatively, dose will be adjusted according to a target blood concentration of 3-
19
20 268 5ng/ml.

21 269 **4.2 Anti-arrhythmic drugs**

22
23
24 270 The following anti-arrhythmic medications will be provided for subjects who
25
26 271 developed accelerated idioventricular rhythm over 100 bpm:

- 27
28 272 1) 450 mg amiodarone hydrochloride, intravenous injection.
29
30 273 2) 200 mg Amiodarone tablet, TID.
31
32 274 3) 5mg Ivabradine tablet, BID.

33 275 **5. Day 1~21 post-operatively**

34
35 276 See Table 1 for different timepoints of assessments to be performed during this
36
37 277 period. Key assessments to be performed include vital sign evaluation, ECG-based
38
39 278 heart rhythm monitoring, and laboratory tests including biochemistry, cardiac injury
40
41 279 markers (NT-Pro-BNP, cardiac troponin, cardiac enzymes, etc.), cytokines (IFN γ ,
42
43 280 TNF α , IL-2, IL-6 and IL-10), PRA and DSA.

44 281 **6. Month 1~12 Visit**

45
46
47 282 See Table 1 for the schedule and assessments to be performed during this period.
48
49 283 Outpatient visits should be completed as close to the scheduled visit dates as possible.
50
51 284 The visit window is ± 7 days from the intended date of the visit (1, 3, 6 and 12 months
52
53 285 post-operatively). Key assessments to be performed include vital sign evaluation, ECG,
54
55 286 echocardiogram-based and MRI-based cardiac function evaluation, NYHA
56
57 287 Classification, 6-minute walk test, chest and abdominal PET scan, and laboratory tests
58
59 288 including biochemistry, cardiac injury markers (NT-Pro-BNP, cardiac troponin, cardiac
60

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

292

293 **Endpoints**

294 **1. Primary Endpoints**

295 1.1. Dose Limiting Toxicity (DLT), the adverse event occurs within 30 days post
296 CABG surgery and is considered to be related to hiPSC-CMs transplantation,
297 including:

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

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

302 (3) Death;

303 1.2. Incidence of newly formed tumor, chest and abdominal CT at 1, 3, 6 months and
304 PET/CT at 6 months post-operatively;

305 1.3. Hemodynamic compromised sustained ventricular tachycardia, from 1-6 months
306 post-operatively.

307

308 **2. Secondary Endpoints**

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

311 including:

312 a. Infarct size;

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

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

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

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

317 f. Left ventricular thickness at sites of injection;

318

- 1
2
3
4 319 (2) Changes in left ventricle function evaluation by Echocardiogram-based evaluation
5
6 320 of left ventricular function at baseline, 1, 3, 6 and 12 months post-operatively.
7
8 321 (3) PET/ECT based evaluation of myocardial perfusion at baseline, 6 months and 12
9
10 322 months post-operatively.
11
12 323 (4) Functional status by 6 Minute Walk Test at baseline, 1, 3, 6 and 12 months post-
13
14 324 operatively.
15
16 325 (5) Functional status by NYHA Classification at baseline, 1, 3, 6 and 12 months post-
17
18 326 operatively.
19
20 327 (6) Functional status by Minnesota Living with Heart Failure Questionnaire (MLHFQ)
21
22 328 at baseline, 1, 3, 6 and 12 months post-operatively.
23
24 329 (7) Incidence of Major Adverse Cardiac Events (MACE) during Month 1-12 visit post-
25
26 330 operatively, including death, non-lethal myocardial infraction and hospitalization
27
28 331 for worsening HF.
29
30 332 (8) Changes in penal reactive antibodies (PRA), donor specific antibodies (DSA) and
31
32 333 NT-pro BNP at baseline, 1, 3, 6 and 12 months post-operatively.
33
34 334

335 **Statistical Considerations**

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

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

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

1
2
3
4 349 enumeration data and rating data, while Miettinen's method will be employed to
5
6 350 determine the 95% confidence intervals of measurement data. Where appropriate,
7
8 351 differences between low dose and high dose groups will be calculated and significance
9
10 352 tests will be performed. A bilateral P value less than or equal to 0.05 is considered
11
12 353 significant.

13
14 354

15 355 **Data collection, management and monitoring**

16
17 356 The schedule of data collection is shown in Table 1. An electronic data capture
18
19 357 (EDC) system will be established for this study. A database manager (DM) will be
20
21 358 appointed, who will be responsible for the design of the EDC system. Data will be
22
23 359 collected from medical notes and hospital records in Nanjing Drum Tower Hospital.
24
25 360 Before freezing the database, the DM will compose the data validation report based on
26
27 361 the study plan, data validation standards and database contents. The Sponsor, Principal
28
29 362 Investigator, Statistician and DM should engage in a meeting to validate the data and
30
31 363 come to a resolution regarding database freezing. Once approved, the DM will be
32
33 364 responsible for the freezing of the database and the Statistician will conduct statistical
34
35 365 analysis afterwards.

36
37 366 Data monitoring and validation will be regularly conducted throughout the study.
38
39 367 The frequency of monitoring will be once a year by the Medical Ethics Committee of
40
41 368 Affiliated Nanjing Drum Tower Hospital, Nanjing University Medical College, starting
42
43 369 from the beginning of this study.

44
45 370

46 371 **Quality control**

47
48 372 The clinical trial investigators will implement a quality assurance and quality
49
50 373 control system based on the standard operating procedures prescribed by the
51
52 374 investigators. Implementation of clinical trial, data creation, recording, monitoring and
53
54 375 reporting will be conducted in compliance with "Administrative Measures for Clinical
55
56 376 Studies of Stem Cell-based Therapeutics". The study will be monitored by a third-party
57
58 377 Data and Safety Monitoring Board.

59
60 378

379 **Patient and public involvement**

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

385

386 **DISCUSSION**

387 Loss of cardiomyocytes in the myocardium contributes to severe impairment of
388 cardiac function and may lead to heart failure. The implantation of cardiomyocytes
389 presents an alternative treatment to heart transplantation¹¹⁻¹³. After a roll-in experience
390 as part of the “Treating Heart Failure With hPSC-CMs (HEAL-CHF)” study
391 (NCT03763136), we now initiate a dose-escalation Phase I/IIa trial to evaluate the
392 safety and efficacy of epicardial injection of hiPSC-CMs during CABG surgery in
393 patients with advanced heart failure. This study will be undertaken with sufficient safety
394 considerations and based on the implementation plan and relevant laws. The allogenic
395 approach can bring down the cost for iPSC-based cell therapy compared to the
396 autologous approach and will also obviate the need for approval of individual patient-
397 derived products by regulatory authorities²⁷. This clinical trial will shed the light on the
398 hiPSC-CMs cell therapy for the unmet clinical needs for advanced heart failure patients.

399

400 **ETHICS AND DISSEMINATION**

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

1
2
3
4 410 reviewed publications. We will inform the public through patient organizations and a
5 411 newsletter to participants.
6
7 412

9 413 **Acknowledgement**

10 414 The authors would like to thank Dr Ph Menasché for his help during preparation
11
12 415 of this manuscript.
13
14 416

15 417 **Author contributions**

16
17 418 D.W. and J.W. designed the whole protocol, reviewed and approved the paper. H.
18
19 419 Z & Y.X wrote the paper and prepared the Figure and Table. T.P, X.Z, H.C, C.X, F.F,
20
21 420 H.C, B.Z, J.P, Q.Z & G.Y reviewed the paper and provided valuable suggestions.
22

23 421 **Funding**

24
25 422 The dose escalation study is fully sponsored by HELP Therapeutics.
26
27 423

28 424 **Competing interests**

29
30 425 J.W. is a full-time employee of HELP Therapeutics. All other authors declare no
31
32 426 competing financial interests or personal relationships that could have appeared to
33
34 427 influence the work reported in this paper.
35
36 428

37 429 **REFERENCES**

- 38
39 430 1. Ziaeeian B, Fonarow GC. Epidemiology and aetiology of heart failure. *Nature reviews*
40
41
42 431 *Cardiology* 2016;13(6):368-78. doi: 10.1038/nrcardio.2016.25 [published Online First:
43
44 432 2016/03/05]
- 45
46
47 433 2. Peng H, Abdel-Latif A. Cellular Therapy for Ischemic Heart Disease: An Update. *Advances*
48
49
50 434 *in experimental medicine and biology* 2019;1201:195-213. doi: 10.1007/978-3-030-
51
52 435 31206-0_10 [published Online First: 2020/01/04]
- 53
54
55 436 3. Elgendy IY, Mahtta D, Pepine CJ. Medical Therapy for Heart Failure Caused by Ischemic
56
57
58 437 Heart Disease. *Circulation research* 2019;124(11):1520-35. doi:
- 59
60

- 1
2
3
4 438 10.1161/circresaha.118.313568 [published Online First: 2019/05/24]
5
6
7 439 4. Rana JS, Khan SS, Lloyd-Jones DM, et al. Changes in Mortality in Top 10 Causes of Death
8
9 440 from 2011 to 2018. *Journal of general internal medicine* 2021;36(8):2517-18. doi:
10 441 10.1007/s11606-020-06070-z [published Online First: 2020/07/25]
11
12
13
14 442 5. Rossignol P, Hernandez AF, Solomon SD, et al. Heart failure drug treatment. *Lancet*
15
16 443 (*London, England*) 2019;393(10175):1034-44. doi: 10.1016/s0140-6736(18)31808-7
17
18 444 [published Online First: 2019/03/13]
19
20
21
22 445 6. Normand C, Kaye DM, Povsic TJ, et al. Beyond pharmacological treatment: an insight into
23
24 446 therapies that target specific aspects of heart failure pathophysiology. *Lancet*
25
26 447 (*London, England*) 2019;393(10175):1045-55. doi: 10.1016/s0140-6736(18)32216-5
27
28 448 [published Online First: 2019/03/13]
29
30
31
32 449 7. Willerson JT. The Medical and Device-Related Treatment of Heart Failure. *Circulation*
33
34 450 *research* 2019;124(11):1519. doi: 10.1161/circresaha.119.315268 [published Online
35
36 451 First: 2019/05/24]
37
38
39
40 452 8. González A, Schelbert EB, Díez J, et al. Myocardial Interstitial Fibrosis in Heart Failure:
41
42 453 Biological and Translational Perspectives. *Journal of the American College of*
43
44 454 *Cardiology* 2018;71(15):1696-706. doi: 10.1016/j.jacc.2018.02.021 [published Online
45
46 455 First: 2018/04/14]
47
48
49
50 456 9. Uygur A, Lee RT. Mechanisms of Cardiac Regeneration. *Developmental cell*
51
52 457 2016;36(4):362-74. doi: 10.1016/j.devcel.2016.01.018 [published Online First:
53
54 458 2016/02/26]
55
56
57
58 459 10. Ponnusamy M, Liu F, Zhang YH, et al. Long Noncoding RNA CPR (Cardiomyocyte
59
60

- 1
2
3
4 460 Proliferation Regulator) Regulates Cardiomyocyte Proliferation and Cardiac Repair.
5
6 461 *Circulation* 2019;139(23):2668-84. doi: 10.1161/circulationaha.118.035832 [published
7
8
9 462 Online First: 2019/03/06]
10
11 463 11. Bertero A, Murry CE. Hallmarks of cardiac regeneration. *Nature reviews Cardiology*
12
13
14 464 2018;15(10):579-80. doi: 10.1038/s41569-018-0079-8 [published Online First:
15
16
17 465 2018/09/08]
18
19 466 12. Nakamura K, Murry CE. Function Follows Form - A Review of Cardiac Cell Therapy.
20
21
22 467 *Circulation journal : official journal of the Japanese Circulation Society*
23
24 468 2019;83(12):2399-412. doi: 10.1253/circj.CJ-19-0567 [published Online First:
25
26
27 469 2019/11/15]
28
29 470 13. Murry CE, MacLellan WR. Stem cells and the heart-the road ahead. *Science (New York,*
30
31
32 471 *NY)* 2020;367(6480):854-55. doi: 10.1126/science.aaz3650 [published Online First:
33
34
35 472 2020/02/23]
36
37 473 14. Burrige PW, Matsa E, Shukla P, et al. Chemically defined generation of human
38
39
40 474 cardiomyocytes. *Nature methods* 2014;11(8):855-60. doi: 10.1038/nmeth.2999
41
42
43 475 [published Online First: 2014/06/16]
44
45 476 15. Lian X, Bao X, Zilberter M, et al. Chemically defined, albumin-free human cardiomyocyte
46
47
48 477 generation. *Nature methods* 2015;12(7):595-6. doi: 10.1038/nmeth.3448 [published
49
50
51 478 Online First: 2015/07/01]
52
53 479 16. Liu YW, Chen B, Yang X, et al. Human embryonic stem cell-derived cardiomyocytes
54
55
56 480 restore function in infarcted hearts of non-human primates. *Nature biotechnology*
57
58
59 481 2018;36(7):597-605. doi: 10.1038/nbt.4162 [published Online First: 2018/07/04]
60

- 1
2
3
4 482 17. Ilic D, Ogilvie C. Concise Review: Human Embryonic Stem Cells-What Have We Done?
5
6 483 What Are We Doing? Where Are We Going? *Stem cells (Dayton, Ohio)*
7
8
9 484 2017;35(1):17-25. doi: 10.1002/stem.2450 [published Online First: 2016/06/29]
10
11 485 18. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult
12
13
14 486 human fibroblasts by defined factors. *Cell* 2007;131(5):861-72. doi:
15
16
17 487 10.1016/j.cell.2007.11.019 [published Online First: 2007/11/24]
18
19 488 19. Yu J, Vodyanik MA, Smuga-Otto K, et al. Induced pluripotent stem cell lines derived from
20
21
22 489 human somatic cells. *Science (New York, NY)* 2007;318(5858):1917-20. doi:
23
24
25 490 10.1126/science.1151526 [published Online First: 2007/11/22]
26
27 491 20. Menasché P, Vanneaux V, Hagege A, et al. Human embryonic stem cell-derived cardiac
28
29
30 492 progenitors for severe heart failure treatment: first clinical case report. *European*
31
32
33 493 *heart journal* 2015;36(30):2011-7. doi: 10.1093/eurheartj/ehv189 [published Online
34
35 494 First: 2015/05/21]
36
37 495 21. Menasché P, Vanneaux V, Hagege A, et al. Transplantation of Human Embryonic
38
39
40 496 Stem Cell-Derived Cardiovascular Progenitors for Severe Ischemic Left Ventricular
41
42
43 497 Dysfunction. *Journal of the American College of Cardiology* 2018;71(4):429-38. doi:
44
45
46 498 10.1016/j.jacc.2017.11.047 [published Online First: 2018/02/02]
47
48 499 22. Guan X, Xu W, Zhang H, et al. Transplantation of human induced pluripotent stem cell-
49
50
51 500 derived cardiomyocytes improves myocardial function and reverses ventricular
52
53
54 501 remodeling in infarcted rat hearts. *Stem cell research & therapy* 2020;11(1):73. doi:
55
56 502 10.1186/s13287-020-01602-0 [published Online First: 2020/02/23]
57
58 503 23. Kawamura M, Miyagawa S, Miki K, et al. Feasibility, safety, and therapeutic efficacy of
59
60

- 1
2
3
4 504 human induced pluripotent stem cell-derived cardiomyocyte sheets in a porcine
5
6 505 ischemic cardiomyopathy model. *Circulation* 2012;126(11 Suppl 1):S29-37. doi:
7
8
9 506 10.1161/circulationaha.111.084343 [published Online First: 2012/09/22]
10
11 507 24. Shiba Y, Gomibuchi T, Seto T, et al. Allogeneic transplantation of iPS cell-derived
12
13
14 508 cardiomyocytes regenerates primate hearts. *Nature* 2016;538(7625):388-91. doi:
15
16
17 509 10.1038/nature19815 [published Online First: 2016/10/21]
18
19 510 25. Mallapaty S. Revealed: two men in China were first to receive pioneering stem-cell
20
21
22 511 treatment for heart disease. *Nature* 2020;581(7808):249-50. doi: 10.1038/d41586-
23
24
25 512 020-01285-w [published Online First: 2020/05/15]
26
27 513 26. Chan AW, Tetzlaff JM, Altman DG, et al. SPIRIT 2013 statement: defining standard
28
29
30 514 protocol items for clinical trials. *Annals of internal medicine* 2013;158(3):200-7. doi:
31
32
33 515 10.7326/0003-4819-158-3-201302050-00583 [published Online First: 2013/01/09]
34
35 516 27. Eschenhagen T, Weinberger F. Heart Repair With Myocytes. *Circulation research*
36
37
38 517 2019;124(6):843-45. doi: 10.1161/circresaha.118.314336 [published Online First:
39
40 518 2019/03/15]
41
42
43 519

520 **Figure Legend**

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

524
525
526
527
528

1
2
3
4 529
5
6 530
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

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 |

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46

| | | | | | | | | | |
|--------------------------------------|---|--|---|---|---|---|---|---|---|
| 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 |

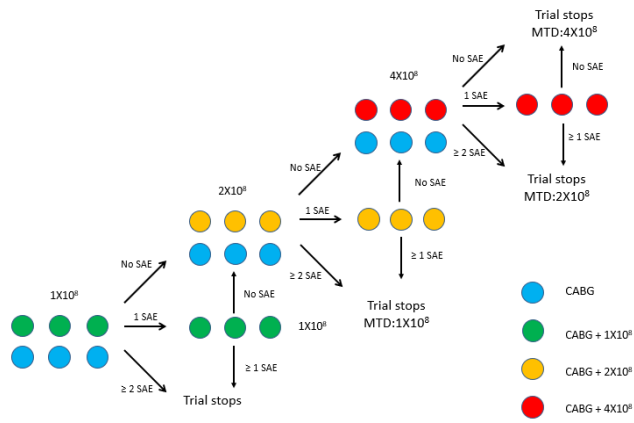
| | | | | | | | | | |
|---|---|---|----------|---|---|---|---|---|---|
| Tumor marker | X | | | | | X | X | X | X |
| Immunoassay (C3, C4, IgA, IgG, IgM) | X | | | X | | X | X | X | X |
| Infectious test | X | | | | | X | X | X | X |
| Coagulation function | X | | | X | X | X | X | X | X |
| HLA typing | 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 |

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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46

535

For peer review only



For peer review only

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 SPIRIT reporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. *BMJ*. 2013;346:e7586

| | | | Page |
|-----------------------------------|---------------------|--|--------|
| | | Reporting Item | 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 |

| | | | |
|----|--------------------------|--|-------|
| 1 | | name of intended registry | |
| 2 | | | |
| 3 | | | |
| 4 | Trial registration: data | #2b All items from the World Health Organization Trial | 4 |
| 5 | | | |
| 6 | set | Registration Data Set | |
| 7 | | | |
| 8 | | | |
| 9 | Protocol version | #3 Date and version identifier | 4 |
| 10 | | | |
| 11 | | | |
| 12 | Funding | #4 Sources and types of financial, material, and other support | 17 |
| 13 | | | |
| 14 | | | |
| 15 | Roles and | #5a Names, affiliations, and roles of protocol contributors | 2 |
| 16 | | | |
| 17 | responsibilities: | | |
| 18 | | | |
| 19 | contributorship | | |
| 20 | | | |
| 21 | | | |
| 22 | | | |
| 23 | Roles and | #5b Name and contact information for the trial sponsor | 2 |
| 24 | | | |
| 25 | responsibilities: | | |
| 26 | | | |
| 27 | sponsor contact | | |
| 28 | | | |
| 29 | information | | |
| 30 | | | |
| 31 | | | |
| 32 | | | |
| 33 | Roles and | #5c Role of study sponsor and funders, if any, in study design; | 17-18 |
| 34 | | | |
| 35 | responsibilities: | collection, management, analysis, and interpretation of | |
| 36 | | | |
| 37 | sponsor and funder | data; writing of the report; and the decision to submit the | |
| 38 | | | |
| 39 | | report for publication, including whether they will have | |
| 40 | | | |
| 41 | | ultimate authority over any of these activities | |
| 42 | | | |
| 43 | | | |
| 44 | | | |
| 45 | Roles and | #5d Composition, roles, and responsibilities of the coordinating | 14 |
| 46 | | | |
| 47 | responsibilities: | centre, steering committee, endpoint adjudication | |
| 48 | | | |
| 49 | committees | committee, data management team, and other individuals | |
| 50 | | | |
| 51 | | or groups overseeing the trial, if applicable (see Item 21a | |
| 52 | | | |
| 53 | | for data monitoring committee) | |
| 54 | | | |
| 55 | | | |
| 56 | | | |
| 57 | Introduction | | |
| 58 | | | |
| 59 | | | |
| 60 | | | |

| | | | | |
|----|---------------------------|---------------------|---|-----|
| 1 | Background and | #6a | Description of research question and justification for | 5-6 |
| 2 | | | | |
| 3 | rationale | | undertaking the trial, including summary of relevant studies | |
| 4 | | | (published and unpublished) examining benefits and harms | |
| 5 | | | for each intervention | |
| 6 | | | | |
| 7 | | | | |
| 8 | | | | |
| 9 | | | | |
| 10 | | | | |
| 11 | Background and | #6b | Explanation for choice of comparators | 6 |
| 12 | | | | |
| 13 | rationale: choice of | | | |
| 14 | | | | |
| 15 | comparators | | | |
| 16 | | | | |
| 17 | | | | |
| 18 | Objectives | #7 | Specific objectives or hypotheses | 6 |
| 19 | | | | |
| 20 | | | | |
| 21 | | | | |
| 22 | Trial design | #8 | Description of trial design including type of trial (eg, parallel | 6 |
| 23 | | | group, crossover, factorial, single group), allocation ratio, | |
| 24 | | | and framework (eg, superiority, equivalence, non-inferiority, | |
| 25 | | | exploratory) | |
| 26 | | | | |
| 27 | | | | |
| 28 | | | | |
| 29 | | | | |
| 30 | | | | |
| 31 | Methods: | | | |
| 32 | | | | |
| 33 | Participants, | | | |
| 34 | | | | |
| 35 | interventions, and | | | |
| 36 | | | | |
| 37 | outcomes | | | |
| 38 | | | | |
| 39 | | | | |
| 40 | | | | |
| 41 | Study setting | #9 | Description of study settings (eg, community clinic, | 4 |
| 42 | | | academic hospital) and list of countries where data will be | |
| 43 | | | collected. Reference to where list of study sites can be | |
| 44 | | | obtained | |
| 45 | | | | |
| 46 | | | | |
| 47 | | | | |
| 48 | | | | |
| 49 | | | | |
| 50 | | | | |
| 51 | Eligibility criteria | #10 | Inclusion and exclusion criteria for participants. If | 7-8 |
| 52 | | | applicable, eligibility criteria for study centres and | |
| 53 | | | individuals who will perform the interventions (eg, | |
| 54 | | | | |
| 55 | | | | |
| 56 | | | | |
| 57 | | | | |
| 58 | | | | |
| 59 | | | | |
| 60 | | | | |

| | | | |
|----|----------------------|---|-------|
| | | surgeons, psychotherapists) | |
| 1 | | | |
| 2 | | | |
| 3 | | | |
| 4 | Interventions: | #11a Interventions for each group with sufficient detail to allow | 9 |
| 5 | | | |
| 6 | description | replication, including how and when they will be | |
| 7 | | | |
| 8 | | administered | |
| 9 | | | |
| 10 | | | |
| 11 | Interventions: | #11b Criteria for discontinuing or modifying allocated | 11 |
| 12 | | | |
| 13 | modifications | interventions for a given trial participant (eg, drug dose | |
| 14 | | | |
| 15 | | change in response to harms, participant request, or | |
| 16 | | | |
| 17 | | improving / worsening disease) | |
| 18 | | | |
| 19 | | | |
| 20 | | | |
| 21 | Interventions: | #11c Strategies to improve adherence to intervention protocols, | N/A |
| 22 | | | |
| 23 | adherence | and any procedures for monitoring adherence (eg, drug | |
| 24 | | | |
| 25 | | tablet return; laboratory tests) | |
| 26 | | | |
| 27 | | | |
| 28 | | | |
| 29 | Interventions: | #11d Relevant concomitant care and interventions that are | N/A |
| 30 | | | |
| 31 | concomitant care | permitted or prohibited during the trial | |
| 32 | | | |
| 33 | | | |
| 34 | Outcomes | #12 Primary, secondary, and other outcomes, including the | 10-11 |
| 35 | | | |
| 36 | | specific measurement variable (eg, systolic blood | |
| 37 | | | |
| 38 | | pressure), analysis metric (eg, change from baseline, final | |
| 39 | | | |
| 40 | | value, time to event), method of aggregation (eg, median, | |
| 41 | | | |
| 42 | | proportion), and time point for each outcome. Explanation | |
| 43 | | | |
| 44 | | of the clinical relevance of chosen efficacy and harm | |
| 45 | | | |
| 46 | | outcomes is strongly recommended | |
| 47 | | | |
| 48 | | | |
| 49 | | | |
| 50 | | | |
| 51 | Participant timeline | #13 Time schedule of enrolment, interventions (including any | 21 |
| 52 | | | |
| 53 | | run-ins and washouts), assessments, and visits for | |
| 54 | | | |
| 55 | | participants. A schematic diagram is highly recommended | |
| 56 | | | |
| 57 | | (see Figure) | |
| 58 | | | |
| 59 | | | |
| 60 | | | |

| | | | | |
|----|----------------------------------|----------------------|--|----|
| 1 | 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 |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |
| 6 | | | | |
| 7 | | | | |
| 8 | | | | |
| 9 | | | | |
| 10 | | | | |
| 11 | Recruitment | #15 | Strategies for achieving adequate participant enrolment to reach target sample size | 12 |
| 12 | | | | |
| 13 | | | | |
| 14 | | | | |
| 15 | | | | |
| 16 | Methods: Assignment | | | |
| 17 | of interventions (for | | | |
| 18 | controlled trials) | | | |
| 19 | | | | |
| 20 | | | | |
| 21 | | | | |
| 22 | | | | |
| 23 | | | | |
| 24 | 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 |
| 25 | | | | |
| 26 | | | | |
| 27 | | | | |
| 28 | | | | |
| 29 | | | | |
| 30 | | | | |
| 31 | | | | |
| 32 | | | | |
| 33 | | | | |
| 34 | | | | |
| 35 | | | | |
| 36 | | | | |
| 37 | | | | |
| 38 | | | | |
| 39 | | | | |
| 40 | | | | |
| 41 | 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 |
| 42 | | | | |
| 43 | | | | |
| 44 | | | | |
| 45 | | | | |
| 46 | | | | |
| 47 | | | | |
| 48 | | | | |
| 49 | | | | |
| 50 | | | | |
| 51 | Allocation: implementation | #16c | Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions | 8 |
| 52 | | | | |
| 53 | | | | |
| 54 | | | | |
| 55 | | | | |
| 56 | | | | |
| 57 | | | | |
| 58 | | | | |
| 59 | | | | |
| 60 | | | | |

| | | | | |
|----|------------------------|----------------------|--|-------|
| 1 | Blinding (masking) | #17a | Who will be blinded after assignment to interventions (eg, | 8 |
| 2 | | | trial participants, care providers, outcome assessors, data | |
| 3 | | | analysts), and how | |
| 4 | | | | |
| 5 | | | | |
| 6 | | | | |
| 7 | | | | |
| 8 | Blinding (masking): | #17b | If blinded, circumstances under which unblinding is | 8 |
| 9 | emergency | | permissible, and procedure for revealing a participant's | |
| 10 | | | allocated intervention during the trial | |
| 11 | unblinding | | | |
| 12 | | | | |
| 13 | | | | |
| 14 | | | | |
| 15 | | | | |
| 16 | Methods: Data | | | |
| 17 | collection, | | | |
| 18 | management, and | | | |
| 19 | analysis | | | |
| 20 | | | | |
| 21 | | | | |
| 22 | | | | |
| 23 | | | | |
| 24 | | | | |
| 25 | | | | |
| 26 | Data collection plan | #18a | Plans for assessment and collection of outcome, baseline, | 12-13 |
| 27 | | | and other trial data, including any related processes to | |
| 28 | | | promote data quality (eg, duplicate measurements, training | |
| 29 | | | of assessors) and a description of study instruments (eg, | |
| 30 | | | questionnaires, laboratory tests) along with their reliability | |
| 31 | | | and validity, if known. Reference to where data collection | |
| 32 | | | forms can be found, if not in the protocol | |
| 33 | | | | |
| 34 | | | | |
| 35 | | | | |
| 36 | | | | |
| 37 | | | | |
| 38 | | | | |
| 39 | | | | |
| 40 | | | | |
| 41 | | | | |
| 42 | | | | |
| 43 | Data collection plan: | #18b | Plans to promote participant retention and complete follow- | N/A |
| 44 | retention | | up, including list of any outcome data to be collected for | |
| 45 | | | participants who discontinue or deviate from intervention | |
| 46 | | | protocols | |
| 47 | | | | |
| 48 | | | | |
| 49 | | | | |
| 50 | | | | |
| 51 | | | | |
| 52 | | | | |
| 53 | Data management | #19 | Plans for data entry, coding, security, and storage, | N/A |
| 54 | | | including any related processes to promote data quality | |
| 55 | | | (eg, double data entry; range checks for data values). | |
| 56 | | | | |
| 57 | | | | |
| 58 | | | | |
| 59 | | | | |
| 60 | | | | |

| | | | |
|----|----------------------------|--|-------|
| 1 | | Reference to where details of data management | |
| 2 | | | |
| 3 | | procedures can be found, if not in the protocol | |
| 4 | | | |
| 5 | | | |
| 6 | Statistics: outcomes | #20a Statistical methods for analysing primary and secondary | 13 |
| 7 | | | |
| 8 | | outcomes. Reference to where other details of the | |
| 9 | | | |
| 10 | | statistical analysis plan can be found, if not in the protocol | |
| 11 | | | |
| 12 | | | |
| 13 | Statistics: additional | #20b Methods for any additional analyses (eg, subgroup and | N/A |
| 14 | | | |
| 15 | analyses | adjusted analyses) | |
| 16 | | | |
| 17 | | | |
| 18 | Statistics: analysis | #20c Definition of analysis population relating to protocol non- | 12 |
| 19 | | | |
| 20 | population and | adherence (eg, as randomised analysis), and any statistical | |
| 21 | | | |
| 22 | missing data | methods to handle missing data (eg, multiple imputation) | |
| 23 | | | |
| 24 | | | |
| 25 | | | |
| 26 | Methods: Monitoring | | |
| 27 | | | |
| 28 | | | |
| 29 | Data monitoring: | #21a Composition of data monitoring committee (DMC); | 12-13 |
| 30 | | | |
| 31 | formal committee | summary of its role and reporting structure; statement of | |
| 32 | | | |
| 33 | | whether it is independent from the sponsor and competing | |
| 34 | | | |
| 35 | | interests; and reference to where further details about its | |
| 36 | | | |
| 37 | | charter can be found, if not in the protocol. Alternatively, an | |
| 38 | | | |
| 39 | | explanation of why a DMC is not needed | |
| 40 | | | |
| 41 | | | |
| 42 | | | |
| 43 | | | |
| 44 | Data monitoring: | #21b Description of any interim analyses and stopping | 14 |
| 45 | | | |
| 46 | interim analysis | guidelines, including who will have access to these interim | |
| 47 | | | |
| 48 | | results and make the final decision to terminate the trial | |
| 49 | | | |
| 50 | | | |
| 51 | Harms | #22 Plans for collecting, assessing, reporting, and managing | 11-12 |
| 52 | | | |
| 53 | | solicited and spontaneously reported adverse events and | |
| 54 | | | |
| 55 | | other unintended effects of trial interventions or trial | |
| 56 | | | |
| 57 | | | |
| 58 | | | |
| 59 | | | |
| 60 | | | |

| | | | |
|----|----------------------|--|-----|
| 1 | | conduct | |
| 2 | | | |
| 3 | | | |
| 4 | Auditing | #23 Frequency and procedures for auditing trial conduct, if any, | 13 |
| 5 | | and whether the process will be independent from | |
| 6 | | | |
| 7 | | | |
| 8 | | investigators and the sponsor | |
| 9 | | | |
| 10 | | | |
| 11 | Ethics and | | |
| 12 | | | |
| 13 | dissemination | | |
| 14 | | | |
| 15 | | | |
| 16 | Research ethics | #24 Plans for seeking research ethics committee / institutional | 13 |
| 17 | | review board (REC / IRB) approval | |
| 18 | approval | | |
| 19 | | | |
| 20 | | | |
| 21 | | | |
| 22 | Protocol | #25 Plans for communicating important protocol modifications | N/A |
| 23 | | (eg, changes to eligibility criteria, outcomes, analyses) to | |
| 24 | amendments | relevant parties (eg, investigators, REC / IRBs, trial | |
| 25 | | participants, trial registries, journals, regulators) | |
| 26 | | | |
| 27 | | | |
| 28 | | | |
| 29 | | | |
| 30 | | | |
| 31 | | | |
| 32 | Consent or assent | #26a Who will obtain informed consent or assent from potential | 9 |
| 33 | | trial participants or authorised surrogates, and how (see | |
| 34 | | Item 32) | |
| 35 | | | |
| 36 | | | |
| 37 | | | |
| 38 | | | |
| 39 | Consent or assent: | #26b Additional consent provisions for collection and use of | N/A |
| 40 | | participant data and biological specimens in ancillary | |
| 41 | ancillary studies | studies, if applicable | |
| 42 | | | |
| 43 | | | |
| 44 | | | |
| 45 | | | |
| 46 | | | |
| 47 | Confidentiality | #27 How personal information about potential and enrolled | 15 |
| 48 | | participants will be collected, shared, and maintained in | |
| 49 | | order to protect confidentiality before, during, and after the | |
| 50 | | | |
| 51 | | | |
| 52 | | trial | |
| 53 | | | |
| 54 | | | |
| 55 | | | |
| 56 | | | |
| 57 | Declaration of | #28 Financial and other competing interests for principal | 17 |
| 58 | | | |
| 59 | | | |
| 60 | | | |

| | | | | |
|----|-----------------------|----------------------|---|-----|
| 1 | interests | | investigators for the overall trial and each study site | |
| 2 | | | | |
| 3 | | | | |
| 4 | Data access | #29 | Statement of who will have access to the final trial dataset, | 18 |
| 5 | | | and disclosure of contractual agreements that limit such | |
| 6 | | | access for investigators | |
| 7 | | | | |
| 8 | | | | |
| 9 | | | | |
| 10 | | | | |
| 11 | Ancillary and post | #30 | Provisions, if any, for ancillary and post-trial care, and for | N/A |
| 12 | | | compensation to those who suffer harm from trial | |
| 13 | trial care | | participation | |
| 14 | | | | |
| 15 | | | | |
| 16 | | | | |
| 17 | | | | |
| 18 | | | | |
| 19 | Dissemination policy: | #31a | Plans for investigators and sponsor to communicate trial | 15 |
| 20 | | | results to participants, healthcare professionals, the public, | |
| 21 | trial results | | and other relevant groups (eg, via publication, reporting in | |
| 22 | | | results databases, or other data sharing arrangements), | |
| 23 | | | including any publication restrictions | |
| 24 | | | | |
| 25 | | | | |
| 26 | | | | |
| 27 | | | | |
| 28 | | | | |
| 29 | | | | |
| 30 | | | | |
| 31 | Dissemination policy: | #31b | Authorship eligibility guidelines and any intended use of | N/A |
| 32 | | | professional writers | |
| 33 | authorship | | | |
| 34 | | | | |
| 35 | | | | |
| 36 | Dissemination policy: | #31c | Plans, if any, for granting public access to the full protocol, | N/A |
| 37 | | | participant-level dataset, and statistical code | |
| 38 | reproducible research | | | |
| 39 | | | | |
| 40 | | | | |
| 41 | | | | |
| 42 | Appendices | | | |
| 43 | | | | |
| 44 | | | | |
| 45 | Informed consent | #32 | Model consent form and other related documentation given | N/A |
| 46 | | | to participants and authorised surrogates | |
| 47 | materials | | | |
| 48 | | | | |
| 49 | | | | |
| 50 | Biological specimens | #33 | Plans for collection, laboratory evaluation, and storage of | N/A |
| 51 | | | biological specimens for genetic or molecular analysis in | |
| 52 | | | the current trial and for future use in ancillary studies, if | |
| 53 | | | applicable | |
| 54 | | | | |
| 55 | | | | |
| 56 | | | | |
| 57 | | | | |
| 58 | | | | |
| 59 | | | | |
| 60 | | | | |

1 None The SPIRIT Explanation and Elaboration paper is distributed under the terms of the Creative
2 Commons Attribution License CC-BY-NC. This checklist can be completed online using
3 <https://www.goodreports.org/>, a tool made by the [EQUATOR Network](#) in collaboration with
4 [Penelope.ai](#)
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60