Efficacy and Safety of Sequential Infusion of Hypoxic 3D-Cultured Umbilical Cord Mesenchymal Stem Cells in Haploidentical Hematopoietic Stem Cell Transplantation for Severe Aplastic Anemia: A Multicenter, Randomized, Phase 1 Trial

Methods

Study Design and Participants

This trial was a multicenter, randomized, phase 1 clinical trial was conducted across in China. The study evaluated the safety and preliminary efficacy of conventional haplo-HSCT combined with UC-MSCs cultured under 3D hypoxic conditions in the treatment of SAA. Patients were recruited after receiving approval from the Institutional Review Board of the People's Liberation Army General Hospital. Written informed consent was provided by the participants, their legal guardians, or next of kin.
The inclusion criteria were (1) age 6 to 60 years; (2) diagnosis of SAA or very SAA according to the International Aplastic Anemia Study Group (3) without any severe pulmonary, cardiac, liver, or renal diseases or any active infection; and (4) adequate performance status (Eastern Cooperative Oncology Group score 0-2). Fanconi anemia was excluded after evaluation of diepoxybutane-stimulated peripheral blood (PB) chromosome breakages. Patients with congenital aplastic anemia like dyskeratosis or Diamond-Blackfan anemia were also excluded.

Preparation of UC-MSCs and 3D dynamic culture of UC-MSCs.

UC-MSCs were purchased from Daji Kanghua Biotechnology Co., Ltd. (Beijing, China). The 3D aggregate culture method of UC-MSCs in this study is similar to the 3D culture method of BMSCs in previous articles. Briefly, UC-MSCs at passages 3 to 4 were suspended in ACF, and 5.0×10^5 UC-MSCs in 2 mL ACF were seeded in a 60 mm ultra-low-attachment (ULA) culture dish (Corning) at 37 °C in a 5 % CO_2 humidified atmosphere. The ULA plates were placed on a rocking base of a Digital OrbitaI Shaker in a standard humidified incubator (37 °C, 5 % CO_2) under controlled rocking speeds (i.e., 40–100 rockings/min, or rpm) for 18–48 h. H-3D- UC-MSCs were incubated at 37 °C under 3 % O_2, 5 % CO_2, and 92 % N_2 for 24 h in a gas-tight humidified chamber (modular incubator chamber; Billups Rothenberg, Del Mar, CA, USA). N-3D- UC-MSCs were incubated under 21% O_2, 5 % CO2.

Procedures

The enrolled patients with an HLA-haploidentical relative for HSCT received the fludarabine (Flu) + cyclophosphamide (Cy) + antithymocyte globulin (ATG) conditioning regimen. For the patients with acute SAA (SAA-I), intravenous administration of 30 mg/(m^2 day) of Flu and 500 – 800 mg/(m^2 day) of Cy was performed from days -5 to -2, and 5 μ g/(kg day) of ATG was administered from days -4 to -1. For the patients with chronic SAA (SAA-II), the same treatment of ATG and Cy was applied as above with the supplement of 0.6 mg/(kg 6 h) of busulfan (BU) from days -8 to -5 prior to transplantation. Donor selection and hematopoietic stem cell mobilization and collection were conducted based on the consensus of The Chinese Society of Hematology regarding indications, conditioning regimens, and donor selection for allogeneic hematopoietic stem cell transplantation. On day 0, HSCs were infused intravenously. Both groups received 5×10^5/kg UC-MSCs at 4 h before HSC infusion. The control group was transfused with conventional 2D-cultured UC-MSCs, whereas the experimental group received 3D hypoxia-preconditioned UC-MSCs. Standard GVHD prophylaxis consisted of mycophenolate mofetil, cyclosporine A, and methotrexate (MTX).

End Points

The primary end point was incidence and severity of adverse events (AEs) within the first 150 days after haplo-HSCT. Secondary end points included the 1-year cumulative incidence of severe cGVHD, the incidence and severity of aGVHD, rates of overall survival (OS), and GVHD-free and relapse-free survival (GRFS; survival without III to IV aGVHD, cGVHD requiring systematic treatment, AA relapse, or death) and AA relapse. Organ scoring and global assessment of cGVHD were conducted based on the 2014 National Institutes of Health consensus criteria, whereas acute GVHD was assessed based on the Mount Sinai Acute GVHD International Consortium criteria. AEs were graded according to the Common Terminology Criteria for Adverse Events version 5.0. For the OS analysis, death was counted as death of any reason after transplantation.

Random Assignment and Statistical Analysis

At each participating center, consented participants meeting the inclusion criteria underwent 1:1 randomization to either the 3D hypoxia-preconditioned UC-MSC group or the control group. The randomization sequence was generated and implemented by dedicated statisticians who remained independent of patient recruitment, clinical management, and endpoint evaluation, thereby maintaining allocation concealment throughout the study.

The Mann-Whitney U test, $\chi 2$ test, and Fisher's exact test were used to compare the baseline patients' characteristics and AEs between the 3D hypoxia-preconditioned UC-MSC and control groups. The competing risk model (Fine and Gray model) was used to estimate the Secondary end point (1 year severe cGVHD cumulative incidence completed by death and relapse) and hazard ratios (HRs) with 95% CIs, and the incidence and severity of cGVHD, aGVHD and AA relapse. The rates of GRFS and OS were analyzed using Kaplan-Meier analysis and presented as percentages with 95% CIs and tested by log-rank test between the two groups. Statistical analyses were conducted using R (version 4.4.2, R Foundation for Statistical Computing, Vienna, Austria).