PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Safety and efficacy of amnion-derived mesenchymal stem cells (AM01) in patients with steroid-refractory acute graft-versus-host disease after allogeneic haematopoietic stem cell transplantation: A study protocol for a phase I/II Japanese trial
AUTHORS	Yamahara, Kenichi; Hamada, Akiko; Soma, Toshihiro; Okamoto, Rika; Okada, Masaya; Yoshihara, Satoshi; Yoshihara, Kyoko; Ikegame, Kazuhiro; Tamaki, Hiroya; Kaida, Katsuji; Inoue, Takayuki; Ohsugi, Yuko; Nishikawa, Hiroki; Hayashi, Hiroshi; Ito, Yoichi; Iijima, Hiroaki; Ohnishi, Shunsuke; Hashimoto, Daigo; Isoe, Toshiyuki; Teshima, Takanori; Ogawa, Hiroyasu; Sato, Norihiro; Fujimori, Yoshihiro

VERSION 1 - REVIEW

REVIEWER	Nikolas von Bubnoff UNIVERSITÄTSKLINIKUM FREIBURG, Klinik für Innere Medizin I Hämatologie, Onkologie und Stammzelltransplantation, Hugstetter Str. 55 79106 Freiburg, Germany
REVIEW RETURNED	30-Oct-2018

GENERAL COMMENTS	Yamahara et al. report a first-in-human, multicenter, single arm, open label trial of amnion-derived MSC (AM01) in patients with steroid-refractory aGVHD. The primary endpoint will be safety of intravenous infusion therapy of AM01 within 24 hours after intravenous infusion, the secondary endpoint included exploratory efficacy of AM01. The trial is IRB approved and registered at UMIN. The trial addresses SR-aGvHD with up to one treatment line in addition to steroids. Dose escalation will follow a two-step 3 + 3 design without dose expansion at the second (high) dose step. Safety analysis will be performed after 3 pts in each dose group. Rules for continuation of other immunosuppressive medications including steroids are specified. Comments: − The trial should be registered at clinicaltrials.gov − Time of safety analysis after patient three in each group should be specified − Process of isolation of amnion-derived MSC from volunteer donors including pre-specified quality criteria for clinical application as IMP should be specified − Why did the authors decide to NOT include a dose expansion at the high dose? 12 patients max might not be sufficient to generate preliminary estimate of efficacy − Manuscript might benefit from proof-reading by native speaker

REVIEWER	Vincent F La Russa, PhD University of Louisville USA
REVIEW RETURNED	21-Nov-2018

GENERAL COMMENTS	NEED INFORMATION AND CORRECTIONS ON ALL OF GTHE FOLLOWING: No Abstract was provided The writing is in the future tense and overall needs to be rewritten by a translator with full english grammar there are no data provided the manuscript needs to conform to journal format THERE IS NO PATIENT OUTCOME DATA ONLY A FLOW DIAGRAM WAS THE STUDY COMPLETED? NEED MORE REFERENCES NO INFORMATION ON MSCS PROCUREMENT METHODS, MANUFACTURING, STORAGE, NUMBER OF PASSAGES, ..., ETC Characterization data of product with positive and negative controls More references on pre-clinical and clinical studies on the Amniotic Mesenchymal Stem, Cells, Viability of cells prior to iv administration.

REVIEWER	Shahrukh Hashmi KFSHRC & Mayo Clinic, USA
REVIEW RETURNED	29-Nov-2018

GENERAL COMMENTS	Yamihara et al. present the plan for their phase I/II study with amnion derived MSCs for GVHD. I have the following concerns: Stopping rules: Why is rapid progression of GVHD after infusion not a stopping rule? Selection: It is unclear if cords and haploidentical transplants will be included or not

VERSION 1 – AUTHOR RESPONSE

To Reviewer 1

We are grateful for your helpful comments on our manuscript. We have addressed all of your comments and revised the paper accordingly. The changes and additions to the manuscript are shown in red font. We believe that all the points you raised have been clarified by the revised manuscript.

Your comments and our replies are shown below:

>1. The trial should be registered at clinicaltrials.gov

Following your advice, we will arrange to register our trial at clinicaltrials.gov.

>2. Time of safety analysis after patient three in each group should be specified

To specify the time of safety analysis after patient three (more than 28 days), we modified our manuscript in the study protocol section (line 11 page 10)

>3. Process of isolation of amnion-derived MSC from volunteer donors including pre-specified quality criteria for clinical application as IMP should be specified

Following your comment, we revised our manuscript to add the section for the preparation of amnion-derived MSCs (page 16-17)

>4. Why did the authors decide to NOT include a dose expansion at the high dose? 12 patients max might not be sufficient to generate preliminary estimate of efficacy.

Based on the result of the toxicity test (page 15), we decided the administration dose of the high-dose group as 4.0×106 cells/kg. We confirmed that intravenous administration of less than 40×106 cells/kg in SCID mice is safe and the regulatory authority advised us to set 1/10 of the maximum safety dose as the upper limit of clinical dose.

As you pointed out, 12 patients max might not be sufficient to generate preliminary estimate of efficacy. This is the first clinical trial to use human amnion-derived MSCs (AM01) in human and we intended to confirm the safety for intravenous administration of AM01 in this clinical protocol, its primary endpoint is the occurrence of adverse events related to acute infusion toxicity.

>5. Manuscript might benefit from proof-reading by native speaker

Following your comment, our manuscript is now proof-read by native speaker.

Manuscript ID bmjopen-2018-026403

Title: Safety and efficacy of amnion-derived mesenchymal stem cell (AM01) in patients with steroid-refractory acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation

To Reviewer 2

We are grateful for your helpful comments on our manuscript. We have addressed all of your comments and revised the paper accordingly. The changes and additions to the manuscript are shown in red font. We believe that all the points you raised have been clarified by the revised manuscript.

Your comments and our replies are shown below:

>1. No Abstract was provided.

Please find the Abstract of our manuscript on page 4.

>2. The writing is in the future tense and overall needs to be rewritten by a translator with full English grammar.

Following your comment, our manuscript is now proof-read by native speaker.

>3. There are no data provided. The manuscript needs to conform to journal format. There is no patient outcome data only a flow diagram. Was the study completed?

Following your comment, we revised our manuscript with the Journal's format. BMJ Open will consider study protocol and our manuscript is written for the detailed protocol of our clinical trial.

>4. Need more references.

Following your comment, we added more references (page 27-29).

>5. No information on MSCs procurement methods, manufacturing, storage, number of passages, ..., etc

Following your comment, we revised our manuscript to add the section for the preparation of amnion-derived MSCs (page 16-17)

>6. Characterization data of product with positive and negative controls

In our previous paper entitled “Comparison of Angiogenic, Cytoprotective, and Immunosuppressive Properties of Human Amnion- and Chorion-Derived Mesenchymal Stem Cells” (PLoS ONE 9(2): e88319), we characterized human amnion MSCs and assessed the therapeutic potential and difference compared to human chorion MSCs. We found that amnion MSCs markedly reduced T-lymphocyte proliferation with the enhanced secretion of PGE2 and improved the pathological situation of a mouse model of acute GVHD. In addition, we previously reported that amnion MSCs exhibited reduced Th1 and Th17 cell-differentiation and proliferation compared to bone marrow MSCs (J Mol Cell Cardiol. 2012 Sep;53(3):420-8).

Following your comments, we added the description including the characteristics of amnion MSCs (page 16-17).

>7. More references on pre-clinical and clinical studies on the Amniotic Mesenchymal Stem, Cells, Viability of cells prior to iv administration.

This is the first clinical trial to use human amnion-derived MSCs (AM01) in human and we intended to confirm the safety for intravenous administration of AM01 in this clinical protocol clinical. Following your comment, we added some references regarding pre-clinical and clinical studies using amnion-derived MSCs. (page 27-29).

Manuscript ID bmjopen-2018-026403

Title: Safety and efficacy of amnion-derived mesenchymal stem cell (AM01) in patients with steroid-refractory acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation

To Reviewer 3

We are grateful for your helpful comments on our manuscript. We have addressed all of your comments and revised the paper accordingly. The changes and additions to the manuscript are shown in red font. We believe that all the points you raised have been clarified by the revised manuscript.

Your comments and our replies are shown below:

>1. Stopping rules: Why is rapid progression of GVHD after infusion not a stopping rule?

As described in the criteria for stopping administration of AM01 in our manuscript (line 6 page 11), the investigators or sharing doctors can stop administration of AM01 if it is necessary withdraw from

the medical point of view. Therefore, if the rapid progression of aGVHD is happened, the administration of AM01 will stop in accordance with this criterion.

>2. Selection: It is unclear if cords and haploidentical transplants will be included or not.

We will intend to use our AM01 for patients with steroid-refractory aGVHD after allogeneic hematopoietic stem cell transplantation, including umbilical cord and haploidentical transplantation.

Following your comment, we added the description concerning umbilical cord and haploidentical transplants in our revised manuscript (line 15 page 5)