Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Broers AEC, de Jong CN, Bakunina K, et al. **Posttransplant Cyclophosphamide for Prevention of Graft-versus-Host Disease: Results of the Prospective Randomized HOVON-96 Trial**

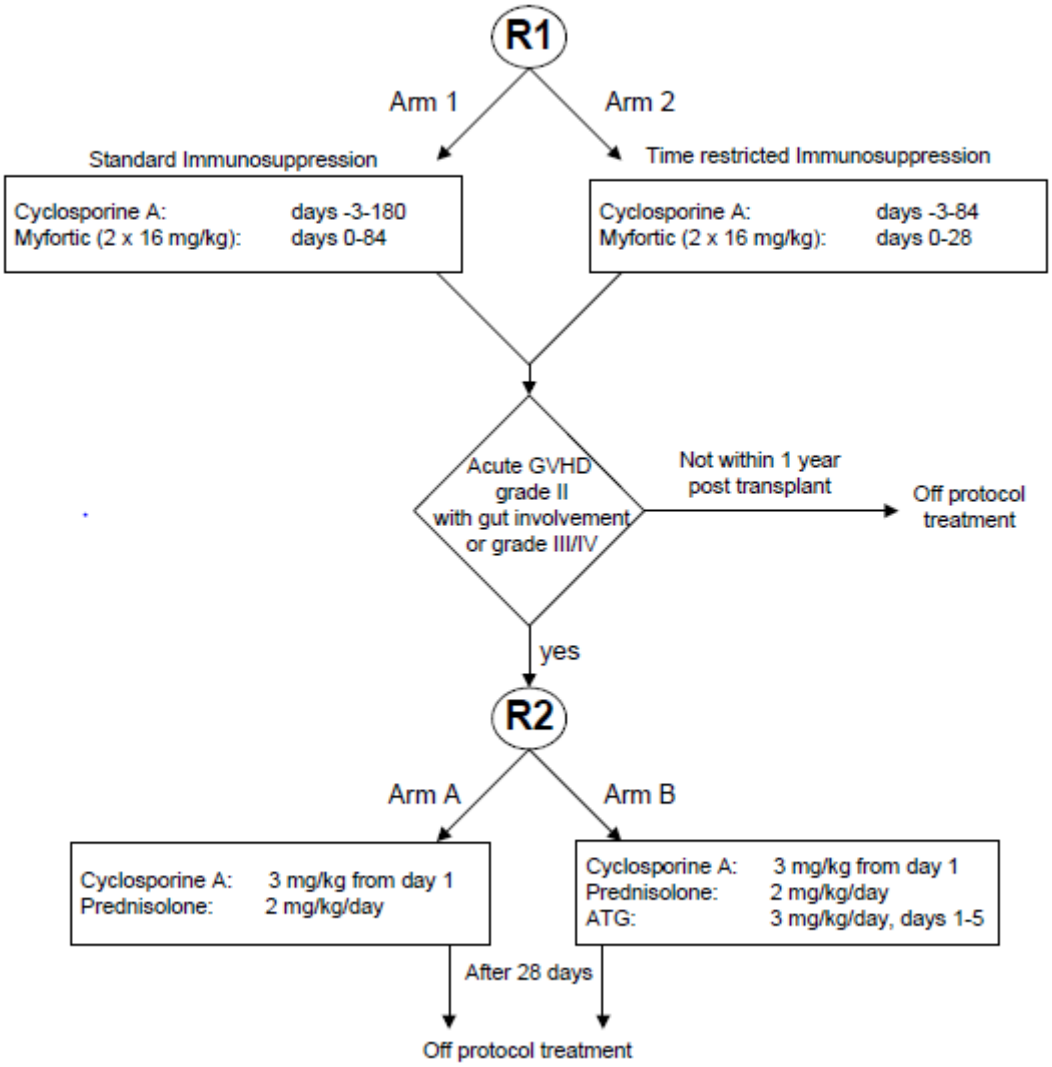
Supplementary material

Table of contents

Supplementary text: Trial design over time and amendments.

Original design, November 12, 2009

This HOVON-96 trial was originally designed as a prospective, open-label, randomized phase III trial with two randomizations, R1 and R2. The sample size of the trial was based on the R2 randomization, and from that it was calculated how many patients should be included in R1. It was planned to include 110 patients in R2, and consequently 500 patients in R1. The aim of these randomizations and the corresponding sample size calculations are specified below.



Immunosuppression randomization (R1):

The aim of the first randomization is to evaluate whether a time-restricted immune suppression (IS) scheme results in a higher proportion of patients with non-severe GVHD (either acute GVHD grade I-II without gut involvement or chronic GVHD not requiring systemic treatment) within 180 days after transplantation (denoted as PG180) compared to the standard IS scheme.

Sample size calculation

For the primary endpoint, patients will be considered a success if only non-severe GVHD has been observed within 180 days post-transplant, without having received any treatment not specified in the protocol (e.g., pre-emptive DLI or reinduction chemotherapy). All other patients will be considered a failure; among those - but not restricted to - patients without any GVHD within 180 days following transplantation, with acute GVHD grade II with gut infiltration or grades III-IV or chronic GVHD requiring systemic treatment or death before any GVHD. As treatment is the same in both treatment arms during the first 28 days, patients with a failure until 28 days posttransplant will be excluded from the primary analysis. It is assumed that about 35% of the patients assigned to the standard IS scheme will attain only non-severe GVHD within 180 days following transplant, i.e., PG_{180} = 35%. In order to detect with 90% power an increase of PG180 to 50% (two-sided significance level $\alpha = 0.05$), 480 patients randomized 1:1 between the standard and time-restricted IS scheme will be required. In order to overcome possible dropout due to non-eligibility, no transplantation or failures before day 28 posttransplant, 500 patients will be randomized.

Acute GVHD treatment randomization (R2)

It is assumed that about 20% of the patients included in R1, will attain acute GVHD grade II with gut infiltration or grades III-IV for which treatment with high-dose steroids is indicated. The aim of the second randomization is to evaluate whether the addition of ATG to standard treatment with high-dose steroids for severe acute GVHD will improve the proportion of patients with a complete (CR_{GVHD}) or partial response (PR_{GVHD}) after treatment for the acute GVHD (i.e., acute GVHD is completely/partially resolved) within 28 days after randomization (CR/PR_{28}).

Sample size calculation

For the primary endpoint, patients will be considered a success if at least a PR is achieved within 28 days after randomization R2, without having received any treatment not specified in the protocol. All other patients will be considered a failure; among those - but not restricted to - patients with steroid-refractory acute GVHD within 28 days following R2, or with any treatment not specified in the protocol, progression or death before day 28. The CR/PR_{28} rate in the high-dose steroids only group is assumed to be about 30%. In order to detect with 80% power (two-sided significance level $\alpha = 0.05$) an improvement of CR/PR_{28} to 60%, 98 patients should be randomized 1:1 between the two treatment arms. This number almost equals the 20% of the 500 patients in R1 supposed to be entered in R2.

Amendment 1, October 10, 2013

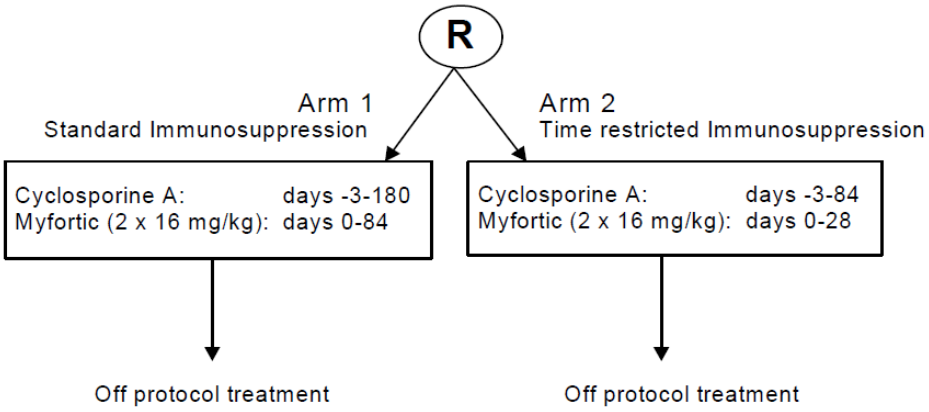
Design has not changed

Amendment 2, May 17, 2011

Design has not changed

Amendment 3, March 13, 2012

Due to poor inclusion in the R2 randomization, this randomization was closed for further accrual. New design:

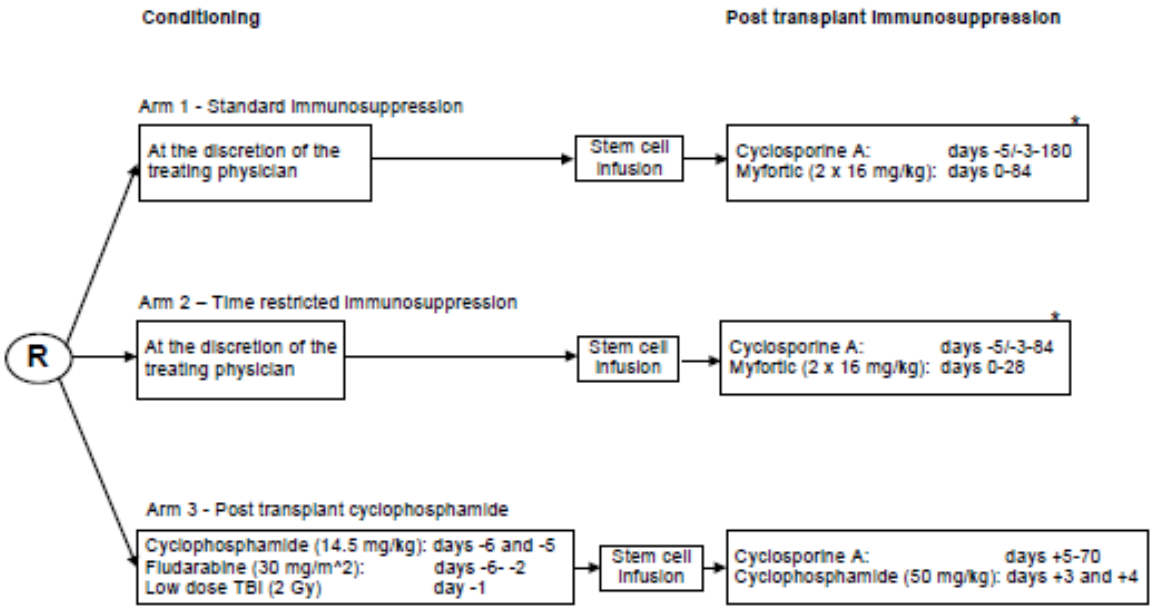


Amendment 4, July 17, 2013

We added a third arm to the R1 randomization, with high dose posttransplant cyclophosphamide combined with a short course of cyclosporine A as GVHD prophylaxis.

Important: the original primary endpoint for the R1 randomization was maintained.

New design:



* Duration of immunosuppressive therapy may vary according to the presence or absence of GVHD and type of donor (related versus unreleated). Information is provided in section 9.2.1.4.

Sample size calculation

For the primary endpoint, patients will be considered a success if only non-severe GVHD has been observed within 180 days post-transplant, without having received any treatment other than preemptive DLI or stem cell boost and not specified in the protocol (e.g., reinduction chemotherapy). All other patients will be considered a failure; among those - but not restricted to - patients without any GVHD within 180 days following transplantation, with acute GVHD grade II with gut infiltration or grades III-IV or chronic GVHD requiring systemic treatment or death before any GVHD. As treatment is the same in treatment arms 1 and 2 during the first 28 days, patients with a failure until 28 days posttransplant will be excluded from the primary analysis. This will therefore also be done for patients in arm 3. It is assumed that about 35% of the patients assigned to the standard IS scheme will attain only non-severe GVHD within 180 days following the allogeneic SCT, i.e., PG_{180} = 35%.

- For the comparison between arms 1 and 2, in order to detect with 80% power an increase of PG_{180} to 50% (two-sided significance level $\alpha = 0.05$), 366 patients randomized 1:1 between the standard and time-restricted IS scheme will be required.
- For the comparison between arms 1 and 3 in order to detect with 80% power an increase of PG_{180} from 35% to 60% (two-sided significance level $\alpha = 0.05$), 156 patients randomized 1:2 between the standard IS scheme (n=52) and the post-transplant cyclophosphamide regimen (n=104) will be required, which will also imply another 52 in arm 2, because the actual randomization will be arm 1 : arm 2 : arm 3 = 1:1:2.

As of June 4, 2013, a total number of 238 patients have been randomized between arms 1 and 2. As soon as the amendment will be approved, patients will be randomized 1:1:2. If this would be achieved around September 2013, then the total number of patients randomized in arms 1 and 2 will be about 270. Together with the 156 + 52 patients, this would sum up to 478. The number of patients for the comparison arm 1 vs arm 2 would then be 270 + (2 x 52) = 374, which is more than the required 366. In order to overcome possible dropout due to non-eligibility, no transplant or failures before day 28 post-transplant, 500 patients will be randomized.

Amendment 5, April 22, 2014

Arm 3 post-transplant cyclophosphamide: Cyclosporine A treatment has been extended to +70 days

Prevention of severe GVHD after allogeneic hematopoietic stem cell transplantation, applied as consolidation immunotherapy in patients with hematological malignancies. A prospective randomized phase III trial.

Substantial or Non-substantial	Date
Substantial	22APR2014

- Arm 3 Post transplant cyclophosphamide: Cyclosporine A treatment is extended to +70 days
- Arm 3: Added option of cyclosporine A p.o. dose.
- Arm 1 and 2: Date start cyclosporine A is changed to day -5 –day -3 (depending on local procedures).
- Period of reporting adverse events is corrected (in accordance to earlier amendment
- Exemptions of SAE reporting are clarified
- Exemption of SAE reporting of chronic GvHD is limited to chronic GvHD not requiring systemic treatment.
- Added is a monitoring of overall mortality to detect possible differences in relapse rate between arm 1 and arm 3.

Substantial or Non-substantial	Date
Substantial	17JUL2013

- A change in adress of HOVON Data Center
- A correction in objective given in synopsis
- Planned start end of recruitment is updated to III 2016
- Addition of an additional treatment arm with a short-course post-transplant GVHD prophylaxis consisting of high-dose cyclophosphamide. (introduction, objectives, study design, treatment, statistical considerations)
- Change of address of E. Meijer, a study coordinator
- Change of one of the members of writing cie.
- Added information about closure of the accrual in the QoL substudy after inclusion of the 200th patient
- Changed inclusion criterion regarding age.
- Added is the use of a Summary of Product Characteristics (SPC) for an authorised medicinal product in the definition of SUSAR.
- Changes in reason for going off protocol treatment with regards to development of GVHD.
- Changed references:
  - Deletion of references regarding treatment of severe GVHD that was removed from protocol before (13 MAR2013)
  - Addition of references regarding the added third treatment arm.

Version	Substantial or Non-substantial	Date
04	Substantial	13MAR2012

- A change in principal investigator is implemented. A.E.C. Broers is the new principal investigator.
- Deleted from protocol is the treatment of severe GVHD.
- The registration of patients receiving a T-cell depleted allogeneic SCT and that will be treated with immunosuppression according to local hospital policy is deleted form protocol. It was added in version 3, but has not been implemented .
- Protocol corrections with regards to reporting of GVHD.
- Information requested at registering patient is corrected from "patient's initials or code" into "local patient code (optional)".

25-4-2014.

Version	Substantial or Non-substantial	Date	Date submitted: EC	Date submitted: CA	Date approved: EC
03	Substantial	17 May 2011			

- A change in principal investigator is implemented. J.J. Cornelissen is the new principal investigator.
- A.E.C. Broers is added as study coordinators.
- Added is the registration of patients receiving a T-cell depleted allogeneic SCT. These patients will be treated with immunosuppression according to local hospital policy. These patients will not participate in quality of life.
- Added inclusion criterium second randomization: In case of gut involvement: an infectious cause of diarrhea has to be excluded, a biopsy specimen should be taken, however the results of a biopsy specimen have not to be awaited.
- Added as dose adjustment of cyclosporine A is that it is allowed to converse to tacrolimus or sirolimus in case of irreversible toxicity.
- Infusion of donor lymphocytes is deleted as reason for going off protocol treatment.
- In the table of required investigations the chimerism analyses is changed to chimerism analyses according to local policy.
- Clinical and laboratory evaluations on day 14 and 28 after allo-SCT are deleted and replaced by 1 month after allo-SCT.
- Giving resolution information of adverse events is deleted from protocol.
- Exemptions for serious adverse events reporting are added to the protocol.
- Information on annual safety reporting is added in chapter 13. To cover this, the chapter title is changed from 'Reporting serious adverse events and SUSARs' to 'Safety'. And the information about the Data and Safety monitoring board is moved to this chapter 'safety' (from 'statistical considerations'').

Version	Substantial or Non-substantial	Date	Date submitted: EC	Date submitted: CA	Date approved: EC
02	Substantial	13 October 2010	03 November 2010	03 November 2010	07 December 2010

Summary of changes

- The number of patients participating in quality of life is limited to 200.
- The study objective and statistical analysis of quality of life assessment are changed.
- The logistics with regards to distribution of quality of life questionnaires is changed.
- Information about reporting of pregnancies is added.
- The processing of adverse events and serious adverse events is updated in accordance with addendum that was previously brought in place.
- Analysis of safety and the installation of a DSMB is added.
- A correction is made in the grading of acute GvHD.
- Changes are made in the information on immune reconstitution and Cryopreservation of viable mononuclear blood cells, plasma, urine and HPC product sample in appendix C and D.

Supplementary text: Disease status at transplantation

All patients diagnosed with acute leukemia were in a complete remission prior to transplant. In case of chronic myeloid leukemia, patients were transplanted in either first or second chronic phase. Patients diagnosed with chronic lymphocytic leukemia, non-Hodgkin's lymphoma and multiple myeloma had to be at least in partial remission upon (re)induction therapy whereas responsive disease was sufficient in case of Hodgkin's lymphoma. Patients suffering from myelodysplastic syndrome and myeloproliferative disease were either transplanted upfront without preceding treatment or after one or two courses of chemotherapy depending on the disease burden before transplant.

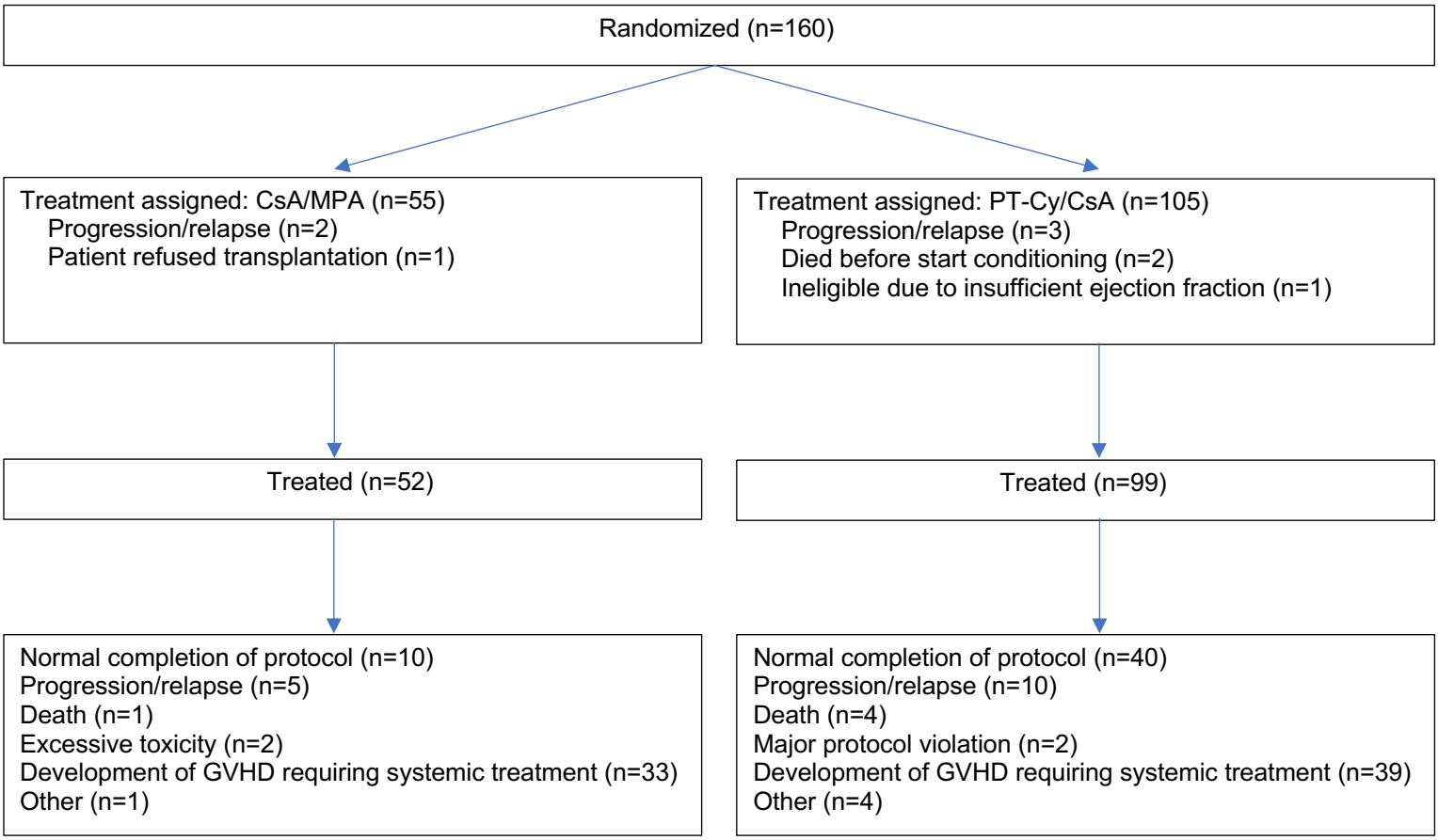
Supplementary text: Supportive care

All patients received transfusions of irradiated red blood cells and platelets. Anti-infectious prophylaxis consisted of trimethoprim-sulfamethoxazole (in case of intolerance: pentamidine inhalation or atovaquone) and (val)acyclovir. In case of prolonged neutropenia and mucositis due to the conditioning regimen, antibacterial and antifungal prophylaxis were administered according to local policy. All cytomegalovirus (CMV) seropositive patients and/or recipients of CMV seropositive transplants were monitored by a plasma DNA assay. The threshold level to start preemptive therapy was according to center policy.

Supplementary text: Serial measurements

Evaluation of treatment response were performed at 3, 6, 12 and 24 months after transplantation. Evaluation of acute and chronic graft-versus-host disease (GVHD), and adverse events was performed every month during the first year after transplantation and every six months in the subsequent years until five years after transplantation.

Supplementary Figure S1: Patient disposition flow chart



CsA denotes cyclosporine A, MPA mycophenolic acid, PT-Cy posttransplant cyclophosphamide, and GVHD graft-versus-host disease

Supplementary Table S1: Acute GVHD-grading according to the updated Glucksberg classification (1) (2)

Stage	Skin rash	Liver Total bilirubin (μmol/L)	Intestinal tract Diarrhea (mL/day)
1	<25%	34-50	500-1000*
2	25-50%	50-102	1000-1500
3	>50%	102-255	>1500
4	Generalized erythroderma with bullae	>255	Severe pain/ileus

*or persistent nausea with histological evidence of GVHD in the stomach or duodenum

Grade	
I	Skin: stage 1-2; no liver or gut involvement
II	Skin: stage 3 or liver: stage 1 or gut; stage 1
III	Liver: stage 2-3 or gut: stage 2-4
IV	Skin or liver: stage 4

GVHD denotes graft-versus-host disease

Supplementary Table S2: Seattle classification for limited and extensive chronic GVHD (3)

Limited Either or both: 1. Localized skin involvement 2. Hepatic dysfunction due to chronic GVHD
Extensive Either 1. Generalized skin involvement, or 2. Localized skin involvement and/or hepatic dysfunction, due to chronic GVHD, plus: a. Liver histology showing chronic aggressive hepatitis, bridging necrosis, or cirrhosis, or b. Involvement of eye (Schirmer's test with < 5mm wetting), or c. Involvement of minor salivary glands or oral mucosa demonstrated on labial biopsy, or d. Involvement of any other target organ

GVHD denotes graft-versus-host disease

Supplementary Table S3: Conditioning regimens

Conditioning regimen	**CsA/MPA (N=52)**	**PT-Cy/CsA (N=99)**
Bu/Flu	1 (2%)	1 (1%) *
Cy/Flu	6 (12%)	-
Cy/Flu/TBI	2 (4%)	98 (99%)
Cy/TBI	2 (4%)	-
Flu/TBI	41 (79%)	-

Bu denotes busulfan, Cy cyclophosphamide, TBI total body irradiation, Flu fludarabine, CsA cyclosporine A, MPA mycophenolic acid, and PT-Cy posttransplant cyclophosphamide

*Conditioning regimen adapted to busulfan and fludarabine because of a history of high-dose radiotherapy of the nasopharynx prohibiting additional TBI.

Conditioning regimens:

Bu/Flu
busulfan 1.6 mg/kg day -7 and 3.2 mg/kg days -6 and -5
fludarabine 30 mg/m2 days -7 to -3
or
busulfan 0.8 mg/kg qid days -3 and -2
fludarabine 40 mg/m2 days -5 to -2

Cy/Flu
cyclophosphamide 500 mg/m2 days -6 to -2
fludarabine 25 mg/m2 days -6 to -2

Cy/Flu/TBI
cyclophosphamide 14.5 mg/kg days -6 and -5
fludarabine 30 mg/m2 days -6 to -2
total body irradiation 2 Gy day -1

Cy/TBI
cyclophosphamide 60 mg/kg days -5 and -4
total body irradiation 5 or 6 Gy days -2 and -1

Flu/TBI
fludarabine 30 mg/m2 days -5 to -3
total body irradiation 2 Gy day -1

Supplementary Table S4: CTCAE grade 3-5 adverse events within six months posttransplantation

	CsA/MPA (N=52)		PT-Cy/CsA (N=99)	
Adverse event - no. of patients (%) CTCAE*	grade 3	grade 4-5†	grade 3	grade 4-5‡
Any	18 (35)	4 (8)	44 (44)	16 (16)
Infection	11 (21	-	36 (36)	5 (5)
Metabolic/laboratory	5 (10	3 (6)	13 (13)	3 (3)
Gastrointestinal	9 (17)	-	6 (6)	-
Cardiac	2 (4)	-	1 (1)	2 (2)
Pain	1 (2)	-	1 (1)	-
Neurology	2 (4)	-	4 (4)	1 (1)
Blood/bone marrow	3 (6)	1 (2)	4 (4)	3 (3)
Constitutional symptoms	2 (4)	-	2 (2)	-
Pulmonary/upper respiratory	2 (4)	1 (2)	2 (2)	3 (3)
Dermatology/skin	-	-	1 (1)	-
Ocular/visual	-	-	1 (1)	-
Vascular	-	-	2 (2)	-
Musculoskeletal/soft tissue	-	-	1 (1)	-
Renal/genitourinary	-	-	2 (2)	1 (1)
Allergy/immunology	-	-	2 (2)	-
Hemorrhage/bleeding	1 (2)	-	-	1 (1)
Lymphatics	-	-	-	-
Syndromes	-	-	1 (1)	1 (1)
Auditory/ear	1 (2)	-	-	-
Endocrine	-	-	1 (1)	-
Hepatobiliary/pancreas	1 (2)	-	-	-
Coagulation	-	-	1 (1)	-
Sexual/reproductive function	-	-	-	-

CTCAE denotes Common Toxicity Criteria for Adverse Events, CSA cyclosporine A, MPA mycophenolic acid, and PT-Cy posttransplant cyclophosphamide.
*Adverse events were scored according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0.
†No CTCAE grade 5 events were reported.
‡Three CTCAE grade 5 events were reported including septic shock, CNS hemorrhage and encephalopathy.

Supplementary Table S5: Causes of death

	CsA/MPA (N=52)	**PT-Cy/CsA (N=99)**
Dead – no.	16	37
Cause of death – no. (%)		
Hematological disease	9 (56)	23 (62)
GVHD	5 (31)	4 (11)
Infection	-	2 (5)
Organ failure	1 (6)	1 (3)
Secondary malignancy	-	1 (3)
Hemorrhage	-	1 (3)
Other	1 (6)	5 (14)

Supplementary references

1. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation 1974;18:295-304.

2. Przepiorka D, Weisdorf D, Martin P, et al. Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant 1995;15:pp. 825-8.

3. Lee SJ, Vogelsang G, Flowers MED. Chronic graft-versus-host disease. Biol Blood Marrow Transplant 2003;9:215-33.