


BMJ Open Randomised controlled trial of conditioning regimen for cord blood transplantation for adult myeloid malignancies comparing high-dose cytarabine/cyclophosphamide/total body irradiation with versus without G-CSF priming: G-CONCORD study protocol

Seitaro Terakura ¹, Takaaki Konuma,² Masatsugu Tanaka,³ Yukiyasu Ozawa,⁴ Makoto Onizuka,⁵ Satoshi Nanno,⁶ Yasushi Onishi,⁷ Nobuyuki Aotsuka,⁸ Tadakazu Kondo,⁹ Toshiro Kawakita,¹⁰ Jun Kato,¹¹ Takeshi Kobayashi,¹² Tetsuya Nishida,¹ Takuhiro Yamaguchi,¹³ Yachiyo Kuwatsuka,¹⁴ Satoshi Takahashi²

To cite: Terakura S, Konuma T, Tanaka M, *et al.* Randomised controlled trial of conditioning regimen for cord blood transplantation for adult myeloid malignancies comparing high-dose cytarabine/cyclophosphamide/total body irradiation with versus without G-CSF priming: G-CONCORD study protocol. *BMJ Open* 2020;**10**:e040467. doi:10.1136/bmjopen-2020-040467

► Prepublication history and additional materials for this paper is available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2020-040467>).

Received 15 May 2020
Revised 06 October 2020
Accepted 28 October 2020



© Author(s) (or their employer(s)) 2020. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

For numbered affiliations see end of article.

Correspondence to

Dr Seitaro Terakura;
tseit@med.nagoya-u.ac.jp

ABSTRACT

Introduction A better long-term quality of life after umbilical cord blood transplantation (CBT) is observed compared with transplants from other alternative donors, whereas graft failure and relapses after CBT are still major issues. To minimise graft failure and relapse after CBT, intensification of conditioning by the addition of high-dose cytosine arabinoside (CA) and concomitant continuous use of granulocyte-colony stimulating factor (G-CSF) are reported to convey a significantly better survival after CBT in some retrospective studies. To confirm the effect of G-CSF plus CA combination, in addition to the standard conditioning regimen, cyclophosphamide (CY)/total body irradiation (TBI), we design a randomised controlled study comparing CA/CY/TBI with versus without G-CSF priming (G-CSF combined conditioned cord blood transplantation [G-CONCORD] study).

Methods and analysis This is a multicentre, open-label, randomised phase III study that aimed to compare G-CSF+CA/CY/TBI as a conditioning regimen for CBT with CA/CY/TBI. Patients with acute myeloid leukaemia or myelodysplastic syndrome, aged 16–55 years, are eligible. The target sample size is 160 and the registration period is 4 years. The primary endpoint is the 2-year disease-free survival rate after CBT. The secondary endpoints are overall survival, relapse, non-relapse mortality, acute and chronic graft-versus-host disease, engraftment rate, time to neutrophil recovery, short-term adverse events, incidence of infections and causes of death.

This study employs a single one-to-one web-based randomisation between the with-G-CSF versus without-G-CSF groups after patient registration. Combination of high-dose CA and CY/TBI in both groups is used for conditioning.

Strengths and limitations of the study

- The G-CONCORD study is a multicentre open-label randomised control study to investigate whether the use of granulocyte-colony stimulating factor (G-CSF), in addition to cytosine arabinoside (CA)/cyclophosphamide (CY)/total body irradiation (TBI) conditioning, facilitates engraftment and reduces relapse of primary disease.
- The randomisation is performed according to the minimisation method, with consideration of transplant centre, disease, age of patients, disease status at transplantation and FMS-like tyrosine kinase 3 mutational status as adjustment factors to reduce the potential risk of bias.
- Sample size is calculated as 80 patients per group, who are required for a superiority test to detect whether the 2-year disease-free survival rate of the G-CSF+CA/CY/TBI combination group exceeds that of the CA/CY/TBI group by 20% or more in a significant level $\alpha=0.05$ (one side); the detection power was 80%.
- This is a truly myeloablative conditioning regimen; hence, it cannot be extrapolated to the majority of patients with acute myeloid leukaemia and myelodysplastic syndrome who are predominantly over the age of 60 years.

Ethics and dissemination The study protocol was approved by the central review board, Nagoya University Certified Review Board, after the enforcement of the Clinical Trials Act in Japan. The manuscripts presenting data from this study will be submitted for publication

in quality peer-reviewed medical journals. Study findings will be disseminated via presentations at national/international conferences and peer-reviewed journals.

Trial registration numbers UMIN000029947 and jRCTs041180059.

INTRODUCTION

Haematopoietic stem cell transplantation from alternative donors has now become a standard of care for high-risk haematopoietic malignancies.^{1–3} There are mainly three types of alternative donors, namely, adult volunteer unrelated donor (UD),^{2,4} umbilical cord blood (CB)^{3,5} and human leucocyte antigen (HLA)-mismatched related donor, that may often involve one haploidentical donor (HID) (one haplomismatched).^{2,6} UD-transplant and CB-transplant outcomes have been compared to determine the optimal donor choice, and such studies repeatedly demonstrate similar results between UD transplantation and cord blood transplantation (CBT).^{7–9} Comparing HID transplantation with CBT, prospective studies are now ongoing to reveal whether the preference of HID in patients who lack HLA-well-matched UD is appropriate or not.¹⁰ Thus, CB as an alternative graft source is still a relevant donor selection.^{2,3,11,12}

Advantages of choosing CB are the quick and wide availability of the graft¹³ and the low risk of severe graft-versus-host disease (GVHD),^{3,5} which is shown to be associated with better long-term quality of life.^{14,15} In contrast, the biggest disadvantage of CB is the limited number of total nucleated cell (TNC) or stem cell (CD34⁺ cell), which is associated with delayed engraftment and higher risk of graft failure.^{5,16} To reduce risk of graft failure and disease relapse, high-dose cytarabine arabinoside (CA) and granulocyte-colony stimulating factor (G-CSF) are added to the conditioning regimen for myeloid haematological malignancies, such as acute myeloid leukaemia (AML) and myelodysplastic syndrome (MDS).^{17–19} The principle of the conditioning regimen is to enhance cytotoxicity against tumour cells and to reduce antigraft immunoreaction by host-derived immune cells by the addition of high-dose CA to cyclophosphamide (CY) plus total body irradiation (TBI) (CY/TBI), which has been the standard myeloablative conditioning regimen. A continuous infusion of G-CSF during high-dose CA administration is adopted to purge leukaemia stem cells from its supporting environment (leukaemia niche) to make it more susceptible without support by niche and to further reduce the risk of relapse.^{20,21} When used as conditioning treatment for CBT, G-CSF combined with CA plus CY/TBI (G-CSF+CA/CY/TBI combination) is reported to significantly accelerate engraftment and to improve long-term survival.^{19,22}

This study is designed to compare CA/CY/TBI with G-CSF+CA/CY/TBI combination to investigate whether combined infusion of G-CSF with high-dose CA can induce faster engraftment, decrease the relapse risk and lead to better long-term survival. The conditioning regimen is truly myeloablative; hence, the eligible

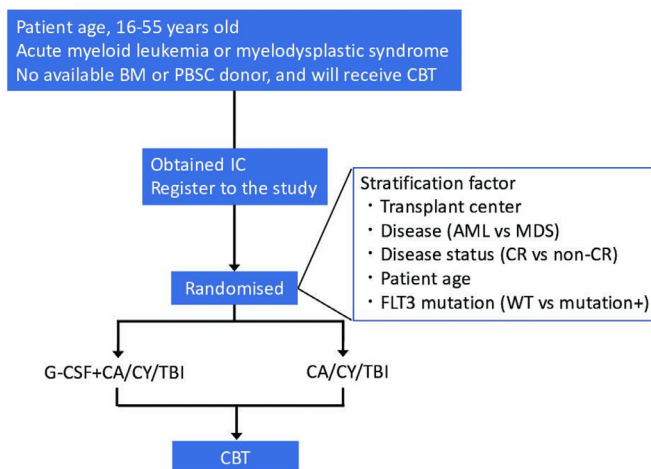


Figure 1 Outline of the G-CSF combined conditioned cord blood transplantation (G-CONCORD) study. AML, acute myeloid leukaemia; BM, bone marrow; CA, cytosine arabinoside; CBT, cord blood transplantation; CR, complete remission; CY, cyclophosphamide; G-CSF, granulocyte colony-stimulating factor; IC, informed consent; MDS, myelodysplastic syndrome; PBSC, peripheral blood stem cell; TBI, total body irradiation; WT, wild type.

patients are relatively younger adult patients between the ages of 16 and 55 years with haematological malignancies. The target diseases are AML and MDS, because an excellent transplant outcome has already been reported in previous retrospective analyses.^{19,22} Therefore, we aimed to investigate whether G-CSF+CA/CY/TBI combination will further promote engraftment and prevent disease recurrence in an open-label, randomised phase III study.

METHODS AND ANALYSIS

Study design

This is a multicentre prospective open-label, randomised phase III study that compares G-CSF+CA/CY/TBI combination with CA/CY/TBI as conditioning regimen for CBT. This study adopts a single randomisation step after inclusion of eligible patients. Registration for the study is done at least 15 days prior to transplantation. The randomisation is performed according to the minimisation method, with consideration of transplant centre, disease (AML vs MDS), age of patients (<40 vs ≥40 years), disease status at transplantation (complete remission (CR) vs non-CR), and FMS-like tyrosine kinase 3 (*FLT3*) mutational status (*FLT3* wild type vs *FLT3* mutation⁺ vs not tested) as adjustment factors (figure 1). Because we have reported that both cytogenetic risk and remission status are significant predictive factors, however, the remission status is the more powerful one. Accordingly, the remission status is adopted as one of the stratification factors.²³ This study was initiated on 1 March 2018. *FLT3* mutational status has adopted as an additional stratification factor after protocol revision on 15 August 2019 (V.2.1.0), when *FLT3* tyrosine kinase inhibitors (gilteritinib and quizartinib) became available in clinics. This study was registered in

the UMIN Clinical Trials Registry and Japan Registry of Clinical Trials.

Endpoints

The primary endpoint is the 2-year disease-free survival (DFS) rate after CBT and the secondary endpoints are as follows:

1. Time to haematopoietic recovery.
2. Engraftment rate.
3. Treatment-related toxicity until day 28.
4. Mucosal toxicity grade until day 42 (maximum grade and incidence).
5. Maximum dose of narcotic drug per day until day 42.
6. Incidence and severity of acute graft-versus-host disease (aGVHD).
7. Incidence and severity of chronic GVHD.
8. Treatment-related mortality at day 100 and 2 years after transplantation.
9. Relapse rate at 2 years after transplantation.
10. Overall survival (OS) at 2 years after transplantation.
11. Incidence of infectious event (causative bacteria or other microorganisms, site of infection, day of onset and frequency).
12. Causes of deaths.

Ethical consideration and patient registration

Twenty-five hospitals from all parts of Japan agreed to participate in this study at the time of writing (see online supplemental appendix 2, list of participating institutes). The protocol was originally approved by the Nagoya University Hospital Ethics Committee (approval number 2017-0430 on 11 January 2018; online supplemental appendix 3, approval letter) and all other institutions individually. Thereafter, the Clinical Trials Act was enforced in Japan, and the protocol was approved again by the Nagoya University Certified Review Board (approval number T0011 on 28 February 2019; online supplemental appendix 1, Certified Review Board approval letter), and the execution of the protocol was further approved by all participating institutes. Written informed consent was obtained from all patients before registration in accordance with the Declaration of Helsinki.

Patients are registered in this study through web-based registration system. In this system, once the physician inputs all the required data, the study group either with or without G-CSF is instantly informed. For patients assigned to the G-CSF group, lenograstim is delivered to the corresponding hospital before starting conditioning treatment.

Eligibility criteria

The inclusion criteria are as follows:

1. CBT recipient without preceding history of autologous or allogeneic stem cell transplantation.
2. Patient with either AML or MDS (AML or MDS of the WHO Classification of Myeloid Neoplasms and Acute Leukemia, Fourth Edition, 2008).²⁴
 - i. AML regardless of remission or non-remission.

- ii. MDS in EB1 or EB2 with either of the following status:
 - Categorised as International Prognostic Scoring System (IPSS) intermediate-II or high.²⁵
 - Categorised as WHO classification-based Prognostic Scoring System (WPSS) high or very high.²⁶
 - Relapsed after chemotherapy.
3. Aged ≥ 16 and ≤ 55 years: for minors under the age of 20 years, informed consent from the parental authority is obtained at the same time as the consent from the patient.
4. Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1 or 2.
5. Adequate function of key organ systems:
 - i. Cardiac: left ventricular ejection fraction of $\geq 40\%$ on echocardiogram.
 - ii. Hepatic: serum aspartate transaminase and alanine aminotransferase both < 150 U/L.
 - iii. Pulmonary: forced expiratory volume in one second $\geq 60\%$ and % vital capacity $\geq 50\%$.
 - iv. Renal: serum creatinine < 2.5 mg/dL.
6. Voluntary written consent must be obtained before enrolment.

Exclusion criteria are as follows:

1. Patients positive for hepatitis B surface (HBs) antigen.
2. Patients positive for hepatitis C virus (HCV) antibody.
3. Patients positive for human immunodeficiency virus (HIV) antibody.
4. Patients with donor-specific HLA antibody (HLA antibody against incompatible HLA antigen in the donor host-versus-graft [HVG] direction).
5. Patients who have been administered with gemtuzumab ozogamicin (Mylotarg) within the past 6 months.
6. Those who are pregnant or breastfeeding.
7. Those with uncontrolled psychiatric diseases.
8. Those with uncontrolled infectious diseases.
9. Patients with a history of hypersensitivity to drugs used for conditioning treatment and drugs used for GVHD prophylaxis.
10. Patients who, in the judgement of the investigator, would be inappropriate for entry into this study.

CB unit selection criteria

1. HLA-A, B and DR serotypes matched in 4/6 or more.
2. The number of CB TNC at the time of freezing is 2.0×10^7 /kg of recipient body weight or more.
3. From the CB units that meet conditions 1 and 2:
 - i. Select CB unit with more CD34⁺ cells.
 - ii. Avoid combinations where HLA-C locus and HLA-DRB1 locus are inconsistent with both alleles as much as possible.

Definitions

Diagnosis and classification of AML and MDS follow the WHO classification (2008).²⁴ The disease risk of the

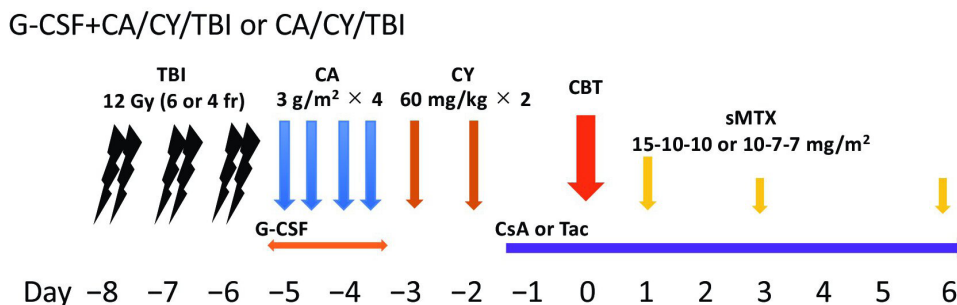


Figure 2 Conditioning regimen and graft-versus-host disease prophylaxis (CA/CY/TBI vs G-CSF+CA/CY/TBI combination regimen). CA, cytosine arabinoside; CBT, cord blood transplantation; CsA, cyclosporine A; CY, cyclophosphamide; G-CSF, granulocyte colony-stimulating factor; sMTX, short-term methotrexate; Tac, tacrolimus; TBI, total body irradiation.

patients is determined according to the refined disease risk index.²⁷

Neutrophil engraftment is defined as three consecutive days with an absolute neutrophil count of $500/\mu\text{L}$, whereas platelet engraftment is defined as a platelet count $>20\,000/\mu\text{L}$ without transfusion support. Graft failure is defined as a lack of absolute neutrophil count of $500/\mu\text{L}$ by day 60. When the bone marrow (BM) is remarkably hypoplastic after day 28 and the donor-type haematopoiesis defined by the $\text{CD}3^+$ T cell fraction is $<50\%$ and decreased during two different time points, the diagnosis of graft failure can be made before day 60. The diagnosis of secondary graft failure can be made once engraftment is confirmed and neutrophil counts fall below $500/\mu\text{L}$ for three consecutive points excluding exacerbation of the original disease and BM suppression due to adverse effects of the drugs.

Conditioning

Conditioning treatment is either CA/CY/TBI (12 Gy) or G-CSF+CA/CY/TBI combination, which are assigned immediately after web-based registration. The treatment schedule can be modified if the total dose is the same, and TBI can be given after chemotherapy. CA/CY/TBI regimen is an intensified regimen consisting of additional use of high-dose CA: four doses of CA $3\text{ g}/\text{m}^2$, two doses of CY $60\text{ mg}/\text{kg}$, and TBI 12 Gy in either four or six fractions (figure 2). G-CSF (lenograstim $5\text{ }\mu\text{g}/\text{kg}/\text{day}$) is continuously intravenously infused from 12 hours before the first dose of CA is administered to the end of CA dosing. If CA is administered four times every 12 hours from days -5 to -4, G-CSF is continuously administered for a total of 50 hours from the night of day -6 to the end of CA administration on day -4 (figure 2). DuBois equation is used to calculate the body surface area of the recipients. Although there is no protocol on the use of antiemetics, they should be used according to the method of each facility. Mesna is used to reduce the incidence of CY-associated haemorrhagic cystitis according to the package insert.²⁸

GVHD prophylaxis

For GVHD prophylaxis after CBT, the combination of cyclosporine A (CsA) and short-term methotrexate

(sMTX) is recommended as follows (but the combination of tacrolimus (Tac) and sMTX is an acceptable alternative method) (figure 2)^{29,30}:

1. CsA (once daily) plus sMTX (recommended method).
CsA $3.0\text{ mg}/\text{kg}/\text{day}$, intravenous infusion over 10 hours starting from day -1.
sMTX $15\text{ mg}/\text{m}^2$ (day 1) and $10\text{ mg}/\text{m}^2$ (days 3 and 6)
2. CsA (twice daily) plus sMTX (alternative method #1)
CsA $1.5\text{ mg}/\text{kg}/\text{dose}$ twice a day, intravenous infusion over three or 4 hours every 12 hours from day -1.
sMTX $15\text{ mg}/\text{m}^2$ (day 1) and $10\text{ mg}/\text{m}^2$ (days 3 and 6).
3. Tac (continuous administration) plus sMTX (alternative method #2)
Tac $0.015\text{ mg}/\text{kg}/\text{day}$, continuous intravenous infusion from day -1.
sMTX $10\text{ mg}/\text{m}^2$ (day 1) and $7\text{ mg}/\text{m}^2$ (days 3 and 6).

Treatment for severe aGVHD

The severity of aGVHD is evaluated according to the revised Glucksberg grading.³¹ If the patient has a clinical grade II or higher aGVHD or aGVHD grade II limited to the skin and does not subside within 1 week with topical steroid therapy, steroid therapy using prednisolone (PSL) $1\text{ mg}/\text{kg}/\text{day}$ or methylprednisolone (mPSL) $1\text{--}2\text{ mg}/\text{kg}/\text{day}$ will be initiated.³² The administration period of the initial dose should be at least 5 days and a maximum of 14 days. The therapeutic effect is determined according to established criteria,³¹ and if a complete response is obtained, PSL/mPSL will be gradually reduced by a maximum of 20% of steroid dose every 7–10 days.

For patients with insufficient effect from the initial treatment or with exacerbation during PSL/mPSL dose reduction, a second-line treatment is considered. Exacerbation of diarrhoea or liver dysfunction (jaundice), despite improvement in the skin, may be associated with thrombotic microangiopathy.³³ Although the necessity and validity of strengthening immunosuppressive therapy is further evaluated and considered, there is no established clinical evidence or recommendation for second-line treatment of steroid-resistant GVHD. Thus, the method of immunosuppressive therapy after first-line GVHD treatment is not specified by this protocol.

Supportive measures

G-CSF will be administered to all patients from day 7 to the day of neutrophil engraftment and then tapered. The following prophylactic measures are recommended for preventive medication for bacterial, fungal and viral infections: Oral ciprofloxacin 200 mg two times per day or levofloxacin 500 mg once a day, oral acyclovir 1000 mg/day, and oral fluconazole 200 mg once a day will be administered from the start of conditioning to the end of immunosuppression. Drugs other than fluconazole, such as voriconazole, can be used when necessary to prevent recurrence of prior deep fungal infection.

To prevent *Pneumocystis carinii* pneumonia, sulfamethoxazole/trimethoprim (400 mg/80 mg) is administered three times per day from the start of conditioning to day -1 and once daily from the time of engraftment to the end of immunosuppression. Alternative therapies such as inhalation of pentamidine or oral administration of atovaquone are allowed in case of allergy to sulfamethoxazole/trimethoprim.

Cytomegalovirus monitoring with antigenemia test (C7-HRP or C10/C11) is performed every 7–10 days after engraftment until day 150. Foscarnet or ganciclovir is administered as appropriate based on the cytomegalovirus antigenemia test results. Letermovir was released in May 2018 and became available in Japan; hence it is at the discretion of each institution to decide whether or not to use this drug.³⁴

Study duration

Patients are followed for at least 24 months after transplant. This study is expected to begin on 11 January 2018 and to end enrolment of the planned number of patients in January 2022.

Grant of study drug (G-CSF, lenograstim)

In implementing this study, we received a grant of G-CSF (lenograstim) from Chugai Pharmaceutical Co. However, we did not receive any other particular funds or goods. Chugai Pharmaceutical Co. is not involved in the planning and implementation of this study. There are donations and research grants from companies that are not specially associated with this research. The data obtained in this study are accumulated in the data centre, which is independent of any companies. In order to maintain the fairness of research results, we make efforts to maintain their accuracy under the data management centre. Therefore, the results of this study do not intentionally lead to favourable results for Chugai Pharmaceutical Co. The Japan Society for Hematopoietic Cell Transplantation, which provides the research grant, is not involved in the analysis of this study and the interpretation and reporting of the results.

Sample size and power calculation

In a retrospective study in Japan, the 2-year DFS rate after CBT in the G-CSF+CA/CY/TBI combination group was 64%,¹⁹ whereas that of the CA/CY/TBI group was 40%.

Accordingly, the control was set to 45% and the difference of 20% was set to be detectable. Based on these data, it was calculated that 76 patients per group are required for a superiority test to detect whether the 2-year DFS rate of the G-CSF+CA/CY/TBI combination group exceeds that of the CA/CY/TBI group by 20% or more in a significant level $\alpha=0.05$ (one side); the detection power was 80%. Including approximately 5% of unqualified patients, the number of patients was calculated as 80 per group, hence a total of 160.³⁵ In this study, both patients in the CR and non-CR groups are included. Therefore, randomisation is performed according to the minimisation method, with adjustment factors of transplant centre, disease (AML vs MDS), disease status at transplantation (CR vs non-CR), patient age (patients of <40 vs ≥ 40 years) and *FLT3* mutation (wild type vs mutation⁺ vs not tested).

Data collection and management

The Research Electronic Data Capture (REDCap) system is the primary data collection instrument and is treated as data source.³⁶ All data entered by the physician are recorded. Data captured through the REDCap system are then reviewed for completeness, accuracy and timeliness by the data managers of data centre (Department of Advanced Medicine, Nagoya University Hospital). If there is any doubt or discrepancy, the physician in charge is questioned by the data managers through REDCap.

All study documentations containing identifiable patient data are available for inspection, monitoring or audit purposes by the sponsor, regulatory authorities or the funder. All electronic data are stored in secure network drives, to which only the relevant study staff have access. All study documents and data are kept for 5 years after completion of the study.

Definition of unevaluable cases

Of all treated cases, patients with serious post-treatment violations, untraceability or postregistration ineligibility are considered non-evaluable cases.

Data monitoring

The study will be monitored internally to ensure data collection procedures and data analyses are accurate. An independent data monitoring committee will not be employed for this study. Central data monitoring will be done once a year or as needed. In the central data monitoring, the principal investigator and the study office will carefully review the adverse event reports in the central data monitoring reports generated by the data centre to ensure that there are no reporting omissions. The presence or absence of reporting omissions should be clearly stated in the central data monitoring report.

Auditing

The study will include two audits. A system audit has already been conducted prior to the start of the study to assess the adequacy of the data collection methods and items. A central audit will be conducted at the end of the

study to assess whether monitoring has been performed as specified.

Planned statistical analysis

Regarding the treatment success rate of the primary endpoint, a survival curve will be estimated for each group using the Kaplan-Meier method for all evaluable patients, and a p value for group comparison will be calculated using the log-rank test. Adjustment factors used in the randomisation with minimisation method will be considered as the covariate of the multivariate analysis. The difference of DFS rate between groups and its 95% CI will be calculated. A p value of <0.05 will be considered as statistically significant.

For analysis of patients' background, analysis methods such as the χ^2 , t- and Wilcoxon tests will be appropriately selected for all evaluable cases based on the type of data. Regarding safety (toxicity), frequency of adverse events will be tabulated for each treatment group according to the Common Terminology Criteria for Adverse Events (CTCAE) grade. For data with competitive events such as engraftment, haematopoietic recovery, aGVHD and chronic GVHD, the incidence will be estimated using the cumulative incidence method,³⁷ and the p value for comparison between groups will be calculated using Gray's test. Because the primary endpoint of this study is 2-year DFS, the data collection for an interim analysis will take 2 years. Therefore, no interim analysis is planned for this study as it is unlikely to be performed in a timely manner.

Patient and public involvement

Patients are 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.

ETHICS AND DISSEMINATION

Research ethics approval

To participate in this study, the study protocol and explanatory documents (including informed consent and assent documents) must be approved by the certified review board of the Nagoya University Hospital, and then permission to conduct the study will be obtained from the director of each institution.

Protocol amendments

The principal investigator will revise the protocol and the REDCap Case Report Form by consensus among the protocol development committee members if the amendments are deemed necessary. When the protocol is revised, all revisions and the reasons for the amendments should be reported to the certified review board, and recorded to the Japan Registry of Clinical Trials (JRCT).

Consent or assent

The patient and/or the patient's legally authorised guardian must acknowledge in writing (consent) to become a study participant on the study. Consent will be obtained jointly by the local investigator or co-investigator and the local research coordinator.

Confidentiality

Study data will be stored in reidentifiable (coded) format, with the master code list only provided to principal investigator. All study files will be stored in password-protected folders in soft copy, with access provided only to principal investigator.

Declaration of interests

The authors and investigators have no relevant conflicts of interests.

Access to data

Only the study office and the principal investigator will have authorisation to transfer data to the statistician for study analysis.

Ancillary and post-trial care

There will be no specific post-trial care, where medical care will follow local institutional practices. In the event of a study-related injury or illness, the participant will be provided with appropriate medical treatment and care. Financial compensation for lost wages, disability or discomfort due to an injury or illness will not be available. The legal rights of the participant will not be waived as a result of participation in the study. The investigators and their respective institutions will still have their legal and professional responsibilities.

Dissemination policy

The manuscripts presenting data from this study will be submitted for publication in quality peer-reviewed medical journals. Study findings will be disseminated via presentations at national/international conferences and peer-reviewed journals. The project is registered with University Hospital Medical Information Network (UMIN) and JRCTs. At the time of writing, there is no specific plans to grant public access to the full protocol, participant-level data set or statistical code.

DISCUSSION

This is a multicentre randomised control trial to investigate the safety and efficacy of conditioning intensification by G-CSF+CA combination in addition to CY/TBI before CBT. It is well recognised that simple conditioning intensification by the additional use of chemotherapeutic agents usually results in the reduction of relapse and increase of NRM simultaneously; ultimately, OS after intensified conditioning is similar to that after standard regimen.^{38–40} However, superior OS is observed in some retrospective studies if the clinical study is conducted by inclusion of mainly young and fit patients.^{17 41} In BM transplantation

and peripheral blood stem cell transplantation, intensified conditioning is more likely to be associated with the increase of severe GVHD triggered by mucosal injury due to the adverse effect of drugs; such severe GVHD often results in mortality. In contrast, although the incidence of severe GVHD after CBT is similar to that of other graft sources,⁴² steroid treatment response to GVHD is usually better in CBT.^{43–45} Thus, development of severe GVHD is less likely to lead to the deterioration of OS in CBT.^{44–45} We believe this is one possible explanation that we observe relatively better survival after CBT after full-intensity conditioning compared with CBT after reduced-intensity conditioning.^{30–46}

An intensified conditioning regimen usually employs either the addition of busulfan, etoposide or CA to CY/TBI.^{17–40–41} Whereas the addition of busulfan and etoposide is associated with the increase of veno-occlusive disease and mucosal injury, respectively, the addition of CA is most unlikely associated with certain organ damages or specific complications. Furthermore, G-CSF may increase the susceptibility of myeloid leukaemia clones and primary leukaemia blasts to CA *in vitro*⁴⁷ and *in vivo* by acting on leukemia-niche and expelling leukaemia stem cells from it.⁴⁸ Particularly, a combination of G-CSF with a relatively high-dose CA resulted in improved survival of patients with AML.^{49–50} Thus, we can expect enhancement of susceptibility of tumour cells to chemotherapy. In humans, because it is practically impossible to directly observe the purging effect of leukaemia stem cells from the BM niche, investigation of the hypothetical effect requires a randomised controlled trial. Therefore, in this study, we choose the CA/CY/TBI regimen for CBT, which is the most promising conditioning regimen for CBT in Japan, and investigate the strategy to further combine it with G-CSF.

The major limitation of this study is the extrapolation of the results to the practice. AML and MDS are diseases with a peak age of onset in the 60s and beyond. They are rare in young and fit patients, such as those included in the present study. Therefore, it is expected that the number of subjects to whom the results can be applied will be small.

This study will provide a conclusive result about safety and efficacy of G-CSF+CA/CY/TBI combination regimen for CBT. G-CSF-combined intensification of conditioning may lead to further development of superior conditioning that can result in reduced relapse and a better safety profile by using the effect of interrupting leukaemia stem cells and niche interactions.

Author affiliations

¹Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan

²Department of Hematology/Oncology, The Institute of Medical Science The University of Tokyo, Tokyo, Japan

³Department of Hematology, Kanagawa Cancer Center, Yokohama, Japan

⁴Department of Hematology and Oncology, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan

⁵Department of Hematology and Oncology, Tokai University School of Medicine Graduate School of Medicine, Isehara, Japan

⁶Department of Hematology, Osaka City University Graduate School of Medicine School of Medicine, Osaka, Japan

⁷Department of Hematology and Rheumatology, Tohoku University Hospital, Sendai, Japan

⁸Division of Hematology-Oncology, Japanese Red Cross Society Narita Hospital, Narita, Japan

⁹Department of Hematology and Oncology, Kyoto University Graduate School of Medicine, Kyoto, Japan

¹⁰Department of Hematology, National Hospital Organisation Kumamoto Medical Center, Kumamoto, Japan

¹¹Division of Hematology, Keio University School of Medicine, Tokyo, Japan

¹²Division of Hematology, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo, Japan

¹³Department of Biostatistics, Graduate School of Medicine, Tohoku University, Sendai, Japan

¹⁴Department of Advanced Medicine, Nagoya University Hospital, Nagoya, Japan

Acknowledgements The authors are grateful to Yuri Amano, Chiho Nishimura, Yasuko Watarai and Chikako Yamada for their excellent help with data management and handling of drugs. Dr Kohei Ueda takes the labour of auditing for this study.

Contributors STe and STa devised the research, secured the funding and drafted this article. TKonu, MT, YOz, MO, SN, YOo, NA, TKond, TKa, JK, TKob and YK are members of the protocol writing committee who discussed the development of this protocol. TN and TY advised monitoring and statistical analyses of this protocol. All authors have read and approved the final manuscript.

Funding This study is supported in part by grants from Nagoya University Hospital Funding for Clinical Development grant number (71004228) to STe, Practical Research Project for Allergic Diseases and Immunology (grant number 19ek0510020s0103) to STa and Clinical Research Committee of the Japan Society for Hematopoietic Cell Transplantation.

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

ORCID iD

Seitaro Terakura <http://orcid.org/0000-0002-1194-8046>

REFERENCES

- 1 Cornelissen JJ, Blaise D. Hematopoietic stem cell transplantation for patients with AML in first complete remission. *Blood* 2016;127:62–70.
- 2 Shouval R, Fein JA, Labopin M, *et al.* Outcomes of allogeneic haematopoietic stem cell transplantation from HLA-matched and alternative donors: a European Society for blood and marrow transplantation registry retrospective analysis. *Lancet Haematol* 2019;6:e573–84.
- 3 Ballen KK, Gluckman E, Broxmeyer HE. Umbilical cord blood transplantation: the first 25 years and beyond. *Blood* 2013;122:491–8.
- 4 Anasetti C, Logan BR, Lee SJ, *et al.* Peripheral-blood stem cells versus bone marrow from unrelated donors. *N Engl J Med* 2012;367:1487–96.

- 5 Dehn J, Spellman S, Hurley CK, *et al.* Selection of unrelated donors and cord blood units for hematopoietic cell transplantation: guidelines from the NMDP/CIBMTR. *Blood* 2019;134:924–34.
- 6 McCurdy SR, Luznik L. How we perform haploidentical stem cell transplantation with posttransplant cyclophosphamide. *Blood* 2019;134:1802–10.
- 7 Laughlin MJ, Eapen M, Rubinstein P, *et al.* Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. *N Engl J Med* 2004;351:2265–75.
- 8 Rocha V, Labopin M, Sanz G, *et al.* Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *N Engl J Med* 2004;351:2276–85.
- 9 Terakura S, Nishida T, Sawa M, *et al.* Prospective evaluation of alternative donor from unrelated donor and cord blood in adult acute leukemia and myelodysplastic syndrome. *Bone Marrow Transplant* 2020;55:1399–409.
- 10 Ballen KK, Koreth J, Chen Y-B, *et al.* Selection of optimal alternative graft source: mismatched unrelated donor, umbilical cord blood, or haploidentical transplant. *Blood* 2012;119:1972–80.
- 11 Milano F, Gooley T, Wood B, *et al.* Cord-blood transplantation in patients with minimal residual disease. *N Engl J Med* 2016;375:944–53.
- 12 Yamamoto H. Single cord blood transplantation in Japan; expanding the possibilities of CBT. *Int J Hematol* 2019;110:39–49.
- 13 Barker JN, Boughan K, Dahi PB, *et al.* Racial disparities in access to HLA-matched unrelated donor transplants: a prospective 1312-patient analysis. *Blood Adv* 2019;3:939–44.
- 14 Inamoto Y, Kimura F, Kanda J, *et al.* Comparison of graft-versus-host disease-free, relapse-free survival according to a variety of graft sources: antithymocyte globulin and single cord blood provide favorable outcomes in some subgroups. *Haematologica* 2016;101:1592–602.
- 15 Mehta RS, Holtan SG, Wang T, *et al.* GRFS and CRFS in alternative donor hematopoietic cell transplantation for pediatric patients with acute leukemia. *Blood Adv* 2019;3:1441–9.
- 16 Konuma T, Kanda J, Inamoto Y, *et al.* Improvement of early mortality in single-unit cord blood transplantation for Japanese adults from 1998 to 2017. *Am J Hematol* 2019.
- 17 Arai Y, Takeda J, Aoki K, *et al.* Efficiency of high-dose cytarabine added to CY/TBI in cord blood transplantation for myeloid malignancy. *Blood* 2015;126:415–22.
- 18 Takahashi S, Ooi J, Tomonari A, *et al.* Comparative single-institute analysis of cord blood transplantation from unrelated donors with bone marrow or peripheral blood stem-cell transplants from related donors in adult patients with hematologic malignancies after myeloablative conditioning regimen. *Blood* 2007;109:1322–30.
- 19 Konuma T, Ooi J, Uchida N, *et al.* Granulocyte colony-stimulating factor combined regimen in cord blood transplantation for acute myeloid leukemia: a nationwide retrospective analysis in Japan. *Haematologica* 2014;99:e264–8.
- 20 Tafuri A, Andreeff M. Kinetic rationale for cytokine-induced recruitment of myeloblastic leukemia followed by cycle-specific chemotherapy in vitro. *Leukemia* 1990;4:826–34.
- 21 Tosi P, Visani G, Ottaviani E, *et al.* Fludarabine + Ara-C + G-CSF: cytotoxic effect and induction of apoptosis on fresh acute myeloid leukemia cells. *Leukemia* 1994;8:2076–82.
- 22 Konuma T, Takahashi S, Uchida N, *et al.* Effect of granulocyte colony-stimulating factor-combined conditioning in cord blood transplantation for myelodysplastic syndrome and secondary acute myeloid leukemia: a retrospective study in Japan. *Biol Blood Marrow Transplant* 2015;21:1632–40.
- 23 Terakura S, Wake A, Inamoto Y, *et al.* Exploratory research for optimal GVHD prophylaxis after single unit CBT in adults: short-term methotrexate reduced the incidence of severe GVHD more than mycophenolate mofetil. *Bone Marrow Transplant* 2017;52:423–30.
- 24 Vardiman JW, Thiele J, Arber DA, *et al.* The 2008 revision of the world Health organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood* 2009;114:937–51.
- 25 Greenberg P, Cox C, LeBeau MM, *et al.* International scoring system for evaluating prognosis in myelodysplastic syndromes. *Blood* 1997;89:2079–88.
- 26 Malcovati L, Germing U, Kuendgen A, *et al.* Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes. *J Clin Oncol* 2007;25:3503–10.
- 27 Armand P, Kim HT, Logan BR, *et al.* Validation and refinement of the disease risk index for allogeneic stem cell transplantation. *Blood* 2014;123:3664–71.
- 28 Shepherd JD, Pringle LE, Barnett MJ, *et al.* Mesna versus hyperhydration for the prevention of cyclophosphamide-induced hemorrhagic cystitis in bone marrow transplantation. *J Clin Oncol* 1991;9:2016–20.
- 29 Takahashi S, Iseki T, Ooi J, *et al.* Single-institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation for adult patients with hematologic malignancies. *Blood* 2004;104:3813–20.
- 30 Terakura S, Wake A, Inamoto Y, *et al.* Exploratory research for optimal GVHD prophylaxis after single unit CBT in adults: short-term methotrexate reduced the incidence of severe GVHD more than mycophenolate mofetil. *Bone Marrow Transplant* 2017;52:423–30.
- 31 Przepiorka D, Weisdorf D, Martin P, *et al.* 1994 consensus conference on acute GVHD grading. *Bone Marrow Transplant* 1995;15:825–8.
- 32 MacMillan ML, Robin M, Harris AC, *et al.* A refined risk score for acute graft-versus-host disease that predicts response to initial therapy, survival, and transplant-related mortality. *Biol Blood Marrow Transplant* 2015;21:761–7.
- 33 Nishida T, Hamaguchi M, Hirabayashi N, *et al.* Intestinal thrombotic microangiopathy after allogeneic bone marrow transplantation: a clinical imitator of acute enteric graft-versus-host disease. *Bone Marrow Transplant* 2004;33:1143–50.
- 34 Marty FM, Ljungman P, Chemaly RF, *et al.* Letermovir prophylaxis for cytomegalovirus in hematopoietic-cell transplantation. *N Engl J Med* 2017;377:2433–44.
- 35 Shih WJ, Li G, Wang Y. Methods for flexible sample-size design in clinical trials: likelihood, weighted, dual test, and promising zone approaches. *Contemp Clin Trials* 2016;47:40–8.
- 36 Harris PA, Taylor R, Minor BL, *et al.* The REDCap consortium: building an international community of software platform partners. *J Biomed Inform* 2019;95:103208.
- 37 Fine JP, Gray RJ. A proportional hazards model for the Subdistribution of a competing risk. *J Am Stat Assoc* 1999;94:496–509.
- 38 Arai Y, Kondo T, Shigematsu A, *et al.* Increased non-relapse mortality due to high-dose cytarabine plus CY/TBI in BMT/PBSCT for acute lymphoblastic leukaemia in adults. *Br J Haematol* 2017;178:106–11.
- 39 Martino R, de Wreede L, Fiocco M, *et al.* Comparison of conditioning regimens of various intensities for allogeneic hematopoietic SCT using HLA-identical sibling donors in AML and MDS with <10% BM blasts: a report from EBMT. *Bone Marrow Transplant* 2013;48:761–70.
- 40 Anderson JE, Appelbaum FR, Schoch G, *et al.* Allogeneic marrow transplantation for myelodysplastic syndrome with advanced disease morphology: a phase II study of busulfan, cyclophosphamide, and total-body irradiation and analysis of prognostic factors. *J Clin Oncol* 1996;14:220–6.
- 41 Arai Y, Kondo T, Shigematsu A, *et al.* Improved prognosis with additional medium-dose VP16 to CY/TBI in allogeneic transplantation for high risk ALL in adults. *Am J Hematol* 2018;93:47–57.
- 42 Murata M, Nakasone H, Kanda J, *et al.* Clinical factors predicting the response of acute graft-versus-host disease to corticosteroid therapy: an analysis from the GVHD Working group of the Japan Society for hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 2013;19:1183–9.
- 43 MacMillan ML, DeFor TE, Weisdorf DJ. The best endpoint for acute GVHD treatment trials. *Blood* 2010;115:5412–7.
- 44 Kanda J, Umeda K, Kato K, *et al.* Effect of graft-versus-host disease on outcomes after pediatric single cord blood transplantation. *Bone Marrow Transplant* 2020;55:1430–7.
- 45 Kanda J, Morishima Y, Terakura S, *et al.* Impact of graft-versus-host disease on outcomes after unrelated cord blood transplantation. *Leukemia* 2017;31:663–8.
- 46 Terakura S, Kuwatsuka Y, Yamasaki S, *et al.* GvHD prophylaxis after single-unit reduced intensity conditioning cord blood transplantation in adults with acute leukemia. *Bone Marrow Transplant* 2017;52:1261–7.
- 47 Takahashi S, Okamoto SI, Shirafuji N, *et al.* Recombinant human glycosylated granulocyte colony-stimulating factor (rhG-CSF)-combined regimen for allogeneic bone marrow transplantation in refractory acute myeloid leukemia. *Bone Marrow Transplant* 1994;13:239–45.
- 48 Saito Y, Uchida N, Tanaka S, *et al.* Induction of cell cycle entry eliminates human leukemia stem cells in a mouse model of AML. *Nat Biotechnol* 2010;28:275–80.
- 49 Pabst T, Vellenga E, van Putten W, *et al.* Favorable effect of priming with granulocyte colony-stimulating factor in remission induction of acute myeloid leukemia restricted to dose escalation of cytarabine. *Blood* 2012;119:5367–73.
- 50 Löwenberg B, van Putten W, Theobald M, *et al.* Effect of priming with granulocyte colony-stimulating factor on the outcome of chemotherapy for acute myeloid leukemia. *N Engl J Med* 2003;349:743–52.