PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	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
AUTHORS	Zhang, He; Xue, Yunxing; Pan, Tuo; Zhu, xiyu; Chong, Hoshun; Xu, Can; Fan, Fudong; Cao, Hailong; Zhang, bomin; Pan, Jun; Zhou, Qing; Yang, Gang; Wang, Jiaxian; Wang, Dong-Jin

VERSION 1 – REVIEW

REVIEWER	Zimmermann, Wolfram-Hubertus Georg August University Gottingen University Medical Center, Pharmacology and Toxicology
REVIEW RETURNED	16-Oct-2021

GENERAL COMMENTS	Zhang et al. present a clinical trial protocol for the to my knowledge first placebo-controlled clinical trial on iPSC-derived cardiomyocyte implantation. Such studies are without doubt important and timely. I have a couple of suggestions which may help to clarify the rationale and endpoint selection: 1) This is a follow-up to the HEAL-CHF trial (NCT03763136). Please add to the statement on the outcome of this trial (lines 142-146) the number of patients treated. In addition, add whether or not arrhythmia were observed. This is the key safety concern related to cardiomyocyte implantation according to preliminary data such as cited in ref [24]. 2) Placebo-control, single blinding is indicated. It is no clear who is blinded. I assume the patient and not the clinical investigator. If so, briefly state why the clinical investigator was or cannot be blinded. Along these lines, I did not find a statement on solvent injection in the placebo group and assume that placebo control refers to a randomization into a standard of care (CAGB) arm. In essence, patient number is too small and outcome will be too variable to support efficacy claims. A short statement summarizing limitations and a rationale for the placebo-control, single-blinding should be included. 3) Please, clarify that no reliable efficacy date will be retrieved from this early clinical trial. The outcome will be safety only. “Preliminary efficacy” (line 299) may be better labeled as “Exploratory efficacy data”. 4) The authors should clearly state that the withdrawal of immune suppression after 28 days will result in a complete rejection of the implanted allograft cells and that all outcome data beyond this

	time-point will not be related to retained cellular grafts. Thus, the goal of this trial is not sustainable remuscularization, but disease modification by up-to-date undefined mechanisms, which may include immune modulation, paracrine activity, exosome release, reverse remodelling. The question will be whether iPSC-derived cardiomyocytes will exhibit differential and potentially better effects compared to non-myocyte implants. 5) Line 268: please specify that you are referring to trough levels of Tacrolimus and not that 3-5 ng/mL are very low. Please provide a rationale for the Tacrolimus dosing strategy. 6) Line 173-175: please clarify that baseline investigation is apparently within 6-month before treatment; this is at least how I understand this. This is a quite long time period for patients with advanced heart failure. The statement on “exclusion of 1-month data after MI” can be deleted” in the inclusion criteria section since it is clearly stated in the exclusion criteria section (line 188). 7) The clinicaltrials.gov registration number should be included.

REVIEWER	Hare, Joshua University of Miami School of Medicine, Interdisciplinary Stem Cell Institute
REVIEW RETURNED	08-Nov-2021

GENERAL COMMENTS	Zhang et al. wrote a detailed description of their methodologies being used in their phase I clinical trial on pericardial injections of allogeneic human stem cells during CABG. The information provided give readers enough information to adequately assess the strengths and weakness of this particular clinical trial. In the methodology section, the authors discuss a dose escalation approach for subsequent test subjects. The language in the body of the paragraph did not make it readily apparent that future injections were separate cohorts of patients, instead of the same patients receiving a second injection at a higher dose. The figure should more clearly indicate that each step is a separate cohort. Since this is a methodology paper, more details needs to be provided on why allogeneic, instead of autologous stem cells were chosen, and a description of the population or individual they were derived from. As many published phase 1 and 2 clinical trials currently utilize endocardial injections, with injection sites determined by cardiac mapping catheters to find borderzone areas to inject. Given that this study differs considerably from the methodologies of recent cardiac stem cell injections, it would be instructive for your paper to discuss how the epicardial site locations are chosen at the time of surgery. In the methodology section, it is indicated that this is a placebo controlled trial. However it is unclear if the control group will undergo sham injections at the time of surgery. If they do undergo a sham injection please elaborate in your methodology. Andrew Sundin, MD assisted with this review

VERSION 1 – AUTHOR RESPONSE

Responds to the reviewer's comments:
Reviewer#1
We thank the reviewer for the positive comments and suggestions on our work to improve the manuscript.

Comment 1: This is a follow-up to the HEAL-CHF trial (NCT03763136). Please add to the statement on the outcome of this trial (lines 142-146) the number of patients treated. In addition, add whether or not arrhythmia were observed. This is the key safety concern related to cardiomyocyte implantation according to preliminary data such as cited in ref [24].
Response 1: It is really true as Reviewer suggested that arrhythmia is very important to evaluate the safety of cardiomyocyte implantation. In our FIH study, we observed arrhythmias including accelerated ventricular tachycardia, premature ventricular contractile, supraventricular tachycardia and atrial fibrillation in two weeks post-operation. However, those arrhythmias were benign without unstable hemodynamic status and could be control with anti-arrhythmic drugs. (Please see Line 146-152).

Comment 2: Placebo-control, single blinding is indicated. It is no clear who is blinded. I assume the patient and not the clinical investigator. If so, briefly state why the clinical investigator was or cannot be blinded. Along these lines, I did not find a statement on solvent injection in the placebo group and assume that placebo control refers to a randomization into a standard of care (CAGB) arm. In essence, patient number is too small and outcome will be too variable to support efficacy claims. A short statement summarizing limitations and a rationale for the placebo-control, single-blinding should be included.
Response 2: Thanks for your comments. The description of single-blind is not correct. In our trial, it is very hard to blind both the patient and clinical investigators. Because if patients have allocated in study group, the short term immunosuppressors should be administrated to them. Otherwise, no immunosuppressors could be given in control group. As we all known, immunosuppressors could bring new risk in such severe patients such as incisional infection, anorexia and kidney injury etc. It is not reasonable and ethical for subjects without cell therapy to have immunosuppressors even though for a short term. On the other hand, it is impossible for the cardiac surgeon was blinded for epicardial injection during the surgery. He knows exactly what dosage was injected in patient and is also clear about the prescription of immunosuppressor to them. Only the physician to evaluate the outcomes is blinded for the randomization. So to avoid misunderstanding, we deleted the word of single-blinded. We agree that the patient number is small to support efficacy claims. The primary endpoint of this trial is safety and maximum tolerated dose (MTD) of epicardial injection of hIPSC-CMs for severe heart failure patient (NYHA III/IV). Therefore, this trial was defined as Phase IB/IIA trial. Based the outcome of this trial, a larger scale Phase IIB trial will be required to study the clinical efficacy. We have summarized these points in Strengths and limitations of this study.

Comment 3: Please, clarify that no reliable efficacy date will be retrieved from this early clinical trial. The outcome will be safety only. "Preliminary efficacy" (line 299) may be better labeled as "Exploratory efficacy data".
Response 3: Yes, we agree. We correct the description of outcome as safety only. We have revised the "Preliminary efficacy" as "Exploratory efficacy data".

Comment 4: The authors should clearly state that the withdrawal of immune suppression after 28 days will result in a complete rejection of the implanted allograft cells and that all outcome data

beyond this time-point will not be related to retained cellular grafts. Thus, the goal of this trial is not sustainable remuscularization, but disease modification by up-to-date undefined mechanisms, which may include immune modulation, paracrine activity, exosome release, reverse remodelling. The question will be whether iPSC-derived cardiomyocytes will exhibit differential and potentially better effects compared to non-myocyte implants.
Response 4: It is true that the discontinuation of immunosuppressors could result in a rejection for the allograft cells. Actually, we have no idea how to track those rejection and to assess the survival status of implanted cells. As you mentioned, all outcome data beyond 28 days will not be surely related to retained cells directly or other potential benefits indirectly. From this trial, it is still hard to answer the question whether iPSC-derived cardiomyocytes will exhibit differential and potentially better effects compared to non-myocyte implants. So we set the primary endpoint is saft only. We have revised the discussion section.
Change 4: We have added this part in Strengths and limitations of this study.

Comment 5: Line 268: please specify that you are referring to trough levels of Tacrolimus and not that 3-5 ng/mL are very low. Please provide a rationale for the Tacrolimus dosing strategy.
Response 5: We are very sorry for our unclear expressions. It is really true that we were referring to level of Tacrolimus with 3-5 ng/ml. We made our immunosuppressive strategy based on heart transplant protocol and the work of Philippe Menasché (2017 JACC).

Comment 6: Line 173-175: please clarify that baseline investigation is apparently within 6-month before treatment; this is at least how I understand this. This is a quite long time period for patients with advanced heart failure. The statement on “exclusion of 1-month data after MI” can be deleted” in the inclusion criteria section since it is clearly stated in the exclusion criteria section (line 188).
Response 6: It is very sorry to bring you unclearness about the baseline investigation duration. Our intention is that the echocardiogram within 6 months before treatment is valid for screening. In fact, baseline information and data required should be collected from all enrolled subjects within 4 weeks before the operation. we have correct the description. We thought these patients with acute myocardial infarction within 1 month are not suitable for iPSC-derived cardiomyocyte implantation because the cardiac function could be recovery after acute phase. Those words have been deleted.

Comment 7: The clinicaltrials.gov registration number should be included.
Response 7: Thanks for your suggestion. We updated our manuscript with the clinicaltrials.gov registration number.

Reviewer#2
We thank the reviewer for the helpful comments and suggestions on our work.

Comment 1: In the methodology section, the authors discuss a dose escalation approach for subsequent test subjects. The language in the body of the paragraph did not make it readily apparent that future injections were separate cohorts of patients, instead of the same patients receiving a second injection at a higher dose. The figure should more clearly indicate that each step is a separate cohort.
Response 1: Thanks for your suggestion. We described our dose-escalation design in Line 241-256.

Comment 2: Since this is a methodology paper, more details needs to be provided on why allogeneic, instead of autologous stem cells were chosen, and a description of the population or individual they were derived from.
Response 2: The allogeneic HiPSC-CMs were selected in this trial for following reasons: firstly, the cost of autologous hIPSC-CMs is much higher than the allogeneic hIPSC-CMs. Especially, it is not realistic for cardiac repair in a large patient populations; Secondly, It is very time-consuming about 1 year for autologous HiPSC-CMs production, which limited the clinical application for severe heart

failure patients; Thirdly, the ideal therapeutic HiPSC-CMs should be screened as healthy status. A potential genetic susceptibility of heart failure is hard to excluded for every patient. So universal allogeneic HiPSC-CMs were preferred in our trial. At last, the allogeneic HiPSC-CMs derived from heathy donor with critical criteria was selected for clinical trial.
Change 2: We added these statements in the discussion section.

Comment 3: As many published phase 1 and 2 clinical trials currently utilize endocardial injections, with injection sites determined by cardiac mapping catheters to find borderzone areas to inject. Given that this study differs considerably from the methodologies of recent cardiac stem cell injections, it would be instructive for your paper to discuss how the epicardial site locations are chosen at the time of surgery.
Response 3: Thank you for your advice. All subjects in this study will receive CMR to determine the area of myocardial infarction. Surgeon will also determine the area of myocardial infarction by wall motion abnormalities. The hiPSC-CMs will be injected into cardiac muscle under direct vision after completion of CABG and before close chest. About 10 points in the central and border areas of all infarcted region will be chosen as injection sites.
Change 3: We have added this part in 3. Dose and treatment method.

Comment 4: In the methodology section, it is indicated that this is a placebo-controlled trial. However, it is unclear if the control group will undergo sham injections at the time of surgery. If they do undergo a sham injection please elaborate in your methodology.
Response 4: Thanks for your suggestion. It is not acceptable for the placebo-control patient to do the sham epicardial injection. We will clarify this point.
Change 4: We have revised placebo-control as randomized control (Line62, Line83 and Line159).

We tried our best to improve the manuscript and made some changes in the manuscript. These changes will not influence the content and framework of the paper. And here we did not list the changes but marked in red in revised paper.
We appreciate for Editors/Reviewers' warm work earnestly, and hope that the correction will meet with approval. Once again, thank you very much for your comments and suggestions.

VERSION 2 – REVIEW

REVIEWER	Zimmermann, Wolfram-Hubertus Georg August University Gottingen University Medical Center, Pharmacology and Toxicology
REVIEW RETURNED	23-Jan-2022

GENERAL COMMENTS	The described Phase I trial is important and timely. Publication of the study protocol with reference to the previously treated 2 patients is important (lines 142-147). The main concern of the trial is arrhythmia induction by the cardiomyocyte implants. The following points should be addressed: Method section, lines 250-256 and Fig 1: authors refer to AEs of different grade. In Fig. 1, authors refer to SAEs. AEs of different grade, for example 2 or higher, may not automatically classify as SAEs. In addition, SAEs may not be therapy related, e.g., hospitalization because of accident related trauma. The authors should distinguish between cell implant related and unrelated SAEs and clarify this in the methods section.

	Related to the comments above: the authors state that in the 2 treated patients in the pilot trial different types of arrhythmia (VT, SVT, Afib) were observed. If they occured for the first time in the study, these would very likely be considered SAEs, in case of a VT probably cell-implant related. Per study exclusion criteria, patients with arrhythmia would not be allowed to participate (lines 191-193). I assume similar exclusion criteria applied to the first 2 patients. In this case, there would be evidenced for arrhythmia induction in all of the so far treated patients, which would be in line with observations in preclinical studies in large animals. The finding of no malignant arrhythmia is important, but the concern that VTs are not innocent bystander observations remains, especially in patients without an ICD remains. Finally, exclusion of patients with ICD and CRT (lines 181-183) is not aligned with the inclusion criteria of HFrEF with EF ≤35% and optimal standard of care (lines 171-172). Current guidelines for the treatment of patients with advanced heart failure with an EF ≤35% recommend ICD implantation. SPIRITreporting guidelines should be mentioned and Chan et al. paper cited as requested in the Instructions to authors.

REVIEWER	Hare, Joshua University of Miami School of Medicine, Interdisciplinary Stem Cell Institute
REVIEW RETURNED	25-Jan-2022

GENERAL COMMENTS	In this manuscript, Zhang et al. provide a detailed description of the methodologies being used in their phase I clinical trial on pericardial injections of allogeneic human stem cells during CABG. Thank you for your response regarding the single-blinded comment, where you indicated that you would remove “single-blinded” from the submitted manuscript. However, under study design, "single-blinded" remains in the body of the text. If you plan to keep this terminology, you please provide as detailed an explanation within in the body of your paper as in your response to the reviewer. In your response to autologous vs. allogenic stem cell therapy you wrote, "Especially, it is not realistic for cardiac repair in a large patient population[s]; Secondly, It is very time-consuming about 1 year for autologous HiPSC-CMs production, which limited the clinical application for severe heart failure patients". These statements are slightly misleading, and do not properly appreciate the breadth of previous studies such as the CHART-1 study regarding autologous stem cells injections and their feasibility. Teerlink, J. R., et al. (2017). "Benefit of cardiopoietic mesenchymal stem cell therapy on left ventricular remodelling: results from the Congestive Heart Failure Cardiopoietic Regenerative Therapy (CHART-1) study." European Journal of Heart Failure 19(11): 1520-1529. Additionally, please provide the relevant citations in the discussion section regarding the choice of using allogenic over autologous stem cell injections. Under the subheading "preparation of hiPSC-CMs" Please elaborate if stem cells are derived from a single donor or pooled donors, and if available, a brief description about the

	donor(s), i.e., were they young, genetically tested, their age, and sex.

VERSION 2 – AUTHOR RESPONSE

Responds to the reviewer's comments:

Reviewer#1

We thank the reviewer for the positive comments and suggestions on our work to improve the manuscript.

Comment 1: Method section, lines 250-256 and Fig 1: authors refer to AEs of different grade. In Fig. 1, authors refer to SAEs. AEs of different grade, for example 2 or higher, may not automatically classify as SAEs. In addition, SAEs may not be therapy related, e.g., hospitalization because of accident related trauma. The authors should distinguish between cell implant related and unrelated SAEs and clarify this in the methods section.

Response 1: It is really important as Reviewer suggested to clarify cell implant related and unrelated SAEs. In fact, we had a third-party Data and Safety Monitoring Board to judge whether SAEs were related to cell implant therapy (Line 376-377). We are very sorry for our incorrect descriptions of AEs grade and have corrected them as "grade 4 or above cell implant related adverse event". It now reads: If no grade 4 or above cell implant related adverse event occurs within 1 month post-operatively in the CABG + 1×10^8 group, dose escalation will proceed to 2×10^8 cells. If one grade 4 or above cell implant related adverse event occurs within 1 month post-operatively, three more patients will be enrolled and injected with 1×10^8 cells during CABG surgery. If no grade 4 or above cell implant related adverse event occurs in the second three-patient cohort, dose escalation will then proceed to 2×10^8 cells. Otherwise, the trial will be stopped. The dose escalation design is depicted in Figure 1(Line 248-255).

Comment 2: Related to the comments above: the authors state that in the 2 treated patients in the pilot trial different types of arrhythmia (VT, SVT, Afib) were observed. If they occured for the first time in the study, these would very likely be considered SAEs, in case of a VT probably cell-implant related. Per study exclusion criteria, patients with arrhythmia would not be allowed to participate (lines 191-193). I assume similar exclusion criteria applied to the first 2 patients. In this case, there would be evidenced for arrhythmia induction in all of the so far treated patients, which would be in line with observations in preclinical studies in large animals. The finding of no malignant arrhythmia is important, but the concern that VTs are not innocent bystander observations remains, especially in patients without an ICD remains. Finally, exclusion of patients with ICD and CRT (lines 181-183) is not aligned with the inclusion criteria of HFrEF with EF ≤35% and optimal standard of care (lines 171-172). Current guidelines for the treatment of patients with advanced heart failure with an EF ≤35% recommend ICD implantation.

Response 2: We gratefully appreciate for your valuable suggestion. As we all known, ICD and CRT treatments are recommended as an optimal treatment for patients with EF ≤35%. The previous description of "optimal standard of care" included the medical therapy and device implantation. It was inconsistent with the exclusion criteria. Now, this description has been replaced by "guideline-directed medical therapy", which only emphasize medical therapy. As we designed, cardiac MRI was a very essential assessment for all subjects. However, the ICD or CRT implantation was one

of contraindications for MRI examination. We have revised the inclusion and exclusion criterias of patients.

Comment 3: SPIRIT reporting guidelines should be mentioned and Chan et al. paper cited as requested in the Instructions to authors.

Response 3: Thank you for your introduction to these wonderful research work. According to your suggestion, we properly cite this article as: [26] Chan AW, Tetzlaff JM, Altman DG, et al. SPIRIT 2013 statement: defining standard protocol items for clinical trials. Annals of internal medicine 2013;158(3):200-7. doi: 10.7326/0003-4819-158-3-201302050-00583 [published Online First: 2013/01/09] (Line151-154)

Reviewer#2

We thank the reviewer for the helpful comments and suggestions on our work.

Comment 1: Thank you for your response regarding the single-blinded comment, where you indicated that you would remove “single-blinded” from the submitted manuscript. However, under study design, "single-blinded" remains in the body of the text. If you plan to keep this terminology, you please provide as detailed an explanation within in the body of your paper as in your response to the reviewer.

Response 1: We are very sorry for our negligence of this question. We have removed “single-blinded” from the new submitted manuscript.

Comment 2: In your response to autologous vs. allogenic stem cell therapy you wrote, "Especially, it is not realistic for cardiac repair in a large patient population[s]; Secondly, It is very time-consuming about 1 year for autologous HiPSC-CMs production, which limited the clinical application for severe heart failure patients". These statements are slightly misleading, and do not properly appreciate the breadth of previous studies such as the CHART-1 study regarding autologous stem cells injections and their feasibility. Teerlink, J. R., et al. (2017). "Benefit of cardiopoietic mesenchymal stem cell therapy on left ventricular remodelling: results from the Congestive Heart Failure Cardiopoietic Regenerative Therapy (CHART-1) study." European Journal of Heart Failure 19(11): 1520-1529. Additionally, please provide the relevant citations in the discussion section regarding the choice of using allogenic over autologous stem cell injections.

Response 2: Thank you for your positive and constructive comments. The choice of using allogenic over autologous stem cell injections had following main reasons: (1) biobanking of a limited number of approved iPSC lines from various human leukocyte antigen (HLA)-homozygous donors that would match the majority of the population will be more efficient to provide large quantities of transplantable cells as an off-the-shelf-product from a quality controlled and rigorously tested production process; (2) regulatory clearance would be easier since any single line of such an iPSC bank could be thoroughly tested to be free of viral contamination, tumorigenicity and genome instability; (3) the patient could more effectively benefit from the off-the-shelf product more readily in critical subacute conditions such as advanced ischemic heart failure. We have provided relevant citations in the discussion section, it now reads: The allogenic approach can bring down the cost for iPSC-based cell therapy compared to the autologous approach and will also obviate the need for approval of individual patient-derived products by regulatory authorities[27] (Line 394-397).

Comment 3: Under the subheading "preparation of hiPSC-CMs". Please elaborate if stem cells are derived from a single donor or pooled donors, and if available, a brief description about the donor(s), i.e., were they young, genetically tested, their age, and sex.

Response 3: Thank you for your comment. In our study, allogeneic human induced-pluripotent stem cell-derived cardiomyocytes are derived from a single donor. We have provided a brief description about donors. It now reads: Donors were screened and tested for relevant communicable disease agents and diseases, including HIV-1 (antigen and nucleic acid), HIV-2, hepatitis B virus (HBV, nucleic acid and surface and core antigen), hepatitis C virus (HCV, antigen and nucleic acid) and Treponema pallidum (syphilis), according to “Guidance for Industry: Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)” by FDA. In order to prevent promotion of delayed carcinogenesis, donors were also screened and tested by exome sequencing for target genes presumably responsible for primary somatic cell mutation in cancer, according to COSMIC, an existing cancer genome mutation database. A health, 28 years old, Chinese female, who met the criteria of donor eligibility tests was selected. Her peripheral mononucleate cells were collected and reprogrammed to induced pluripotent stem cells under current good manufacturing practice (cGMP) condition (Line 225-236).

We tried our best to improve the manuscript and made some changes in the manuscript. These changes will not influence the content and framework of the paper. And here we did not list the changes but marked in yellow in revised paper.

We appreciate for Editors/Reviewers’ warm work earnestly, and hope that the correction will meet with approval. Once again, thank you very much for your comments and suggestions.

VERSION 3 – REVIEW

REVIEWER	Zimmermann, Wolfram-Hubertus Georg August University Gottingen University Medical Center, Pharmacology and Toxicology
REVIEW RETURNED	11-Apr-2022

GENERAL COMMENTS	I would like to thank the authors for addressing my critiques.

REVIEWER	Hare, Joshua University of Miami School of Medicine, Interdisciplinary Stem Cell Institute
REVIEW RETURNED	07-Apr-2022

GENERAL COMMENTS	Revisions are appropriate. Final read through for grammar may be necessary but overall well written protocol.