Author’s response to reviews

Title: Rationale and design of a prospective, randomised study of retrograde application of bone marrow aspirate concentrate (BMAC) through coronary sinus in patients with congestive heart failure of ischemic etiology (The RETRO study)

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Rationale and design of a prospective, randomised study of retrograde application of bone marrow aspirate concentrate (BMAC) through coronary sinus in patients with congestive heart failure of ischemic etiology (The RETRO study)

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BMC Cardiovascular Disorders

Dear Reviewers,

Thank you for your kind advices. We have accepted all your recommendations.

The changes are marked in red in the manuscript.

Technical Comments:

1. Please rename 'Availability of supporting data' to 'Availability of data and material'.
2. Please rename 'Financial disclosure' to 'Funding'.

- We renamed it

Reviewer reports:

Amit Patel (Reviewer 1):

This is well written manuscript from a group who has previously successfully used bone marrow based cell therapy in other clinical applications. The use of retrograde BMAC delivery has been previously described in the REVIVE Trial. However, that was a randomized open label trial in a similar group of patients. The authors describe a prospective blinded study with MRI end-points. The following questions need to be addressed:

1. Have there been statistics calculated for MRI drop out even if the patients are not a drop out to adequately power the trial?

- An expected loss of 10% of patients in the follow-up includes also MRI drop out. We added this information to the Manuscript.

2. Can KCCQ along with Minnesota Questionnaire/NYHA class be added as a clinical end point as KCCQ has been validated and accepted for heart failure trials by the FDA?

- We replaced a Minnesota QoL Questionaire with KCCQ-12.

3. What will be training required to adequately perform the procedures? This is very relevant as in the discussion the authors describe a low retention rate for retrograde. There are other groups which have found up to 30% retention at 24hrs in the heart.

- The procedure will be performed by experienced interventional cardiologist and the training of coronary sinus canulation and retrograde delivery was performed on animal (pig) model.

4. Can patients with BiV-ICD be included in the trial due to the lead being in the coronary sinus?

- No, due to safety reasons. We included this information into the Discussion section.
5. Can a baseline holter be performed as there is only one other time in the trial and should have a reference to compare?

- We agree and we added a baseline ECG Holter monitoring in the Study Schedule.

6. There are many other clinical trial which have use retrograde coronary sinus delivery but they have not been used in the introduction of discussion to demonstrate there potential benefits or shortfalls as related to this trial.

- We added further clinical trials into the Discussion section.

Amish Raval (Reviewer 2):

The authors provide an interesting trial design manuscript, that will test a novel therapy/administration approach in a desperate patient population with tremendous unmet needs. The trial will test the safety and possibly efficacy of retrograde transcoronary sinus administration of autologous bone marrow mononuclear cell treatment in chronic ischemic HF with NYHA class 3-4 patients. Few items were noticed:

1. NYHA class 3 patients are fine, but NYHA class 4 patients may not yield data for 6 minute walk distance, and are a rather unstable patient cohort. Further, this can be a challenging patient population to enroll in HF trials. Please discuss the rationale for including class 4 patients.

- We agree and we removed the class 4 patients from the study.

2. HF stability for 1 month is should be better defined (does this mean no outpatient diuretic variation?). 1 month is rather short to prove stability - perhaps this rationale should be discussed further.

- We added this information into the Discussion section.

3. The authors should provide the parameters of what would constitute "standard HF treatment", citing recent National Guideline statements, and indicating efforts to optimize standard dosing. Furthermore, how long after a CRT device can a subject be enrolled?

- We added this information into the Discussion section.
4. There is no effort to blind subjects, which makes the trial results highly susceptible to placebo effect and potential subject drop out. This will be a serious limitation to the study conclusions. Please discuss.

- We discussed it in the Discussion section.

5. What is the sedation/anesthesia protocol for 240mL aspirate?

- We added this information into the Manuscript.

6. Will right heart catheterization be done before and after treatment?

- No, right heart catheterization will be only as a sham procedure in the placebo group.

7. Comment on the feasibility and published experience of coronary sinus infusion and cardiac resynchronisation LV lead.

- We included this information into the Discussion section.

8. How will the investigators evaluate for pulmonary embolism with infusing a platelet rich cell mixture?

- In case of clinical suspicion to pulmonary embolism an angio CT examination would be performed.

9. The study population will be variable consists of recent MI, only excluding patients <1 week post MI. A pt with HF considered for enrollment day 8 post MI would not be chronic heart failure. This should be clarified.

- We added this information into the Discussion section.

10. There are numerous grammatical and spelling errors throughout.

- We corrected it.
11. Is there a GFR cutoff for the exclusion criteria?
- We added this information into the Manuscript.

12. Authors should cite: PMID26217065, PMID29803986 as other relevant un-fractionated BMNC trials completed or ongoing.
- We added this citation into the Discussion section.

Joshua Hare (Reviewer 3):

The RETRO study will evaluate the efficacy of BMAC in patients with Ischemic Cardiomyopathy. This randomized, controlled study will evaluate cardiac function and geometry and 6-minute walk test as primary endpoints. Secondary endpoints will evaluate arrhythmogenic adverse events and quality of life parameters.

The author's novel study is based on the cell characteristics of autologous non-selected bone marrow cells delivered by retrograde methodology.

Major comments:

- Please provide a more updated description of the current donor sources or state of the art in the stem cell field. Your statement on line 37-38 page 6, until last paragraph is not accurate.
  - We corrected it.

- Please provide reference to studies that have shown efficacy and safety of BMAC on line 54-59 page 6.
  - We corrected it.

- In the Background and Discussion, please provide more clinical trial references and fewer reviews.
  - We remade the Discussion section.
- The authors state that cardiac function will be measured by MRI. Please consider including scar size measurement in your parameters as a decrease in fibrosis has been one of the main outcomes in the stem cell field.

- We included these parameters in the Protocol.

- Please include in the Discussion some of previous trials that have utilized retrograde delivery of cells; including the REVIVE Trial: Retrograde Delivery of Autologous Bone Marrow in Patients With Heart Failure (2015), that used a very similar type of cell and methodology of delivery. Others that also used a similar approach albeit in different pathologies: -Silva SA, Sousa AL, Haddad AF, Azevedo JC, Soares VE, Peixoto CM, et al. Autologous bone-marrow mononuclear cell transplantation after acute myocardial infarction: comparison of two delivery techniques. Cell Transplantation. 2009; 18(3):343-352.


- We included them into the Discussion section
Minor comments

- Please review the reference style because references in the discussion appear in a different format.
- We corrected it.

- Page 11 line 44: where the authors mention the increase in dP/dt, are they referring to dp/dt max or min?
- We corrected it.

Line 54 of page 11: Transdifferentiation is missing a letter "d".
- We corrected it.

Khawaja Haider (Reviewer 4):

Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format. Please overwrite this text when adding your comments to the authors.

Dr Pleva and Colleagues have submitted rationale and design of a prospective study entitled "Rationale and design of a prospective, randomised study of retrograde application of bone marrow aspirate concentrate (BMAC) through coronary sinus in patients with congestive heart failure of ischemic etiology (The RETRO study)". The study is intended to include 40 patients who will be divided in to two groups of control (without treatment) and experimental group (who would be treated with bone marrow aspirate by retrograde coronary infusion) for myocardial cell therapy. I have following comments:

1. The inclusion criteria states that the patients included in the study would be 18 years or older in age. Such inclusion criteria will include a diverse age group of patients. How can the researchers would compare autologous transplantation of young autologous cells from a (for example) 30 year old patient injected into a young recipient heart of 30 years as compared to another patient included in the study who would be (for example) 75 years of age (75 years aging donor cells in to a 75 years aging heart). Please refer to a recently published Paper in
Regenerative Medicine 2018; 13(4), 457-75 and discuss your proposed inclusion criteria in the light of this paper. There is plenty of evidence in literature that aging of the donor is associated with declined function of the donor cells in cell therapy (For example, please refer to "Aging is associated with decreased maximal life span and accelerated senescence of bone marrow stromal cells. Bone 33(6), 919-926 (2003).

- We changed the Inclusion criteria and added this informations into the Discussion section

2. Quality of cell preparation remains fundamental to any cell therapy procedure. The authors have not mentioned any quality control of their cell preparation, not even to measure the cell viability. (Please refer to the article: "The modest outcome of clinical trials with bone marrow cells for myocardial repair: is the autologous source of cells the prime culprit? J. Thorac. Dis. 8(10), E1371-E1374 (2016)." Please take into serious consideration how can you have good and uniform quality of cell preparation in all patients included in the study.

- We added this information into the Discussion section

3. The number of cells to be injected is a critical factor that would determine the outcome of the cell therapy procedure (as discussed by the authors in their discussion section). However, the cell preparation for injection would be based solely on volume of cell preparation and not the number of cells. Thus, it is anticipated that the number of cells injected in each patient would be different. Therefore, it would be highly difficult to compare the effect of cell therapy in patients in the treatment group who had received same volume of cell preparation but containing different cell number.

- We added this information into the Protocol and Discussion section

4. The composition of the cell preparation in terms of constituent cell population should be determined in terms of BM cells (surface markers for hematopoietic and non-hematopoietic populations), platelets, granulocytes etc. As the authors of the study design are anticipating the role of cytokine and growth factors (bioactive molecules) secreted by these constituents of their cell preparation, it would be prudent to characterize the cell preparation for each patient.

- We added this information into the Protocol and Discussion section
With best regards,

Leos Pleva, MD, PhD.