BMJ Open

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: A study protocol for phase I/II Japanese trial

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-026403
Article Type:	Protocol
Date Submitted by the Author:	30-Aug-2018

Complete List of Authors:	Yamahara, Kenichi; Hyogo College of Medicine, Laboratory of Medical Innovation, Institute for Advanced Medical Sciences; Hyogo College of Medicine, Center for Transfusion Medicine and Cellular Therapy Hamada, Akiko; Hyogo College of Medicine, Laboratory of Medical Innovation, Institute for Advanced Medical Sciences Soma, Toshihiro; Hyogo College of Medicine, Center for Transfusion Medicine and Cellular Therapy Okamoto, Rika; Hyogo College of Medicine, Center for Clinical Research and Education Okada, Masaya; Hyogo College of Medicine, Division of Hematology, Department of Internal Medicine Yoshihara, Satoshi; Hyogo College of Medicine, Center for Transfusion Medicine and Cellular Therapy Yoshihara, Kyoko; Hyogo College of Medicine, Division of Hematology, Department of Internal Medicine Ikegame, Kazuhiro; Hyogo College of Medicine, Division of Hematology, Department of Internal Medicine Tamaki, Hiroya; Hyogo College of Medicine, Division of Hematology, Department of Internal Medicine Kaida, Katsuji; Hyogo College of Medicine, Division of Hematology, Department of Internal Medicine Inoue, Takayuki; Hyogo College of Medicine, Division of Hematology, Department of Internal Medicine Ohsugi, Yuko; Hyogo College of Medicine, Division of Hematology, Department of Internal Medicine Nishikawa, Hiroki; Hyogo College of Medicine, Center for Clinical Research and Education Hayashi, Hiroshi; Hokkaido Daigaku Byoin Itoh, Yoichi; Hokkaido University, Department of Biostatistics Iijima, Hiroaki; Hokkaido University Hospital, Clinical Research and Medical Innovation Center Ohnishi, Shunsuke Hashimoto, Daigo; Hokkaido University, Department of Hematology Isoe, Toshiyuki; Hokkaido Daigaku Byoin Teshima, Takanori; Hokkaido University, Department of Hematology Ogawa, Hiroyasu; Hyogo College of Medicine, Division of Hematology, Department of Internal Medicine

	Sato, Norihiro; Hokkaido Daigaku Byoin Fujimori, Yoshihiro; Hyogo College of Medicine, Laboratory of Medical Innovation, Institute for Advanced Medical Sciences
Keywords:	Clinical trials < THERAPEUTICS, HAEMATOLOGY, Blood bank & transfusion medicine < HAEMATOLOGY

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: A study protocol for phase I/II Japanese trial

Author names (academic degree) and affiliation

Kenichi Yamahara[1,2,3], Akiko Hamada[1], Toshihiro Soma[2], Rika Okamoto[4], Masaya Okada[3], Satoshi Yoshihara[2,3], Kyoko Yoshihara[3], Kazuhiro Ikegame[3], Hiroya Tamaki[3], Katsuji Kaida[3], Takayuki Inoue[3], Yuko Ohsugi[3], Hiroki Nishikawa[4], Hiroshi Hayashi[5], Yoichi M. Ito[6], Hiroaki Iijima[5], Shunsuke Ohnishi[7], Daigo Hashimoto[8], Toshiyuki Isoe[5], Takanori Teshima[8], Hiroyasu Ogawa[3], Norihiro Sato[5], Yoshihiro Fujimori[1,2,3]

1. Laboratory of Medical Innovation, Institute for Advanced Medical Sciences, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan
2. Center for Transfusion Medicine and Cellular Therapy, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan
3. Division of Hematology, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan

4. Center for Clinical Research and Education (CCRED), Hyogo College of Medicine, Nishinomiya, Hyogo, Japan.

5. Clinical Research and Medical Innovation Center, Hokkaido University Hospital, Sapporo, Hokkaido, Japan.

6. Department of Biostatistics, Hokkaido University Graduate School of Medicine, Sapporo, Hokkaido, Japan.

7. Department of Gastroenterology and Hepatology, Hokkaido University Graduate School of Medicine, Sapporo, Hokkaido, Japan.

8. Department of Hematology, Hokkaido University Faculty of Medicine, Sapporo, Hokkaido, Japan.

Corresponding author: Kenichi Yamahara

Kenichi Yamahara, MD, PhD, Laboratory of Medical Innovation, Institute for Advanced Medical Sciences, Hyogo College of Medicine, Hyogo, Japan. 1-1, Mukogawa-cho, Nishinomiya, Hyogo, 663-8501, Japan.

Tel: +81-798-45-6398; Fax: +81-798-45-6119

E-mail: yamahara@hyo-med.ac.jp

Running title: Amnion-derived mesenchymal stem cell and steroid-refractory acute

GVHD

Abbreviations

aGVHD; acute graft-versus-host disease, HSCT; hematopoietic stem cell transplantation, AHSCT; allogenic hematopoietic stem cell transplantation, ATG; Anti-thymocyte globulin, MMF; mycophenolate mofetil, MSC; mesenchymal stromal cell, ESAC; efficacy and safety assessment committee, AE; adverse event, CR; complete response, PR; partial response, OS; overall survival.

Word count: 2,907 words

◆ Abstract

Introduction and aims

Regenerative medicine and cell therapies have been gaining much attention among clinicians. Therapeutic infusion of mesenchymal stromal cells (MSCs) is now a leading investigational strategy for the treatment of acute graft-versus-host disease (aGVHD). Bone marrow MSC is approved for manufacture and marketing as a product for regenerative medicine for aGVHD. In our non-clinical studies, we confirmed that human amnion-derived MSC had immunomodulatory activity equal to or higher than that of human bone marrow MSC. In this study, we will aim to evaluate the safety and efficacy of amnion-derived MSC (AM01) in patients with steroid-refractory aGVHD.

Methods and analysis

This study will be a multicenter single arm open label trial (interventional study involving invasion to humans). This clinical trial begins with the low dose group, and when the safety is confirmed in at least 3 cases in the low-dose group, it is transferred to the high-dose group and the safety of the high-dose group will be verified. The primary endpoint is to assess the safety of intravenous infusion therapy of AM01 within 24 hours after intravenous infusion of AM01 and the secondary endpoint is to exploratory assess the efficacy of intravenous infusion therapy of AM01.

Ethics

The Institutional Review Board at all participating hospitals approved this study protocol. Final data will be publicly announced. A report releasing the study results will be submitted for publication to an appropriate peer-reviewed journal.

Trial registration

UMIN000029945 (https://upload.umin.ac.jp/); pre-results. No patient is registered at the submission of our manuscript.

Strengths and limitations of this study

(1) This study is the first-in-human clinical trial. Safety of intravenous infusion therapy of amnion-derived mesenchymal stem cell (AM01) for patients with steroid-refractory acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation will be verified.

(2) This study limitations are that our study results will be limited to a Japanese population, and validation studies on other ethnic backgrounds will be needed and that the target sample size is small.

Key words

Amnion-derived mesenchymal stem cell, steroid-refractory acute GVHD, Safety, Efficacy

◆ Introduction

Acute graft-versus-host disease (aGVHD) is a condition in which immune cells from the donor attack recipient tissues. [1] In cases with hematopoietic stem cell transplantation (HSCT), aGVHD is classified into classical aGVHD developed within 100 days after transplantation and atypical aGVHD after 100 days. Classical aGVHD is a clinical entity presenting clinical symptoms such as maculopapular rash, nausea, vomiting, diarrhea, watery diarrhea, ileus and cholestatic hepatitis. [1] aGVHD remains the second leading cause of death following allogeneic HSCT (AHSCT). Over the last decade, the advance in understanding the pathophysiology of this immune based-process helped redefine graft *vs.* host reaction and opened new possibilities for novel preventive and therapeutic approaches. [2]

Preventive measures for aGVHD are commonly undertaken when AHSCT is performed, however, about half of patients develop aGVHD even if preventive measures are adequately undertaken. [3] When aGVHD develops, standard therapy with corticosteroid drugs is performed, however, nearly half of cases with aGVHD are steroid-refractory. [4 5] Anti-thymocyte globulin (ATG), mycophenolate mofetil (MMF) and steroid pulse therapy are selected as second-line therapies for steroid-refractory aGVHD. [4 5] However, none of the clinical trials has shown their usefulness, and in

Japan there is nothing approved except ATG. These second-line therapies may improve the symptoms of aGVHD in some cases. However, there are many treatment related complications such as severe infections due to excessive immunosuppression, and the mortality rate of standard treatment-refractory aGVHD cases is reported to be 70%. [6] Therefore, if an effective therapy for steroid-refractory aGVHD is established, the outcome of AHSCT will improve and it also can be expected to lead to the dramatic improvement for the prognosis of various hematopoietic malignancies and non-malignant diseases. For this reason, new molecular targeted therapies and development of cell therapies are currently being promoted worldwide. [7]

Therapeutic infusion of mesenchymal stromal cells (MSCs) is now a leading investigational strategy for the treatment of aGVHD. [8] MSCs exist in most tissues of human, with bone marrow, adipose and perinatal tissues representing the most common sources of cells utilized for clinical investigation. [9] MSC products have received approvals for steroid-resistant aGVHD in several countries. MSC has an anti-inflammatory effect (i.e., effect of strongly suppressing T cell differentiation and proliferation) and bone marrow MSC (TEMCELL® HS Inj) is approved for manufacture and marketing as a product for regenerative medicine for aGVHD. However, since the sample collection of bone marrow MSC is an invasive procedure, more accessible

source of MSC is ideal.

Amnion-derived MSCs are undifferentiated cells present in the amniotic membrane and have the ability to differentiate into various cells belonging to the mesenchymal system such as muscle, bone, cartilage, fat and the like, self-replication ability and immunosuppressive action. [10-13] In our previous studies, we confirmed that human amnion (amniotic membrane of pregnant women (medical waste))-derived MSC had immunomodulatory activity equal to or higher than that of human bone marrow MSC, demonstrated the effect on aGVHD and inflammatory bowel disease model, and there were no significant safety problems of human amnion-derived MSC in non-clinical safety tests. [10 12 13] Furthermore, we are pursuing clinical research of cell therapy in aGVHD and Crohn's disease using amnion-derived MSCs, which have more stem cells (easy to mass culture), higher proliferative capacity, and less invasiveness to sample collection and less frequency to rejection compared with bone marrow MSC. [14] Using our original cell culture serum NeoSERA@, its main component is adult bovine platelet rich-plasma, we have succeeded in formulating the amnion-derived MSC as the investigational drug for the first time in the world. Based on these backgrounds, in this study, we will aim to evaluate the safety and efficacy of amnion-derived MSC (trial product name, AM01) in patients with steroid-refractory aGVHD after AHSCT.

◆ Patient eligibility criteria

Inclusion criteria

(1) Patients diagnosed as grade 2 or more steroid-refractory acute GVHD after AHSCT. Assessment of the severity of aGVHD is based on the guidelines of Japanese Society for Hematopoietic Cell Transplantation. [15]

(2) Patient between 15 and 80 years old at the consent acquisition.

(3) Patients who have written informed consent under the sufficient understanding for the contents of this trial or those who have written informed consent by substitute (e.g, persons who exercise custody of the subject) if the patient is under 20 years old (written informed consent is also obtained by the subject as much as possible).

Exclusion criteria

1. Patients who have received more than one treatment other than steroid therapy for aGVHD.
2. Patients with elevated liver enzyme other than aGVHD related liver dysfunction (serum total bilirubin >2.0mg/dl or serum aspartate aminotransferase/alanine aminotransferase >3 times upper limit of normal).
3. Patients with renal dysfunction (serum creatinine >2.0mg/dl).
4. Patients with percutaneous oxygen saturation <94% even under oxygen

administration.

5. Patients with active HBV or HBC infection (Serum HBV-DNA >2,000 IU/ml or HCV-RNA >25 IU/ml) within high-risk for hepatitis (positive for any of hepatitis B antigen/antibody including HBs-Ag, HBs-Ab, HBc-Ab, and/or HCV-Ab).
6. Patients positive for HIV-Ab.
7. Patients with uncontrolled severe infection.
8. Patients with severe hypersensitivity to bovine-derived constituents, human serum albumin and gentamicin.
9. Patients with past history for hypersensitivity to iodine or iodine-containing contrast agent.
10. Patients with previous participation in a study of any treatment with regenerative medical products.
11. Patients with participation in any clinical trial within 12 weeks of consent for this study.
12. Patients with pregnancy or breast-feeding.
13. Patients considered unsuitable for the study as determined by the principal investigators or sharing doctors.

◆ Study protocol

Study design and protocol

This study will be a multicenter single arm open label trial (interventional study involving invasion to humans). This clinical trial begins with the low-dose group, and when the safety is confirmed in at least 3 cases in the low-dose group, it is transferred to the high-dose group and the safety of the high-dose group will be verified. At twenty-eight days after the initial administration in the first case of each group, and in the three patients in each group, the efficacy and safety assessment committee (ESAC) will evaluate the safety profile and the validity of the next case registration or dose group transition will be discussed. (Figure 1) After intravenous infusion of AM01, efficacy is evaluated until efficacy is judged to be inadequate and then safety assessment will be undertaken at 52 weeks after initial administration or until discontinuation criteria are reached.

Criteria for stopping administration of AM01

(1) The patient wishes to stop receiving the administration of AM01.

(2) The patient becomes impossible to continue the administration of AM01 because he/she is transferred to another hospital during the trial.

(3) When it turns out that the patient is ineligible as a trial subject after starting the trial.

(4) When administration of AM01 becomes difficult due to the serious adverse events (AEs).

(5) In the event that the investigators or sharing doctors determine that it is necessary to withdraw from the medical point of view.

Criteria for determining as an inadequate effectiveness

(1) When complete response (CR) or partial response (PR) could not be obtained between 4 and 24 weeks after the initial administration of AM01.

◆ Intravenous infusion of AM01

Dose and administration period

Intravenous infusion of AM01 will be performed at day 0, 7, 14, and 21 according to the following dosage. The dose will be calculated based on the body weight in the screening period.

1) Low-dose group: 1.0×10^6 cells/kg (day 0, 7, 14 and 21)

2) High-dose group: 4.0×10^6 cells/kg (day 0, 7, 14 and 21)

Study subjects will receive the same donor's AM01. The AM01 will be infused intravenously with 1 mL/min slowly as a guide. Since there is a possibility that cellular embolism, thrombus formation and intravascular hemolysis may develop as a risk

resulting from intravenous administration of allogeneic cells, it will not exceed 1.5 mL/min.

For intravenous drip infusion of AM01, administration will start within 2 hours after thawing and finish within 3 hours. The following premedication will be administered intravenously 30 minutes to 1 hour prior to the start of administration of AM01.

1. Hydrocortisone sodium succinate (Solu-Cortef® or Succizone®) 100 mg

2. d-Chlorpheniramine maleate (Polaramine®) 5 mg

Rationale for administration dose and administration period

In the repeated intravenous administration test of AM01 using severe combined immunodeficiency (SCID) mice, 0, 4, 20 and 40×10^6 cells/kg once a week, was administered in a total of four times. In these results, deaths during the test period and deterioration of respiratory status after administration of AM01 were not observed. During the administration period and during the drug withdrawal period, there were no significant changes that could be attributed to AM01 in general condition, body weight, food intake, ophthalmological examination, urinalysis, blood biochemical examination and autopsy.

Human bone marrow MSC (TEMCELL® HS Inj) approved in Japan is targeted for grade 2 or more aGVHD and human bone marrow MSC at 2×10^6 cells/kg twice a week

for a total of 8 treatments is the approved protocol. In this study, 4×10^6 cells/kg are administered once a week for a total of 4 times in the high-dose group, which is the same as the number of cells per week, and the total number of cells to be administered in Thamesel. This study is the first-in-human clinical study and the primary endpoint is the safety of AM01. Thus, the current study dose and administration period was set after full consideration of safety data in Human bone marrow MSC (TEMCELL® HS Inj).

Prohibited drug

Between the first administration and 4 weeks after the first administration of AM01, the use of a new drug or therapy including TEMCELL® HS Inj for the treatment of aGVHD will be prohibited (steroid external medicine can be used together).

If study subjects are receiving prophylactic therapy for aGVHD or treatment for aGVHD with the following drugs before administration of AM01, their dose will not be increased between the first administration and 4 weeks after the first administration of AM01;

a）Prophylaxis for aGVHD：methotrexate、cyclosporine、tacrolimus、ATG、MMF、corticosteroid

b) Treatment for aGVHD：corticosteroid

◆ Outcome measure

Primary endpoints

To assess the safety of intravenous infusion therapy of AM01 within 24 hours after intravenous infusion of AM01.

Secondary endpoints

To exploratory assess the efficacy of intravenous infusion therapy of AM01.

(1) The proportion of patients with CR that lasts 28 days or more.

(2) The proportion of patients with CR or PR at 4 weeks after the first infusion of AM01.

(3) The following items at 8, 12, 16, 20 and 24 weeks after the first intravenous infusion of AM01 will be checked: (i) the severity of aGVHD; (ii) the presence or the severity of chronic GVHD; (iii) overall survival (OS); (iv) presence of the recurrence of the primary disease; (v) presence of severe infection; (vi) the total dosage of steroid therapy.

To exploratory assess the overall safety of intravenous infusion therapy of AM01

The overall assessment of AEs within 52 weeks after the first intravenous infusion of AM01 will be done.

Definition of AEs

AEs indicate all undesirable or unintended diseases or laboratory disorders and symptoms that occurred in subjects after the initial administration and 52 weeks after the initial administration of AM01 or until the withdrawal. It does not matter the causal relationship with AM01. If the primary disease worsens, we will treat it as an AE. Grading of AEs will be based on CTCAE v4.0-JCOG.

Definition of treatment response

In this trial, assessment of the therapeutic response will be made according to the following criteria.

(1) CR: All organ failure due to aGVHD disappears.

(2) PR: Clinical stage in at least one diseased organ improves and the clinical stages of other diseased organs do not worsen.

(3) MR (mixed response): Clinical stage in at least one diseased organ has improved, but the clinical stage of another diseased organ worsened.

(4) PG (Progression): Clinical stage in at least one diseased organ deteriorates and the clinical stages of other diseased organs do not improve.

(5) NC (No Change): In cases where neither improvement nor deterioration is observed in any diseased organ.

◆ Evaluation by ESAC

For the purpose of confirming whether the trial is being carried out safely and appropriately, an ESAC will be established.

Periodical assessment

The chairman of the ESAC will hold a committee to conduct periodic evaluation (every six months) at the time specified in the trial implementation plan after starting the trial.

Extraordinary assessment

The chairman of the ESAC will promptly hold a committee and make a temporary assessment of the validity of trial continuation when receiving a report of serious problem of this trial.

◆ Standard of care (Response at the occurrence of AEs)

If the investigators or sharing doctors know the occurrence of AEs, appropriate measures to study subjects will be undertaken immediately and at the same time an explanation to the subject will be given and it will be reported to relevant departments.

◆ Case registration period

December 11, 2017 to December 31, 2019

◆ Statistical methods

Definition of safety analysis set

Among the subjects who obtained consent, the group excluding the subjects that satisfy the following conditions will be regarded as the safety analysis set.

(1) Subjects in whom AM01 was never administered.

Definition of efficacy analysis set

Among the subjects who obtained consent, the group excluding subjects falling under any of the following conditions will be regarded as the efficacy analysis set.

1) Subjects in whom AM01 was never administered.

2) Subjects whose selection criteria violation or exclusion criteria conflict were found after registration.

3) Subjects missing all data of efficacy of AM01.

Statistical analysis

The data of this study will be summarized using descriptive statistics. As for descriptive statistics, in continuous data, sample size, average value, standard deviation, minimum, median and maximum value will be used, and in categorical data, frequency and

percentage will be used. Details for breakdown of subjects will be presented. The number and percentage of subjects according to events, degree of disease severity, causal relationship with AM01 will be calculated for primary and secondary outcome measures. The number and percentage of CR and PR and OS ratio will also be calculated.

Interim analysis

Interim analysis will not be performed.

◆ Quality control and quality assurance of clinical trials

The investigators of the clinical trial will implement the quality assurance and quality control system based on the standard operating procedure prescribed by investigators of the trial. Implementation of clinical trial, data creation, recording, and reporting will be conducted in compliance with the following items.

1) Study implementation plan

2) Laws on securing quality, effectiveness and safety of pharmaceuticals and medical devices.

3) Ministerial ordinance on standards for clinical trial implementation of regenerative medicine products.

◆ Discussion

Regenerative medicine and cell therapies have been gaining much attention in these days among clinicians. MSCs are a valuable cell source in regenerative medicine and there have been increasing interests in using these cells in critical illness. [2 16-19] Recently, several reports have demonstrated that MSCs can be easily and safely isolated from human amnion while the sample collection of bone marrow MSC is an invasive procedure. [10] In that sense, the rationale and concept for our study can be well accepted. Since this study is the first-in-human trial, this study will be undertaken under sufficient safety considerations and based on the implementation plan and relevant laws.

One of major strong points of this study is that our study (first-in-human trial) protocol is well sophisticated. Limitations of the study are that our study results will be limited to a Japanese population, and validation studies on other ethnic backgrounds will be necessary and that target sample size is small. However, if the safety profile of AM01 in this study is well accepted, beneficial information will be provided for clinicians. At the same time, although the efficacy evaluation is the secondary outcome measure, we are also expecting the efficacy of AM01.

◆ Ethics and dissemination

Research ethics

This study will be conducted in compliance with laws and regulations that regulate this study, including the World Medical Association Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subjects. This study has received approval from the Institutional Review Board at Hyogo College of Medicine (approval no. 217851) and Hokkaido University Hospital (approval no. H29-9). The study protocol, informed consent form and other submitted documents were reviewed and approved. Trial registration number is UMIN000029945 (https://upload.umin.ac.jp/); pre-results. No patient is registered at the submission of our manuscript.

Confidentiality

On recruitment, the research assistant will provide a unique scrambled identification (ID) number to each study participant. Only the ID number will be utilized to identify subjects. Data sheets and any printout of electronic files will be saved in a locked filing cabinet in a secure office in Center for Clinical Research and Education (CCRED), Hyogo College of Medicine, and Clinical Research and Medical Innovation Center, Hokkaido University Hospital, with limited access.

Dissemination policy

Final data will be publicly disseminated irrespective of the study results. A report releasing study results will be submitted for publication in an appropriate peer-reviewed journal after trial closure and completion of data collection.

◆ Author contributions

Kenichi Yamahara designed the study and will write the initial draft of the paper. Rika Okamoto and Toshiyuki Isoe will contribute to the analysis and interpretation of the data, and will assist in the preparation of the paper. The other remaining authors will contribute to the collection and interpretation of trial data, and will critically review the paper.

◆ Acknowledgements

We thank Kunimi Takashima, Noriko Fujita, Masami Kondo, and Shoko Kuroda for technical assistance.

◆ Funding statement

This work was supported by a grant of Project for Japan Translational and Clinical Research Core Centers from Japan Agency for Medical Research and Development (AMED) under Grant Number JP18lm0203001. This work was also supported by research support grants for Two University Collaboration and Multicenter Joint Specific Clinical Research from Hyogo College of Medicine.

◆ Competing interests statement

All authors have declared no conflicts of interest.

◆ Figure legend

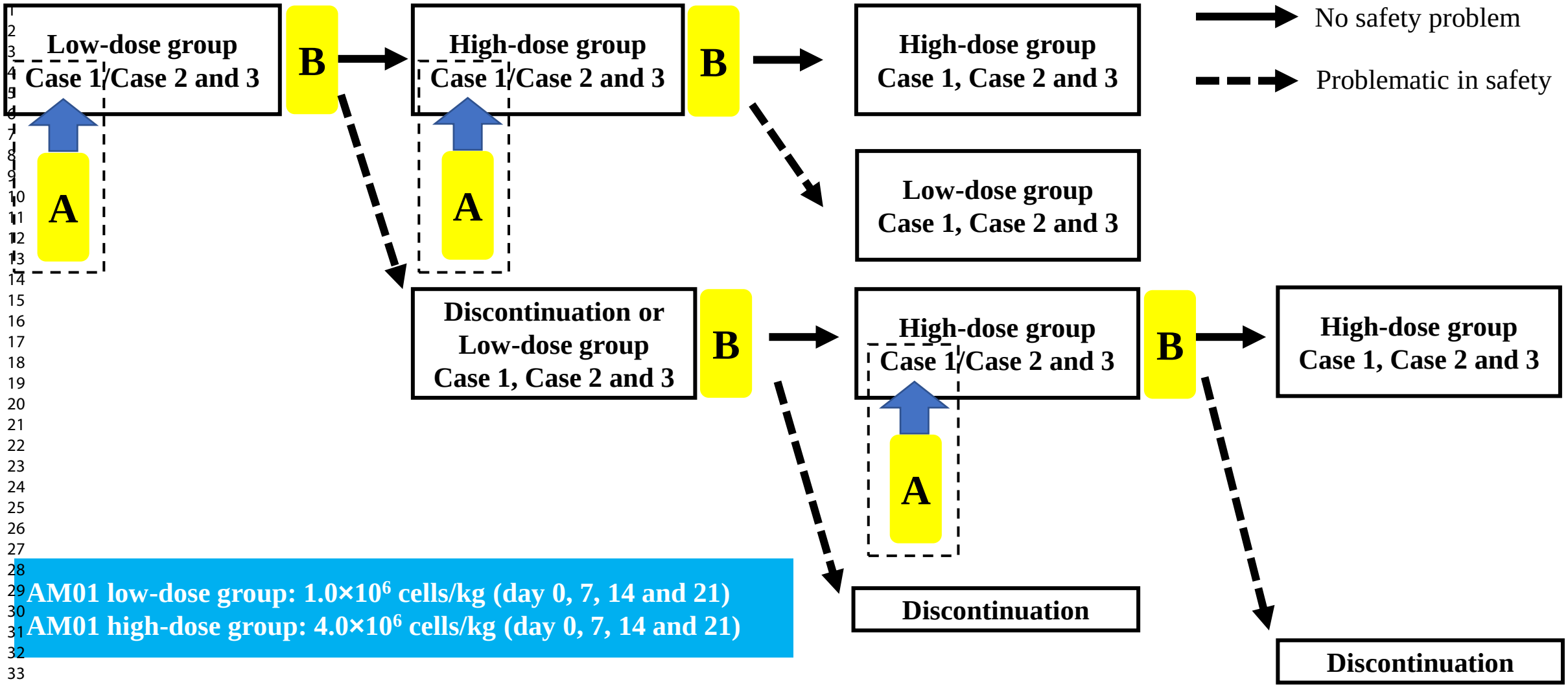
Figure 1. Study flow chart. ESAC; efficacy and safety assessment committee.

◆ References

1. Saliba RM, de Lima M, Giralt S, et al. Hyperacute GVHD: risk factors, outcomes, and clinical implications. *Blood* 2007;109(7):2751–8. doi: 10.1182/blood-2006-07-034348
2. Lalu MM, McIntyre L, Pugliese C, et al. Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials. *PLoS One* 2012;7(10):e47559. doi: 10.1371/journal.pone.0047559
3. Kim JG, Sohn SK, Kim DH, et al. Different efficacy of mycophenolate mofetil as salvage treatment for acute and chronic GVHD after allogeneic stem cell transplant. *Eur J Haematol* 2004;73(1):56–61. doi: 10.1111/j.1600-0609.2004.00247.x
4. Martin PJ, Schoch G, Fisher L, et al. A retrospective analysis of therapy for acute graft-versus-host disease: initial treatment. *Blood* 1990;76(8):1464–72.
5. Martin PJ, Schoch G, Fisher L, et al. A retrospective analysis of therapy for acute graft-versus-host disease: secondary treatment. *Blood* 1991;77(8):1821–8.
6. Hashmi S, Ahmed M, Murad MH, et al. Survival after mesenchymal stromal cell therapy in steroid-refractory acute graft-versus-host disease: systematic review and meta-analysis. *Lancet Haematol* 2016;3(1):e45–52. doi: 10.1016/S2352-3026(15)00224-0
7. Nassereddine S, Rafei H, Elbahesh E, et al. Acute Graft Versus Host Disease: A Comprehensive Review. *Anticancer Res* 2017;37(4):1547–55. doi: 10.21873/anticanres.11483
8. Dunavin N, Dias A, Li M, et al. Mesenchymal Stromal Cells: What Is the Mechanism in Acute Graft-Versus-Host Disease? *Biomedicines* 2017;5(3) doi: 10.3390/biomedicines5030039
9. Covas DT, Panepucci RA, Fontes AM, et al. Multipotent mesenchymal stromal cells obtained from diverse human tissues share functional properties and gene-expression profile with CD146+ perivascular cells and fibroblasts. *Exp Hematol* 2008;36(5):642–54. doi: 10.1016/j.exphem.2007.12.015
10. Yamahara K, Harada K, Ohshima M, et al. Comparison of angiogenic, cytoprotective, and immunosuppressive properties of human amnion- and chorion-derived mesenchymal stem cells. *PLoS One* 2014;9(2):e88319. doi: 10.1371/journal.pone.0088319
11. Ohshima M, Yamahara K, Ishikane S, et al. Systemic transplantation of allogenic fetal membrane-derived mesenchymal stem cells suppresses Th1 and Th17 T cell

responses in experimental autoimmune myocarditis. *J Mol Cell Cardiol* 2012;53(3):420–8. doi: 10.1016/j.yjmcc.2012.06.020

12. Onishi R, Ohnishi S, Higashi R, et al. Human Amnion-Derived Mesenchymal Stem Cell Transplantation Ameliorates Dextran Sulfate Sodium-Induced Severe Colitis in Rats. *Cell Transplant* 2015;24(12):2601–14. doi: 10.3727/096368915X687570
13. Miyamoto S, Ohnishi S, Onishi R, et al. Therapeutic effects of human amnion-derived mesenchymal stem cell transplantation and conditioned medium enema in rats with trinitrobenzene sulfonic acid-induced colitis. *Am J Transl Res* 2017;9(3):940–52.
14. Otagiri S, Ohnishi S, Miura A, et al. Evaluation of amnion-derived mesenchymal stem cells for treatment-resistant moderate Crohn's disease: study protocol for a phase I/II, dual-centre, open-label, uncontrolled, dose-response trial. *BMJ Open Gastroenterol* 2018;5(1):e000206. doi: 10.1136/bmjgast-2018-000206
15. Kuwatsuka Y, Atsuta Y, Horowitz MM, et al. Graft-versus-host disease and survival after cord blood transplantation for acute leukemia: a comparison of Japanese versus White populations. *Biol Blood Marrow Transplant* 2014;20(5):662–7. doi: 10.1016/j.bbmt.2014.01.020
16. Fang B, Song Y, Liao L, et al. Favorable response to human adipose tissue-derived mesenchymal stem cells in steroid-refractory acute graft-versus-host disease. *Transplant Proc* 2007;39(10):3358–62. doi: 10.1016/j.transproceed.2007.08.103
17. Wu KH, Chan CK, Tsai C, et al. Effective treatment of severe steroid-resistant acute graft-versus-host disease with umbilical cord-derived mesenchymal stem cells. *Transplantation* 2011;91(12):1412–6. doi: 10.1097/TP.0b013e31821aba18
18. Gong W, Han Z, Zhao H, et al. Banking human umbilical cord-derived mesenchymal stromal cells for clinical use. *Cell Transplant* 2012;21(1):207–16. doi: 10.3727/096368911x586756
19. Ringdén O, Erkers T, Nava S, et al. Fetal membrane cells for treatment of steroid-refractory acute graft-versus-host disease. *Stem Cells* 2013;31(3):592–601. doi: 10.1002/stem.1314



A: In the first case registered in each group, safety will be evaluated by ESAC at 28 days.
B: In the three cases registered in each group, safety will be evaluated by ESAC.

Figure 1

BMJ Open

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

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-026403.R1
Article Type:	Protocol
Date Submitted by the Author:	07-Mar-2019

Complete List of Authors:	Yamahara, Kenichi; Hyogo College of Medicine, Laboratory of Medical Innovation, Institute for Advanced Medical Sciences; Hyogo College of Medicine, Center for Transfusion Medicine and Cellular Therapy Hamada, Akiko; Hyogo College of Medicine, Laboratory of Medical Innovation, Institute for Advanced Medical Sciences Soma, Toshihiro; Hyogo College of Medicine, Center for Transfusion Medicine and Cellular Therapy Okamoto, Rika; Hyogo College of Medicine, Center for Clinical Research and Education Okada, Masaya; Hyogo College of Medicine, Division of Hematology, Department of Internal Medicine Yoshihara, Satoshi; Hyogo College of Medicine, Center for Transfusion Medicine and Cellular Therapy Yoshihara, Kyoko; Hyogo College of Medicine, Division of Hematology, Department of Internal Medicine Ikegame, Kazuhiro; Hyogo College of Medicine, Division of Hematology, Department of Internal Medicine Tamaki, Hiroya; Hyogo College of Medicine, Division of Hematology, Department of Internal Medicine Kaida, Katsuji; Hyogo College of Medicine, Division of Hematology, Department of Internal Medicine Inoue, Takayuki; Hyogo College of Medicine, Division of Hematology, Department of Internal Medicine Ohsugi, Yuko; Hyogo College of Medicine, Division of Hematology, Department of Internal Medicine Nishikawa, Hiroki; Hyogo College of Medicine, Center for Clinical Research and Education Hayashi, Hiroshi; Hokkaido Daigaku Byoin Ito, Yoichi; Hokkaido University, Department of Biostatistics Iijima, Hiroaki; Hokkaido University Hospital, Clinical Research and Medical Innovation Center Ohnishi, Shunsuke Hashimoto, Daigo; Hokkaido University, Department of Hematology Isoe, Toshiyuki; Hokkaido Daigaku Byoin Teshima, Takanori; Hokkaido University, Department of Hematology Ogawa, Hiroyasu; Hyogo College of Medicine, Division of Hematology, Department of Internal Medicine

	Sato, Norihiro; Hokkaido Daigaku Byoin Fujimori, Yoshihiro; Hyogo College of Medicine, Laboratory of Medical Innovation, Institute for Advanced Medical Sciences
Primary Subject Heading:	Haematology (incl blood transfusion)
Secondary Subject Heading:	Pharmacology and therapeutics
Keywords:	Clinical trials < THERAPEUTICS, HAEMATOLOGY, Blood bank & transfusion medicine < HAEMATOLOGY

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

Author names (academic degree) and affiliation

Kenichi Yamahara[1,2,3], Akiko Hamada[1], Toshihiro Soma[2], Rika Okamoto[4], Masaya Okada[3], Satoshi Yoshihara[2,3], Kyoko Yoshihara[3], Kazuhiro Ikegame[3], Hiroya Tamaki[3], Katsuji Kaida[3], Takayuki Inoue[3], Yuko Ohsugi[3], Hiroki Nishikawa[4], Hiroshi Hayashi[5], Yoichi M. Ito[6], Hiroaki Iijima[5], Shunsuke Ohnishi[7], Daigo Hashimoto[8], Toshiyuki Isoe[5], Takanori Teshima[8], Hiroyasu Ogawa[3], Norihiro Sato[5], Yoshihiro Fujimori[1,2,3]

1. Laboratory of Medical Innovation, Institute for Advanced Medical Sciences, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan
2. Center for Transfusion Medicine and Cellular Therapy, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan
3. Division of Hematology, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan

4. Center for Clinical Research and Education (CCRED), Hyogo College of Medicine, Nishinomiya, Hyogo, Japan

5. Clinical Research and Medical Innovation Center, Hokkaido University Hospital, Sapporo, Hokkaido, Japan

6. Department of Biostatistics, Hokkaido University Graduate School of Medicine, Sapporo, Hokkaido, Japan

7. Department of Gastroenterology and Hepatology, Hokkaido University Graduate School of Medicine, Sapporo, Hokkaido, Japan

8. Department of Hematology, Hokkaido University Faculty of Medicine, Sapporo, Hokkaido, Japan

Corresponding author:

Kenichi Yamahara, MD, PhD

Laboratory of Medical Innovation, Institute for Advanced Medical Sciences, Hyogo College of Medicine, Hyogo, Japan

1-1, Mukogawa-cho, Nishinomiya, Hyogo, 663-8501, Japan

Tel: +81-798-45-6398; Fax: +81-798-45-6119

E-mail: yamahara@hyo-med.ac.jp

Running title: Amnion-derived mesenchymal stem cells and steroid-refractory acute GVHD

Abbreviations

aGVHD; acute graft-versus-host disease, HSCT; haematopoietic stem cell transplantation, AHSCT; allogenic haematopoietic stem cell transplantation, ATG; anti-thymocyte globulin, MMF; mycophenolate mofetil, MSC; mesenchymal stromal cell, ESAC; efficacy and safety assessment committee, AE; adverse event, CR; complete response, PR; partial response, OS; overall survival.

Word count for the main text: 3,286 words

◆ Abstract

Introduction

Regenerative medicine and cell therapies have been gaining much attention among clinicians. Therapeutic infusion of mesenchymal stromal cells (MSCs) is now a leading investigational strategy for the treatment of acute graft-versus-host disease (aGVHD). Bone marrow MSC are approved for manufacture and marketing as a cell therapy for aGVHD. Our non-clinical studies confirmed that human amnion-derived MSC had immunomodulatory activity equal to or higher than that of human bone marrow MSC. This study will aim to evaluate the safety and efficacy of amnion-derived MSC (AM01) in patients with steroid-refractory aGVHD.

Methods and analysis

This study will be a multicentre, single-arm, open-label trial (an interventional study). This clinical trial will begin with a low-dose group, and when safety has been confirmed in at least 3 cases in the low-dose group, treatment will begin for the high-dose group, for which the safety will also be verified. The primary endpoint is to assess the safety of intravenous infusion therapy of AM01 within 24 hours after intravenous infusion of AM01. The secondary endpoint is to explore the efficacy of intravenous infusion therapy with AM01.

Ethics and dissemination

The Institutional Review Boards of all participating hospitals approved this study protocol (latest version 3.3.0, 3 Aug 2018). Final data will be publicly announced. A report releasing the study results will be submitted for publication to an appropriate peer-reviewed journal.

Trial registration

UMIN000029945 (https://upload.umin.ac.jp/); pre-results. No patients are registered at the time of manuscript submission.

Strengths and limitations of this study

- This study is a first-in-human clinical trial.
- This study follows a clear and rigorous protocol, guided by experienced methodologists, and implemented in a clinical trials section.
- The study results will be limited to a Japanese population with a small target sample size, and validation studies on other ethnic backgrounds and larger sample sizes will be needed.

Keywords

Amnion-derived mesenchymal stem cell, steroid-refractory acute GVHD, safety, efficacy

◆ Introduction

Acute graft-versus-host disease (aGVHD) is a condition in which immune cells from the donor attack recipient tissues.[1] In cases with haematopoietic stem cell transplantation (HSCT), aGVHD is classified into two groups: classical aGVHD developed within 100 days after transplantation and atypical aGVHD after 100 days. Classical aGVHD is a clinical entity presenting symptoms such as maculopapular rash, nausea, vomiting, diarrhoea, watery diarrhoea, ileus and cholestatic hepatitis.[1] aGVHD remains the second leading cause of death following allogeneic HSCT (AHSCT). Over the last decade, the advances in understanding the pathophysiology of this immune-based process helped redefine the graft-versus-host reaction and opened new possibilities for novel preventive and therapeutic approaches.[2]

Preventive measures for aGVHD are commonly undertaken when AHSCT is performed; however, about half of patients develop aGVHD, even when preventive measures are adequately followed.[3] When aGVHD develops, corticosteroid drugs are given as the standard therapy; however, nearly half of cases with aGVHD are steroid-refractory.[4 5] Anti-thymocyte globulin (ATG), mycophenolate mofetil (MMF) and steroid pulse therapy are often selected as second-line therapies for steroid-refractory aGVHD.[4 5] However, none of the clinical trials have shown their

usefulness. In some cases, these second-line therapies may improve the symptoms of aGVHD. However, there are many treatment-related complications, such as severe infections due to excessive immunosuppression, and the mortality rate with standard treatment of refractory aGVHD cases is reported to be 70%.[6] Therefore, if an effective therapy for steroid-refractory aGVHD is established, the outcome of AHSCT will improve, which can be expected to lead to the dramatic improvement for the prognosis of various hematopoietic malignancies and non-malignant diseases. For this reason, new molecular-targeted therapies and the development of cell therapies are currently being promoted worldwide.[7]

Therapeutic infusion of mesenchymal stromal cells (MSCs) is now a leading investigational strategy for the treatment of aGVHD.[8] MSCs exist in most human tissues, with bone marrow, adipose and perinatal tissues representing the most common sources of cells utilized for clinical investigation.[9] MSC products have received approvals for steroid-resistant aGVHD in several countries. MSCs have an anti-inflammatory effect (i.e., strongly suppressing T cell differentiation and proliferation) and bone marrow MSCs (TEMCELL® HS Inj) are approved for manufacture and marketing as a cell therapy for aGVHD.[10] However, since bone

marrow collection requires an invasive procedure, a more accessible source of MSCs is ideal.

Amnion-derived MSCs are undifferentiated cells present in the amniotic membrane that have the ability to differentiate into various cells belonging to the mesenchymal system, such as muscle, bone, cartilage, fat and the like, with a self-replication ability and immunosuppressive action.[11-21] In our previous studies, we confirmed that human amnion (amniotic membrane of pregnant women (medical waste))-derived MSCs had immunomodulatory activity equal to or higher than that of human bone marrow MSCs and demonstrated their effects on aGVHD and inflammatory bowel disease models. There were no significant safety problems with human amnion-derived MSC in non-clinical safety tests.[11 13 14] Furthermore, we are pursuing clinical research of cell therapy in aGVHD and Crohn's disease using amnion-derived MSCs, which have more stem cells (making them easy to mass culture), a higher proliferative capacity, less invasiveness of sample collection and less frequency of rejection, compared to bone marrow MSCs.[22] Using our original cell culture serum NeoSERA@, for which the main component is adult bovine platelet-rich plasma, we successfully formulated amnion-derived MSCs as an investigational drug for the first time in the world. Based on these background studies, this clinical study will aim to evaluate the safety and

efficacy of amnion-derived MSCs (trial product name, AM01) in patients with steroid-refractory aGVHD after AHSCT.

◆ Methods and analysis

➢ Study design

This study is a multicentre, single-arm, open-label clinical trial, which will take place at Hokkaido University Hospital in Sapporo, Japan, and at Hyogo College of Medicine College Hospital in Nishinomiya, Japan. This clinical trial will begin with the low-dose group, and when the safety is confirmed in at least 3 cases of the low-dose group, treatment will begin for the high-dose group, for which the safety will also be verified. More than 28 days after the initial administration in the first and third cases of each group, the efficacy and safety assessment committee (ESAC) will evaluate the safety profile and the validity of the next case registration, or dose group transition will be discussed (Figure 1). After intravenous infusion of AM01, efficacy will be evaluated until it is judged to be inadequate. Then, a safety assessment will be carried out 52 weeks after the initial administration, or until discontinuation criteria are reached.

Criteria for stopping administration of AM01

(1) The patient wishes to stop receiving the administration of AM01.

(2) It becomes impossible to continue the administration of AM01 because the patient is transferred to another hospital during the trial.

(3) When a patient is deemed ineligible as a trial subject after starting the trial.

(4) When administration of AM01 becomes difficult due to serious adverse events (AEs).

(5) In the event that the investigators or sharing doctors determine that it is necessary to withdraw the patient when assessed from the medical point of view.

Criteria for determining inadequate effectiveness

(1) When complete response (CR) or partial response (PR) could not be obtained between 4 and 24 weeks after the initial administration of AM01.

➢ Study population

Inclusion criteria

(1) Patients diagnosed as grade 2 or higher steroid-refractory aGVHD after AHSCT. Assessment of the severity of aGVHD will be based on the guidelines of Japanese Society for Hematopoietic Cell Transplantation.[23]

(2) Patients between 15 and 80 years old at the consent acquisition.

(3) Patients who have provided written informed consent with a sufficient understanding of the trial contents, or those who have provided written informed consent by substitute (e.g., persons who exercise custody of the subject) if the patient is under 20 years old (written informed consent will also be obtained by the subject as much as possible).

Exclusion criteria

1. Patients who received more than one treatment other than steroid therapy for aGVHD.
2. Patients with elevated liver enzymes other than aGVHD-related liver dysfunction (serum total bilirubin >2.0 mg/dl or serum aspartate aminotransferase/alanine aminotransferase >3 times the upper limit of normal).
3. Patients with renal dysfunction (serum creatinine >2.0 mg/dl).
4. Patients with percutaneous oxygen saturation <94%, even under oxygen administration.
5. Patients with active hepatitis B virus (HBV) or hepatitis C virus (HVC) infection (serum HBV-DNA >2,000 IU/ml or HCV-RNA >25 IU/ml) with a high-risk for hepatitis (positive for any hepatitis B antigen/antibody including HBs-Ag, HBs-Ab, HBc-Ab and/or HCV-Ab).

6. Patients positive for human immunodeficiency virus (HIV)-Ab.
7. Patients with uncontrolled severe infection.
8. Patients with severe hypersensitivity to bovine-derived constituents, human serum albumin and gentamicin.
9. Patients with a past history for hypersensitivity to iodine or iodine-containing contrast agents.
10. Patients with previous participation in a study involving any treatment with regenerative medical products.
11. Patients who participated in any clinical trial within 12 weeks of consent for this study.
12. Patients who are pregnant or breast-feeding.
13. Patients considered unsuitable for the study as determined by the principal investigators or sharing doctors.

➢ Implementation

Dose and administration period of AM01

Intravenous infusion of AM01 will be performed at day 0, 7, 14, and 21 according to the following dosages, which will be calculated based on the body weight in the screening period:

1) Low-dose group: 1.0×10^6 cells/kg (day 0, 7, 14 and 21)

2) High-dose group: 4.0×10^6 cells/kg (day 0, 7, 14 and 21)

Study subjects will receive one donor's AM01. The AM01 will be infused intravenously slowly at 1 mL/min as a guide. Since there is a risk that cellular embolism, thrombus formation and intravascular haemolysis may develop as a result of intravenous administration of allogeneic cells, infusion should not exceed 1.5 mL/min.

For intravenous drip infusion of AM01, administration will start within 2 hours after thawing of product and will finish within 3 hours. The following premedication is administered intravenously 30 minutes to 1 hour prior to the start of AM01 administration:

(1) Hydrocortisone sodium succinate (Solu-Cortef® or Succizone®) 100 mg

(2) d-Chlorpheniramine maleate (Polaramine®) 5 mg

Rationale for administration dose and administration period

In the repeated intravenous administration test of AM01 using severe combined immunodeficiency (SCID) mice, 0, 4, 20 and 40 × 10^6 cells/kg once a week was administered a total of four times. In these results, deaths during the test period and deterioration of respiratory status after administration of AM01 were not observed. During the administration period and during the drug withdrawal period, no significant changes could be attributed to AM01 in general condition, body weight, food intake, ophthalmological examination, urinalysis, blood biochemical examination and autopsy.

Human bone marrow MSCs (TEMCELL® HS Inj) approved in Japan are targeted for grade 2 or higher aGVHD, and human bone marrow MSCs at 2 × 10^6 cells/kg twice a week for a total of 8 treatments is the approved protocol. In this study, 4 × 10^6 cells/kg will be administered once a week a total of 4 times in the high-dose group, which is the same as the number of cells per week, and the total number of cells to be administered in TEMCELL®. This study is a first-in-human clinical study, and the primary endpoint is the safety of AM01. Thus, the current study dose and administration period was set with full consideration of safety data in human bone marrow MSCs (TEMCELL® HS Inj).

Prohibited drug

Between the first administration and 4 weeks after the first administration of AM01, the use of a new drug or therapy including TEMCELL® HS Inj for the treatment of aGVHD will be prohibited (steroid external medicine can be used together).

If study subjects are receiving prophylactic therapy for aGVHD or treatment for aGVHD with the following drugs before administration of AM01, their dose cannot be increased between the first administration and 4 weeks after the first administration of AM01;

a） Prophylaxis for aGVHD: methotrexate, cyclosporine, tacrolimus, ATG, MMF, corticosteroid

b) Treatment for aGVHD: corticosteroid

➢ Preparation of amnion-derived MSCs (AM01)

In this study, AM01, human amnion-derived MSCs will be prepared according to the manufacturing procedure approved by the Pharmaceuticals and Medical Devices Agency (PMDA, Tokyo, Japan)[11 22]. The phenotypes of AM01 are characterized by their positivity for CD73, CD90 and CD105, and by their negativity for the haematopoietic-associated marker CD45 and the epithelial cell-associated marker

CD326. In addition, AM01 expresses a significant amount of prostaglandin E2 and reduces T-lymphocyte proliferation.

After obtaining informed consent, the human foetal membrane was aseptically obtained during caesarean deliveries, and the amnion was separated from the chorion by manual peeling. Amnion-derived MSCs was isolated and expanded by digestion using several enzymes. The cells were then seeded in plastic cell culture chambers with basal medium supplemented with adult bovine-derived platelet-rich plasma (NeoSERA@, Japan Biomedical, Otofuke, Japan, https://www.japan-biomedical.jp). The cultures were maintained at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Next, the cells were harvested, and AM01 was packaged into frozen bags and stored at -130°C. Cells were further cultured after thawing the frozen bags, passaged, harvested and packaged into frozen bags and stored at -130°C as product doses. Quality testing for AM01 included assessments for cell appearance, purity, viable cell number, viability and presence of bacteria, viruses, mycoplasma and endotoxin contamination.

➢ Outcome measures

Primary endpoint

To assess the safety of AM01 intravenous infusion therapy within 24 hours after administration.

Secondary endpoints

To explore the efficacy of AM01 intravenous infusion therapy, the following will be assessed: (1) the proportion of patients with CR that lasts 28 days or more; (2) the proportion of patients with CR or PR 4 weeks after the first infusion of AM01; and (3) the following items at 8, 12, 16, 20 and 24 weeks after the first intravenous infusion of AM01: (i) the severity of aGVHD, (ii) the presence or the severity of chronic GVHD, (iii) overall survival (OS), (iv) presence of the recurrence of the primary disease, (v) presence of severe infection and (vi) the total dosage of steroid therapy. We will also investigate the overall safety of AM01 intravenous infusion therapy, as well as assess the development of AEs within 52 weeks after the first intravenous infusion of AM01.

Definition of AEs

AEs indicate all undesirable or unintended diseases or laboratory disorders and symptoms that occur in subjects after the initial administration and 52 weeks after the initial administration of AM01, or until the patient withdrawals from the study. If the primary disease worsens, the event will be treated as an AE. AE grading will be based on CTCAE v4.0-JCOG.

Definition of treatment response

In this trial, assessment of the therapeutic response will be carried out according to the following criteria: (1) CR: the disappearance of any organ failure related to aGVHD; (2) PR: the clinical stage of at least one diseased organ improves, and the clinical stages of other diseased organs do not worsen; (3) MR (mixed response): the clinical stage of at least one diseased organ improves, but the clinical stage of another diseased organ worsens; (4) PG (progression): the clinical stage of at least one diseased organ deteriorates, and the clinical stages of other diseased organs does not improve; and (5) NC (no change): cases where neither improvement nor deterioration is observed in any diseased organ.

➢ Evaluation by ESAC

For the purpose of confirming whether the trial is being carried out safely and appropriately, an ESAC will be established.

Periodical assessment

After the trial begins, the chairman of the ESAC will hold a committee meeting at least every six months to conduct periodic evaluations as specified in the trial implementation plan.

Extraordinary assessment

In the event that a serious problem occurs, the chairman of the ESAC will promptly hold a committee meeting and make a temporary assessment of the validity of trial continuation upon receiving the report.

➢ Standard of care (response to the occurrence of AEs)

If the investigators or sharing doctors are aware of the occurrence of AEs, appropriate measures regarding study subjects will be undertaken immediately. At the same time, an explanation will be given to the subject and will be reported to all relevant departments.

➢ Case registration period

This study is registered from December 1, 2017 through March 31, 2021.

➢ Statistical methods

Definition of safety analysis set

Excluding the subjects for whom AM01 was never administered, subjects from whom consent is obtained will be considered the safety analysis set.

Definition of efficacy analysis set

Among the subjects for whom consent is obtained, the group that is referred to as the efficacy analysis set will exclude subjects that fall under any of the following conditions: (1) subjects for whom AM01 was never administered; (2) subjects whose selection criteria violation or exclusion criteria conflict are found after registration; and (3) subjects missing all AM01 efficacy data.

Statistical analysis

The data from this study will be summarized using descriptive statistics. For continuous data, the sample size, average value, standard deviation, minimum, median and maximum value will be used for descriptive statistics; for categorical data, the frequency and percentage will be used. Details of the breakdown of subjects will be presented. The number and percentage of subjects according to events, degree of disease severity and causal relationship with AM01 will be calculated for primary and secondary outcome measures. The number and percentage of CR and PR, as well as OS ratio, will also be calculated.

Interim analysis

Interim analysis will not be performed.

➢ Quality control and quality assurance of clinical trials

The clinical trial investigators will implement a quality assurance and quality control system based on the standard operating procedure prescribed by the investigators. Implementation of clinical trial, data creation, recording, monitoring and reporting will be conducted in compliance with the following items: (1) the study implementation plan; (2) laws for securing quality, effectiveness and safety of pharmaceuticals and medical devices; and (3) the ministerial ordinance on standards for clinical trial implementation of regenerative medical products.

➢ Patient & public involvement

Neither patients nor the public were not involved in the development of the research question, choice of outcome measures, design of the trial, recruitment of participants or conduct of the trial. Results of the trial will be disseminated to study participants through direct consultation with a trial clinician at completion of the trial, as well as through the publication of results.

◆ Discussion

Regenerative medicine and cell therapies have been gaining much attention in recent days among clinicians. MSCs are a valuable cell source in regenerative medicine and

there has been increasing interest in using these cells to treat critical illnesses.[2 24-27] Recently, several reports have demonstrated that MSCs can be easily and safely isolated from human amnion, while bone marrow MSC sample collection requires an invasive procedure.[11] In that sense, the rationale and concept for our study will be well accepted. Since this study is a first-in-human trial, it will be undertaken with sufficient safety considerations and based on the implementation plan and relevant laws.

One of major strong points of this study is that our study protocol is sophisticated and well designed. Limitations of the study include the fact that it will be limited to a Japanese population with a small target sample size. Therefore, validation studies on other ethnic backgrounds and with larger sample sizes will be necessary in the future. However, if the safety profile of AM01 in this study shows that the therapy is well accepted, it will provide clinicians with beneficial information. In addition, although the efficacy evaluation of AM01 is a secondary outcome measure, we expect those results will also be highly beneficial.

◆ Ethics and dissemination

Research ethics

This study will be conducted in compliance with the laws and regulations that regulate this study, including the World Medical Association Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subjects. This study has received approval from the Institutional Review Board at Hyogo College of Medicine (approval no. 217851) and Hokkaido University Hospital (approval no. H29-9). The study protocol, informed consent form and other submitted documents were reviewed and approved. The trial registration number is UMIN000029945 (https://upload.umin.ac.jp/); pre-results. No patients were registered at the time this manuscript was submitted.

Confidentiality

Upon recruitment, the research assistant will provide a unique scrambled identification (ID) number to each study participant. Subjects will only be identified by this ID number. Data sheets and any printouts of electronic files will be saved in a locked filing cabinet in secure offices at the Center for Clinical Research and Education (CCRED), Hyogo College of Medicine, and the Clinical Research and Medical Innovation Center, Hokkaido University Hospital, with limited access.

Dissemination policy

Final data will be publicly disseminated, irrespective of the study results. A report releasing study results will be submitted for publication in an appropriate peer-reviewed journal after trial closure and completion of data collection.

◆ Author contributions

KYa designed the study and wrote the initial draft of the paper as a coordinating and principal investigator. KYa, AH, and TS were involved in manufacturing and quality control of AM01. RO and TI assisted in the preparation of this paper and will contribute to the analysis and interpretation of the data collected from the study. MO, SY, KYo, KI, HT, KK, TI, YO, HN, HH, HI, SO, and DH reviewed all protocol versions and contributed to the start-up of the trial. YMI contributed to the design of the trial and statistical analysis. TT is another principal investigator, responsible for processing of the trial in Hokkaido University Hospital. HO, NS, and YF supervised and edited the protocol.

◆ Acknowledgements

The authors would like to thank Kunimi Takashima, Noriko Fujita, Masami Kondo, Yoshie Takigawa and Shoko Kuroda for technical assistance, and Dr Takahiro Yamada, Dr Kayoko Harada, Dr Hiroyuki Tanaka, Dr Hideaki Sawai, and Dr Hiroaki Shibahara for preparing and performing caesarean deliveries.

◆ Funding statement

This work was supported by a grant from the Project for Japan Translational and Clinical Research Core Centers of the Japan Agency for Medical Research and Development (AMED) under Grant Number JP18lm0203001. This work was also supported by research support grants from Two University Collaboration and Multicentre Joint Specific Clinical Research of Hyogo College of Medicine.

◆ Competing interests statement

All authors have declared no conflicts of interest.

◆ Figure legend

Figure 1. Study flow chart. ESAC; efficacy and safety assessment committee.

◆ References

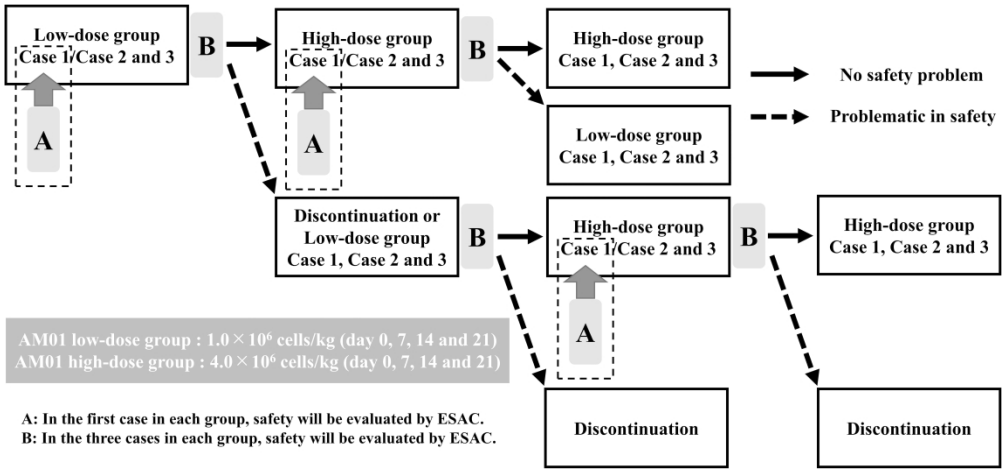
1. Saliba RM, de Lima M, Giralt S, et al. Hyperacute GVHD: risk factors, outcomes, and clinical implications. *Blood* 2007;109(7):2751-8. doi: 10.1182/blood-2006-07-034348
2. Lalu MM, McIntyre L, Pugliese C, et al. Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials. *PLoS One* 2012;7(10):e47559. doi: 10.1371/journal.pone.0047559
3. Kim JG, Sohn SK, Kim DH, et al. Different efficacy of mycophenolate mofetil as salvage treatment for acute and chronic GVHD after allogeneic stem cell transplant. *Eur J Haematol* 2004;73(1):56-61. doi: 10.1111/j.1600-0609.2004.00247.x
4. Martin PJ, Schoch G, Fisher L, et al. A retrospective analysis of therapy for acute graft-versus-host disease: initial treatment. *Blood* 1990;76(8):1464-72.
5. Martin PJ, Schoch G, Fisher L, et al. A retrospective analysis of therapy for acute graft-versus-host disease: secondary treatment. *Blood* 1991;77(8):1821-8.
6. Hashmi S, Ahmed M, Murad MH, et al. Survival after mesenchymal stromal cell therapy in steroid-refractory acute graft-versus-host disease: systematic review and meta-analysis. *Lancet Haematol* 2016;3(1):e45-52. doi: 10.1016/S2352-3026(15)00224-0
7. Nassereddine S, Rafei H, Elbahesh E, et al. Acute Graft Versus Host Disease: A Comprehensive Review. *Anticancer Res* 2017;37(4):1547-55. doi: 10.21873/anticanres.11483
8. Dunavin N, Dias A, Li M, et al. Mesenchymal Stromal Cells: What Is the Mechanism in Acute Graft-Versus-Host Disease? *Biomedicines* 2017;5(3) doi: 10.3390/biomedicines5030039
9. Covas DT, Panepucci RA, Fontes AM, et al. Multipotent mesenchymal stromal cells obtained from diverse human tissues share functional properties and gene-expression profile with CD146+ perivascular cells and fibroblasts. *Exp Hematol* 2008;36(5):642-54. doi: 10.1016/j.exphem.2007.12.015
10. Okada K, Miyata T, Sawa Y. Insurance systems and reimbursement concerning research and development of regenerative medicine in Japan. *Regen Med* 2017;12(2):179-86. doi: 10.2217/rme-2016-0124
11. Yamahara K, Harada K, Ohshima M, et al. Comparison of angiogenic, cytoprotective, and immunosuppressive properties of human amnion- and

chorion-derived mesenchymal stem cells. *PLoS One* 2014;9(2):e88319. doi: 10.1371/journal.pone.0088319

12. Ohshima M, Yamahara K, Ishikane S, et al. Systemic transplantation of allogenic fetal membrane-derived mesenchymal stem cells suppresses Th1 and Th17 T cell responses in experimental autoimmune myocarditis. *J Mol Cell Cardiol* 2012;53(3):420-8. doi: 10.1016/j.yjmcc.2012.06.020
13. Onishi R, Ohnishi S, Higashi R, et al. Human Amnion-Derived Mesenchymal Stem Cell Transplantation Ameliorates Dextran Sulfate Sodium-Induced Severe Colitis in Rats. *Cell Transplant* 2015;24(12):2601-14. doi: 10.3727/096368915X687570
14. Miyamoto S, Ohnishi S, Onishi R, et al. Therapeutic effects of human amnion-derived mesenchymal stem cell transplantation and conditioned medium enema in rats with trinitrobenzene sulfonic acid-induced colitis. *Am J Transl Res* 2017;9(3):940-52.
15. Kawakubo K, Ohnishi S, Fujita H, et al. Effect of Fetal Membrane-Derived Mesenchymal Stem Cell Transplantation in Rats With Acute and Chronic Pancreatitis. *Pancreas* 2016;45(5):707-13. doi: 10.1097/MPA.0000000000000541
16. Ichihara Y, Kaneko M, Yamahara K, et al. Self-assembling peptide hydrogel enables instant epicardial coating of the heart with mesenchymal stromal cells for the treatment of heart failure. *Biomaterials* 2018;154:12-23. doi: 10.1016/j.biomaterials.2017.10.050
17. Kubo K, Ohnishi S, Hosono H, et al. Human Amnion-Derived Mesenchymal Stem Cell Transplantation Ameliorates Liver Fibrosis in Rats. *Transplant Direct* 2015;1(4):e16. doi: 10.1097/TXD.0000000000000525
18. Tsuda H, Yamahara K, Ishikane S, et al. Allogenic fetal membrane-derived mesenchymal stem cells contribute to renal repair in experimental glomerulonephritis. *Am J Physiol Renal Physiol* 2010;299(5):F1004-13. doi: 10.1152/ajprenal.00587.2009
19. Tsuda H, Yamahara K, Otani K, et al. Transplantation of allogenic fetal membrane-derived mesenchymal stem cells protects against ischemia/reperfusion-induced acute kidney injury. *Cell Transplant* 2014;23(7):889-99. doi: 10.3727/096368913X665594
20. Ishikane S, Hosoda H, Yamahara K, et al. Allogeneic transplantation of fetal membrane-derived mesenchymal stem cell sheets increases neovascularization and improves cardiac function after myocardial infarction

in rats. *Transplantation* 2013;96(8):697–706. doi: 10.1097/TP.0b013e31829f753d

21. Imai Y, Yamahara K, Hamada A, et al. Human amnion-derived mesenchymal stem cells ameliorate imiquimod-induced psoriasiform dermatitis in mice. *J Dermatol* 2019;46(3):276–78. doi: 10.1111/1346-8138.14768
22. Otagiri S, Ohnishi S, Miura A, et al. Evaluation of amnion-derived mesenchymal stem cells for treatment-resistant moderate Crohn's disease: study protocol for a phase I/II, dual-centre, open-label, uncontrolled, dose-response trial. *BMJ Open Gastroenterol* 2018;5(1):e000206. doi: 10.1136/bmjgast-2018-000206
23. Kuwatsuka Y, Atsuta Y, Horowitz MM, et al. Graft-versus-host disease and survival after cord blood transplantation for acute leukemia: a comparison of Japanese versus White populations. *Biol Blood Marrow Transplant* 2014;20(5):662–7. doi: 10.1016/j.bbmt.2014.01.020
24. Fang B, Song Y, Liao L, et al. Favorable response to human adipose tissue-derived mesenchymal stem cells in steroid-refractory acute graft-versus-host disease. *Transplant Proc* 2007;39(10):3358–62. doi: 10.1016/j.transproceed.2007.08.103
25. Wu KH, Chan CK, Tsai C, et al. Effective treatment of severe steroid-resistant acute graft-versus-host disease with umbilical cord-derived mesenchymal stem cells. *Transplantation* 2011;91(12):1412–6. doi: 10.1097/TP.0b013e31821aba18
26. Gong W, Han Z, Zhao H, et al. Banking human umbilical cord-derived mesenchymal stromal cells for clinical use. *Cell Transplant* 2012;21(1):207–16. doi: 10.3727/096368911x586756
27. Ringdén O, Erkers T, Nava S, et al. Fetal membrane cells for treatment of steroid-refractory acute graft-versus-host disease. *Stem Cells* 2013;31(3):592–601. doi: 10.1002/stem.1314



Figure

307x142mm (600 x 600 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page
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, name of intended registry	5
	2b	All items from the World Health Organization Trial Registration Data Set	Yes
Protocol version	3	Date and version identifier	5
Funding	4	Sources and types of financial, material, and other support	26
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1-2
	5b	Name and contact information for the trial sponsor	1-2
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	25
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	25
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	7
	6b	Explanation for choice of comparators	n/a
Objectives	7	Specific objectives or hypotheses	9

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	10
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	10
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	11-13
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	13-14
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	10-11
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	10-11
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	15-16
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	17-18
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	13-14
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	10
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	Figure 1
Methods: Assignment of interventions (for controlled trials)			

Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	n/a
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	n/a
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	n/a
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	n/a
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n/a
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	21-22
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	21-22
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	24-25
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	20-21
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	20-21

	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	20-21
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	21-22
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	21-22
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	21-22
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	21-22
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	23-24
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	23-24
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	12
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	12
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	24
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	26
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	24

Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	24-25
	31b	Authorship eligibility guidelines and any intended use of professional writers	25
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	25
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	n/a
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.