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ARTICLE

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Time-restricted versus standard-duration immunosuppression after allogeneic hematopoietic stem cell transplantation: Results of the prospective randomized HOVON-96 trial

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Abstract

Cyclosporine A combined with mycophenolate mofetil (CsA/MMF) has become an established regimen for the prevention of graft-versus-host disease (GVHD) following non-myeloablative (NMA) allogeneic hematopoietic stem cell transplantation (alloHSCT). However, the optimal duration of immunosuppression (IS) has not yet been defined and overtreatment is of concern. We hypothesized that time-restricted IS with CsA/MMF would increase the proportion of patients with non-severe GVHD compared to standard-duration IS, thereby resulting in reduction of the relapse rate and improvement of progression-free survival (PFS) and overall survival (OS). In a prospective randomized, multicenter, phase III trial, patients were allocated (1:1) to standard or time-restricted IS. A total of 389 patients were randomized, of whom 369 were transplanted (184 vs. 185 patients). The primary endpoint, the proportion of patients with non-severe GVHD defined as acute GVHD grades I–II without gut involvement or chronic GVHD not requiring systemic treatment within 180 days posttransplant, was 23% after standard-duration IS versus 24% after time-restricted IS (odds ratio: 1.02; 95% confidence interval (CI) 0.63–1.66, $p = 0.92$). The cumulative incidence of grade III–IV acute GVHD at 6 months posttransplant was not significantly different (14% vs. 18%; $p = 0.20$). The two-year cumulative incidence of chronic extensive GVHD was 50% versus 46% ($p = 0.62$). There were no significant differences in the rates of relapse/progression, non-relapse mortality, PFS, OS, and GVHD-free, relapse-free survival. Time-restricted IS with CsA/MMF did not increase the proportion of patients with non-severe GVHD, and secondary outcomes were not different compared to standard-duration IS following NMA-matched alloHSCT.

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Results of the present study have been presented at ASH: Abstract #371; December 8, 2019. The trial was registered at https://www.trialregister.nl (until June 2022) and https://trialsearch.who.int/ as NTR2252

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (alloHSCT) is an important treatment modality for patients with hematological malignancies. While in the first decades of alloHSCT development, the transplant strategy aimed at complete eradication of the malignant cells by administration of high dose chemo-radiation, emphasis has gradually shifted towards its immunotherapeutic effect against residual malignant cells executed by donor T cells, known as the graft-versus-leukemia (GVL) effect.[1,2] By avoiding the serious toxicity associated with the conventional high-dose conditioning regimen, reduced intensity alloHSCT could be applied to older and otherwise ineligible patients.[3] The non-myeloablative (NMA) minimally toxic preparative regimen combining low-dose chemo-radiation with cyclosporine A (CsA) and mycophenolate mofetil (MMF) as post-transplant immunosuppression (IS) to control graft rejection and graft-versus-host disease (GVHD) was first explored by the Seattle group[4] and many reports have been published since (reviewed by Holtan et al.[5]). The optimal duration of immunosuppressive therapy to protect against GVHD after NMA transplantation using this platform, however, has not yet been defined and overtreatment is of concern as prolonged IS might impair the beneficial GVL effect. A retrospective study by Burroughs et al. showed that a prolonged course of postgrafting CsA significantly decreased the risk of severe acute GVHD without affecting the risks of chronic GVHD or relapse.[6] Baron et al. showed that an extended course of MMF combined with early discontinuation of CsA increased the incidence of acute GVHD suggesting extended use of CsA for better control of GVHD.[7] In a prospective trial evaluating the incidence of chronic extensive GVHD in recipients of NMA alloHSCT, prolonged-duration CsA versus short-duration CsA, however, failed to show a difference in the incidence of chronic GVHD or disease-free survival.[8] Apart from intensification of postgrafting immunosuppression either by prolonged administration or addition of compounds, improved protection against severe GVHD might also be achieved by early and aggressive treatment of GVHD once it appears. Such an approach might prevent prolonged postgrafting immunosuppression and subsequent impairment of the GVL effect in the proportion of patients that will never develop GVHD at all.

Here we report the results of a prospective, randomized, multicenter, phase III trial designed to study whether the use of time-restricted IS with CsA and MMF would increase the proportion of patients with non-severe GVHD within 180 days posttransplant (without compromising this by a substantial increase of severe GVHD) and subsequently reduce the relapse rate and improve PFS and OS, as compared to standard-duration IS after NMA matched alloHSCT.

METHODS

Study design

Patients were randomly assigned in a 1:1 ratio to standard-duration or time-restricted IS. Adults (age 18–70 years inclusive) with a WHO performance status between 0 and 2, diagnosed with a high-risk hematological malignancy and having a matched related donor (MRD) or at least 8 out of 8 HLA (A, B, C, DRB1; DNA based, four digits) matched unrelated donor (MUD) could participate in the trial. Exclusion criteria included renal dysfunction (serum creatinine >150 µmol/L or clearance <50 mL/min), active uncontrolled infection, or progressive disease. Patients receiving anti-thymocyte globulin (ATG) as part of the conditioning regimen, and those who were to receive a cord blood-derived graft were also excluded.

All patients diagnosed with acute leukemia were in complete remission prior to transplant. In the 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 the 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. Conditioning regimens and intensities were at the discretion of the treating physician. Posttransplant IS consisted of CsA at a dose of 4.5 mg/kg twice daily orally or 1.5 mg/kg twice daily intravenously, aiming at trough levels between 250 and 350 µg/L (immunoassay). MMF was replaced by mycophenolic acid (MPA; Myfortic®) as it was assumed that, comparable to convincing data obtained in renal transplant recipients, MPA would be associated with less gastrointestinal toxicity as compared to MMF while being therapeutically equivalent.[9–11] MPA was administered at a dose of 16 mg/kg twice daily with a maximum daily dose of 2160 mg. In recipients of standard-duration IS (arm A), CsA was administered from 3 to 5 days before transplantation (depending on local procedures) and tapered with 10% per week down to zero from Day +120 in patients without GVHD or from Day +180 in patients with a history of GVHD. MPA was administered from transplant and discontinued at Day +84 without tapering. In patients allocated to the time-restricted arm (arm B) and without GVHD, CsA was tapered with 10% per week down to zero from Day +84. MPA was discontinued at Day +28 without tapering in recipients of an MRD. In the case of a MUD, MPA was tapered from Day +28 down to zero in 4 to 6 weeks. In case of GVHD necessitating local or systemic treatment, tapering of IS was to be tailored to the patient's clinical needs, but not earlier or faster than described in the protocol. In case of acute GVHD grade II with gut involvement or grades III–IV, the intention was to include patients in a second randomization comparing standard treatment with high-dose prednisone versus high-dose prednisone combined with ATG. The aim of the second randomization was to evaluate whether the addition of ATG to standard treatment with high-dose steroids for severe acute GVHD would improve the proportion of patients with a complete (CR_{GVHD}) or partial response (PR_{GVHD}) after treatment. However, that randomization failed to accrue a sufficient number of patients. Therefore, the study was amended in October 2013 and approved as such by the review committees involved. In the amended protocol, the second randomization was closed, and, instead, a third arm was added, including the administration of posttransplant cyclophosphamide (PT-Cy). The results of the comparison between PT-Cy and standard-duration immunosuppression with CsA/MPA have been reported separately.[12]

Quality of Life (QoL)

To assess the impact of alloHSCT on the QoL in a prospectively treated cohort of patients, the first 200 randomized patients were evaluated pretransplant, during the first year and during follow-up until five years posttransplant using the EORTC QLQ-C30 questionnaire and the FACT-BMT.[13,14] QoL was measured at entry (prior to the initiation of the conditioning regimen), at 180 days posttransplant and at 1, 2, and 5 years posttransplant. Measurement was stopped at progression.

Study oversight

The trial was designed by the Stem cell transplantation working group of the Dutch-Belgian Cooperative Trial Group for Hematology

Oncology (HOVON) Foundation. Data were collected at HOVON, and HOVON statisticians conducted the analysis. The study protocol was approved by the ethics committee at each participating center and conducted according to the principles of the Declaration of Helsinki. All patients gave written informed consent to enroll in the study. The Data Safety and Monitoring Board (DSMB) met several times to review the progress of the study and advise the investigators.

Study endpoints

The primary endpoint of the study was defined as the proportion of patients with non-severe GVHD within D180 after transplantation (PG_{180}). Non-severe GVHD was defined as acute GVHD grade I, acute GVHD grade II without gut involvement, or chronic GVHD not requiring systemic treatment within 180 days after randomization.

Secondary endpoints related to GVHD were time from transplantation to acute GVHD grade ≥I, ≥II, ≥IIII, and ≥IV, time to limited/extensive and extensive chronic GVHD. Additional secondary endpoints included the incidence of relapse/progression, non-relapse mortality (NRM), progression-free survival (PFS), overall survival (OS), GVHD-free, relapse-free survival (GRFS), adverse events (AEs), and QoL (of the first 200 randomized patients) as defined by the EORTC QLQ-C30 and the FACT-BMT definitions. PFS was defined as time from transplantation until relapse/progression or death whichever came first. OS was defined as time from transplantation until death, irrespective of the cause. GRFS was defined as survival without acute GVHD grade III–IV, chronic GVHD requiring systemic immunosuppressive treatment, or relapse/progression.[15] For GRFS, PFS, and OS, patients without an event were censored at the date last known to be alive. AEs were scored according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. Acute GVHD was graded according to the updated Glucksberg classification.[16,17] Chronic GVHD was graded according to the Seattle classification for limited and extensive GVHD.[18]

Statistical analysis

The primary objective was to evaluate whether time-restricted IS would result in a higher proportion of patients with non-severe GVHD within 180 days posttransplant (PG_{180}) as compared to standard-duration IS. For the sample size calculation, it was assumed that PG_{180} would be about 35% with standard-duration IS. In order to detect with 80% power an increase of PG_{180} from 35% to 50% (two-sided significance level $\alpha = 0.05$), 366 patients should be randomized 1:1 between standard-duration IS and time-restricted IS. Patients could be randomized 24/7 via the Internet via the randomization program TOP of HOVON. Randomizations were balanced with a biased-coin minimization procedure, with the bias dependent on the average imbalance between the numbers of patients already assigned to each group overall, within the participating hospital, within donor type (MRD vs. MUD), and within conditioning regimen (myeloablative, MAC vs. NMA). All analyses were according to the intention-to-treat (ITT) principle, that is, patients were analyzed according to the treatment arm they were assigned to. However, patients initially randomized but considered ineligible afterward based on information that should have been available before randomization were excluded from all analyses (modified-ITT), and data collection for these patients was discontinued. The proportion of transplanted patients with non-severe acute GVHD within 180 days posttransplant was determined per treatment arm with a 95% confidence interval (CI). As primary analysis, PG_{180} was compared between both arms using logistic regression with adjustment for donor type (MRD vs. MUD) and conditioning regimen (MAC vs. NMA). Cumulative incidence curves for time to acute and chronic GVHD were determined per treatment arm. The Fine and Gray model was used to assess the effect of the treatment arm on the cumulative incidence of GVHD in the presence of competing risks via regression on GVHD subdistribution hazard, adjusted for donor type and conditioning regimen. Kaplan–Meier curves for GRFS, PFS, and OS were constructed per treatment arm. A Cox proportional hazards model was used to assess the effect of the treatment arm on each of the survival endpoints, adjusted for donor type and conditioning regimen. Hazard ratios (HRs) and 95% CI were determined.

The treatment toxicity analyses were carried out by tabulating the incidence of AEs with CTCAE grade 3 or more within 180 days posttransplant. QoL analysis was restricted to patients with at least a completed baseline and one follow-up QoL questionnaire. To evaluate the change in QoL over time with respect to the multi-item scales of the QLQ-C30 and the FACT-BMT, the repeated measures should be analyzed using mixed analysis of variance models. However, due to the high drop-out rate, available QoL data were only summarized for the different time points.

All reported p-values are two-sided, and a significance level $\alpha = 0.05$ was used. As there is one primary analysis for endpoint PG_{180}, all other analyses should be considered exploratory, and no correction for multiple testing was done. The data cutoff date was January 19, 2021, when data collection was discontinued and the database was locked. All analyses were performed using Stata software, version 16.1 (StataCorp.).

RESULTS

Patient characteristics

A total of 389 patients were randomized between April 29, 2010, and June 8, 2018, at nine centers. Two patients were ineligible because of AML with more than 5% blasts ($n = 1$) or relapsed AML, and therefore excluded from all analyses. Eventually, 369 eligible patients received an alloHSCT. Figure 1 shows the patient disposition flow chart. As shown in Table 1, baseline characteristics were well balanced between the two groups of transplanted patients. The disease categories at transplantation were equally distributed, with half of the patients diagnosed with acute leukemia in both groups. Forty-nine patients (13%) received MAC, and 320 patients (87%) received an NMA conditioning. Details of the regimens used are shown in Table S3. The median follow-up of the 195 transplanted patients still alive was 61.2 months (inter-quartile range [IQR], 59.5–63.3) from transplantation, i.e., median 61.0 months (IQR, 59.2–63.2) in arm A and 61.3 months (IQR, 59.7–63.3) in arm B. Of note, follow-up data were only required until 5 years after randomization. As during the first 17 months of the trial, only 4/87 (5%) patients had been included in the second randomization, it was decided by the study team following consultation with the DSMB to amend the study and close the second randomization for further accrual.

Acute and chronic GVHD

The proportion of patients with non-severe GVHD within 180 days posttransplant (PG_{180}) was 23% in recipients of standard-duration IS and 24% in recipients of time-restricted IS (odds ratio [OR]: 1.02; 95% CI: 0.63–1.66, $p = 0.92$, adjusted for donor type and conditioning regimen). The difference in the proportion of patients who achieved the primary endpoint was 1%, with 95% CI: −8% to +9%. Results are

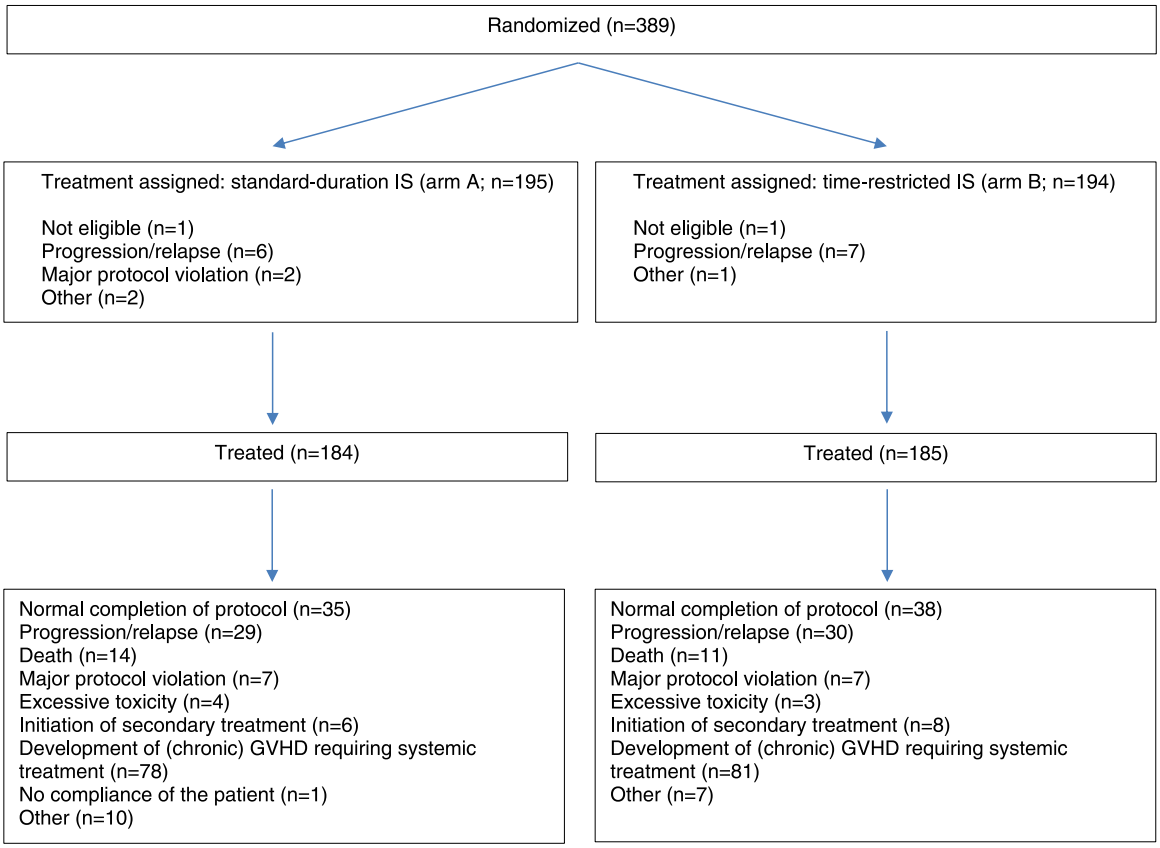

FIGURE 1 **Patient disposition flow chart.** IS, immunosuppression; GVHD, graft-versus-host disease.

shown in Table 2. The OR and 95% CI within the subgroups according to conditioning regimen (MA vs. NMA) and donor type (MRD vs. MUD) are shown in Figure S1. Overall, 64% of patients developed acute GVHD of any grade (62% vs. 65%). In patients experiencing acute GVHD, the skin was most commonly involved (89%), followed by the gut (45%), and the liver (17%). At six months, the cumulative incidence of acute GVHD grade II–IV was 46% (standard error [SE] 4%) in recipients of standard-duration IS and 51% (SE 4%) in recipients of time-restricted IS ($p = 0.70$; Figure 2A). The cumulative incidence of acute GVHD grade III–IV at six months was not significantly different between the two treatment arms: 14% (SE 3%) versus 18% (SE 3%), respectively ($p = 0.20$; Figure 2B). Organ involvement was comparable between the two groups of patients. In multivariable analysis, donor type (MUD vs. MRD) was significantly associated with acute GVHD grade II–IV (HR: 2.08; 95% CI: 1.53–2.82; $p < 0.001$) and acute GVHD grade III–IV (HR: 2.08; 95% CI: 1.17–3.67; $p = 0.012$).

Overall, 67% of patients experienced chronic GVHD of any grade, including 66% of patients in the standard-duration arm and 68% of patients in the time-restricted arm. No difference was observed in the cumulative incidence of limited and extensive chronic GVHD between the two treatment arms at two years: 61% (SE 4%) versus 60% (SE 4%) respectively ($p = 0.94$). The cumulative incidence of extensive chronic GVHD at two years was 50% (SE 4%) after standard-duration IS versus 46% (SE 4%) after time-restricted IS ($p = 0.62$; Figure 2C). In multivariable analysis, no association was observed between the incidence of chronic GVHD and donor type.

Toxicity, nonrelapse mortality, and relapse/progression

The number of patients experiencing at least one CTCAE grade 3–5 AE within six months after transplantation was not different between the two treatment arms: 54% versus 58%, respectively. The type of AEs was similar between the two groups of patients. The proportion of patients with at least one CTCAE grade 3–5 infection was 22% in recipients of standard-duration IS and 29% in recipients of time-restricted IS. One patient in the standard-duration IS arm experienced cytomegalovirus (CMV) disease. Three patients (2%) in both treatment arms developed grade 3 renal complications. Results are shown in Table 2. A full list of all CTCAE grade 3–5 events is available in Table S4. NRM estimated 20% (SE 3%) versus 21% (SE 3%) in the respective study arms at three years posttransplant ($p = 0.80$; Figure 3A). The cumulative incidence of relapse at three years posttransplant was 27% (SE 3%) in both treatment arms ($p = 0.72$; Figure 3B).

QoL

A total of 200 eligible patients (99 in the standard-duration IS arm vs. 101 in the time-restricted IS arm) were included in the QoL part of the trial. In only half of the patients in both groups, baseline and follow-up data were available. At baseline, 40 QoL forms (17 vs. 23) had not been completed because of the following reasons: forgotten to fill out ($n = 1$), forms were not given to the patient

TABLE 1 Baseline characteristics of the patients.[a]

Characteristic	arm A (*N* = 184)	arm B (*N* = 185)
Age, y		
Median	56	55
IQR	46–62	45–61
Male sex, no. (%)	103 (56)	109 (59)
Diagnosis, no (%)		
Acute myeloid leukemia	75 (41)	74 (40)
Acute lymphoblastic leukemia	27 (15)	24 (13)
Myelodysplastic syndrome	20 (11)	18 (10)
Chronic myeloid leukemia	5 (3)	5 (3)
Chronic lymphocytic leukemia	10 (5)	11 (6)
Non-Hodgkin's lymphoma	22 (12)	27 (15)
Hodgkin's lymphoma	6 (3)	4 (2)
Myeloproliferative disease	3 (2)	7 (4)
Multiple myeloma	6 (3)	8 (4)
other	10 (5)	7 (4)
Donor type, no. (%)		
Matched related donor	67 (36)	68 (37)
Matched unrelated donor	117 (64)	117 (63)
Female donor–male recipient pairs, no. (%)	26 (14)	29 (16)
Cytomegalovirus status, no. (%)		
Recipient-positive, donor positive	63 (34)	69 (37)
Recipient negative, donor negative	71 (39)	70 (38)
Recipient positive, donor negative	31 (17)	25 (14)
Recipient negative, donor positive	19 (10)	21 (11)
Conditioning regimen, no. (%)		
Myeloablative	23 (13)	26 (14)
Non-myeloablative	161 (88)	159 (86)
Source of stem cells, no. (%)		
Bone marrow	3 (2)	3 (2)
Peripheral blood	181 (98)	182 (98)
Infused CD34+ cells × 10^6/kg		
Median	6.6	6.3
IQR	4.9–8.9	4.5–8.0
Infused CD3+ cells × 10^6/kg		
Median	234	225
IQR	(172–313)	(150–323)

Abbreviation: IQR, inter-quartile range.

[a]A total of 389 patients were randomized in the study; 20 patients were excluded from the analysis as they did not receive the stem cell transplantation.

TABLE 2 Rates of acute and chronic GVHD and complications after stem cell transplantation.

Variable	arm A (*N* = 184)	arm B (*N* = 185)	*p* Value
Nonsevere GVHD <180 days posttransplant, no (%)	43 (23)	44 (24)	0.92
Only acute GvHD grade 1	12 (7)	6 (3)	
Only acute GVHD grade 2 w/o gut involvement	26 (14)	34 (18)	
Only chronic GVHD w/o systemic treatment	4 (2)	4 (2)	
Acute GVHD grade 1 and chronic GVHD w/o systemic treatment	1 (1)	-	
Acute GVHD, no. (%)			
Maximum overall grade per patient[a]			0.34
Grade 1	19 (10)	19 (10)	
Grade 2	63 (34)	61 (33)	
Grade 3	25 (14)	38 (21)	
Grade 4	7 (4)	3 (2)	
Chronic GVHD, no (%)			
Maximum overall grade per patient[b]			0.65
Limited	18 (10)	24 (13)	
Extensive	104 (57)	101 (55)	
CTCAE grade 3–4 AEs <6 months posttransplant, no (%)[c]			
All events	99 (54)	108 (58)	0.40
Infections	41 (22)	53 (29)	0.19
Febrile neutropenia	26 (14)	29 (16)	0.77
Invasive pulmonary aspergillosis	2 (1)	4 (2)	0.69
Pulmonary infections other	2 (1)	8 (4)	0.11
CMV disease	1 (1)	-	0.50
Graft failure	6 (3)	7 (4)	1.00
Cardiac	7 (4)	5 (3)	0.57

Abbreviations: GVHD, graft-versus-host disease; CMV, cytomegalovirus; CTCAE, common toxicity criteria for adverse events; w/o, without.

[a]Severity of acute GVHD according to the updated Glucksberg classification (see Table S1).

[b]Severity of chronic GVHD according to the Seattle criteria (see Table S2).

[c]The complete list of CTCAE grade 3–4 events <6 months posttransplantation is available in Table S4.

(*n* = 11), too ill (*n* = 4), too much effort (*n* = 2), other reason (*n* = 7), and unknown reason (*n* = 15). At six months, only 101 QoL forms (49 vs. 52) had been completed, while reasons for missing data were as follows: no allo (*n* = 10), forgotten to fill out (*n* = 1), too ill (*n* = 3), too much effort (*n* = 1), relapse (*n* = 29), death (*n* = 17), other reason (*n* = 8), and unknown reason (*n* = 30), with no difference between both arms. At five years, only 45 patients had completed a QoL form (18 vs. 27), while reasons for missing forms were mainly as follows: relapse (31 vs. 23), death (27 vs. 20), and unknown (15 vs. 21). Analysis of the available EORTC QLQ-C30 questionnaires concerning the patients' physical, psychological, and social functions by multiple-item scales and single items showed no difference between the two groups of patients. With respect to physical-, role-, cognitive-, and social functioning, patients experienced a decrease in QoL score at six months posttransplant compared to baseline, which returned to baseline at one year posttransplant. In addition, an increase in appetite loss, dyspnea, and pain were reported at six months compared to baseline. The FACT-BMT score showed a comparable pattern with a decrease in physical- and functional well-being at six months posttransplant. Profile plots of the EORTC QLQ-C30 Global health status and the FACT-BMT Total score are shown in Figure S2.

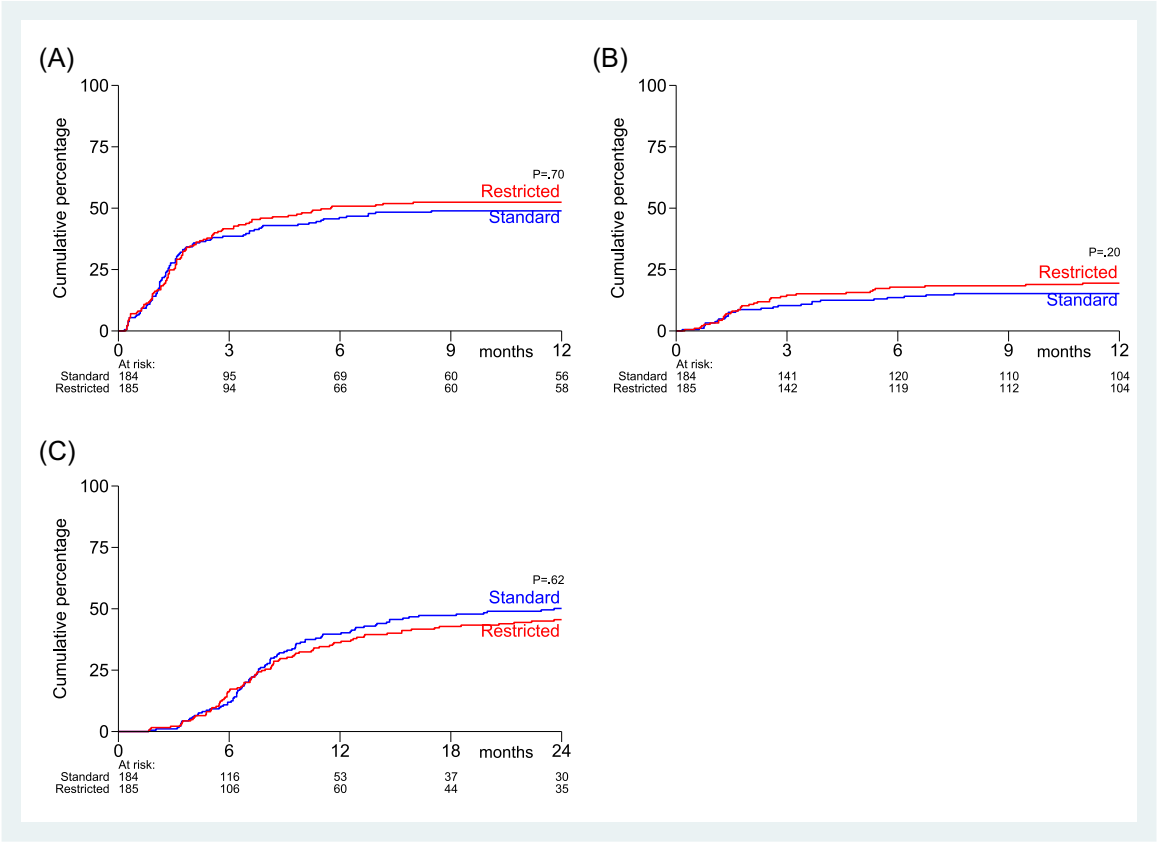

FIGURE 2 **Acute and chronic graft-versus-host disease (GVHD)**. Cumulative incidence of **(A)** acute GVHD grade II–IV, **(B)** acute GVHD grade III–IV, and **(C)** chronic extensive GVHD.

Survival

The three-year estimate of PFS after standard-duration versus time-restricted IS was not different, being 52% (95% CI: 45–59), HR = 0.96 (95% CI: 0.73–1.27). In addition, no difference in the three-year estimate of OS was observed between both treatment arms, being 59% (95% CI: 52–66) in the standard-duration arm versus 57% (95% CI: 50–64) in the time-restricted arm, HR = 0.99 (95% CI: 0.73–1.34). Results are shown in Figure 3C,D. Comparable, in multivariable analysis, no association was observed between PFS or OS and donor type.

The one-year estimate of GRFS was 14% (95% CI: 9–19) after standard-duration IS and 18% (95% CI: 13–24) after time-restricted IS (HR: 0.95 (0.76–1.19; $p = 0.65$, Figure 4A). Multivariable analysis showed that donor type was not associated with GRFS. As shown in the forest plot, the impact of IS duration on GRFS was independent of donor type (Figure 4B).

DISCUSSION

Posttransplant GVHD prophylaxis consisting of CsA and MMF has become a widely accepted immunosuppressive regimen after NMA alloHSCT, resulting in an incidence of 30%–50% acute grade II–IV and 40%–60% of chronic GVHD.[4,19–21] However, the optimal duration of GVHD prophylaxis is still not fully elucidated and over-treatment of patients may be of concern.[5] In the current randomized prospective multicenter phase III trial, we aimed to answer the question of whether early discontinuation of IS would result in a higher incidence of non-severe GVHD (without an increase of severe GVHD) and a subsequent decrease in relapse mortality as compared to prolonged IS. We show that rates of non-severe and severe acute and chronic GVHD after NMA-matched alloHSCT are not different between time-restricted IS and prolonged IS. Subsequently, no difference in rates of NRM, relapse, and OS was observed. In addition, based on the completed questionnaires, the reported QoL was independent of the duration of IS and similar between the two groups of patients. As only half of the included patients could be analyzed, these QoL data should be interpreted with caution.

The optimal duration of IS after alloHSCT has been a subject of study and debate for decades. Whereas some studies suggest early discontinuation of IS to be safe, others show a significantly increased risk of severe GVHD.[6–8] Preceding acute GVHD seems to be an important factor in the success rate of early discontinuation. While IS may be safely discontinued early in patients without prior acute GVHD, patients with preceding acute GVHD might benefit from prolonged administration.[22] The influence of early discontinuation of IS on the risk of relapse after alloHSCT is also not unambiguous and dependent on several factors, including type of IS, preceding GVHD, conditioning regimen, and underlying disease. Although early withdrawal of IS may be associated with a decreased risk of relapse, it only seems applicable to patients without prior GVHD and during the first 18 months posttransplant.[23]

Donor type was significantly and independently associated with the incidence of acute GVHD grade II–IV and grade III–IV. AlloHSCT using HLA-matched unrelated donors is a well-known risk factor for

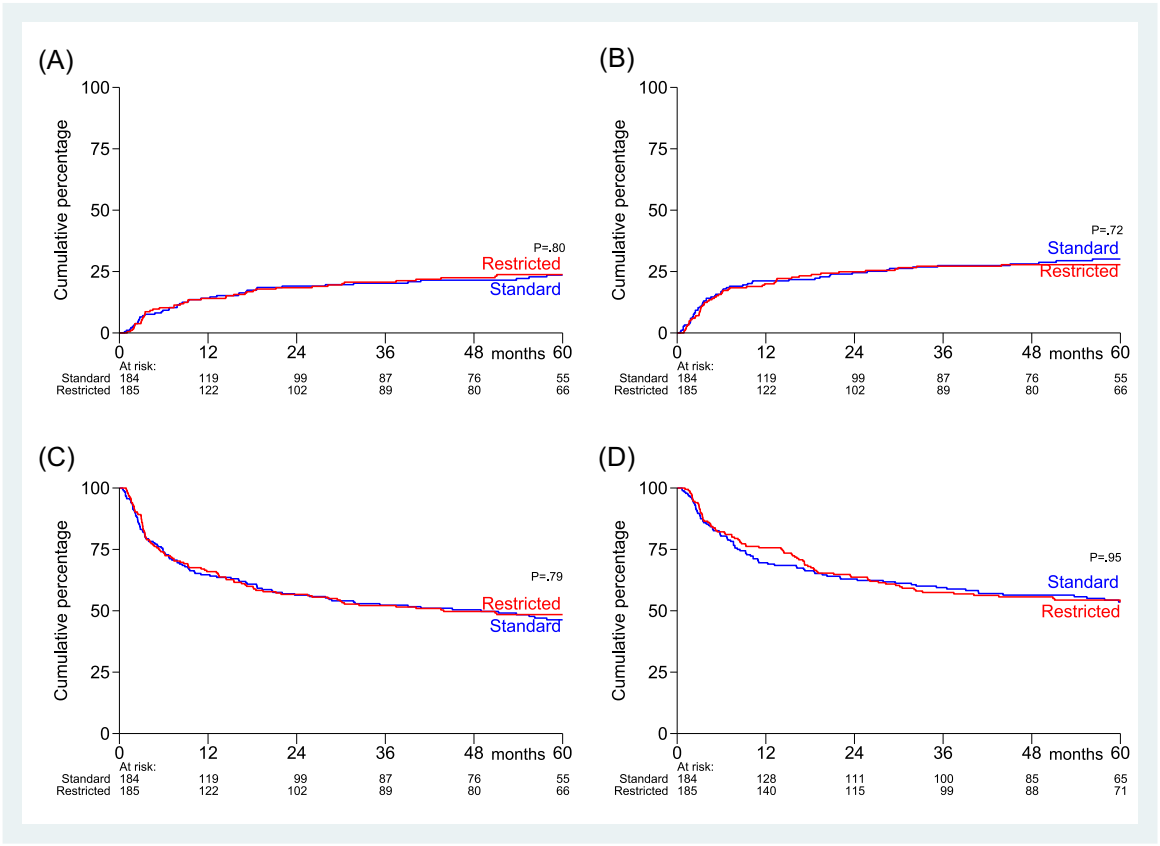

FIGURE 3 **Non-relapse mortality (NRM), relapse/progression, progression-free survival (PFS), and overall survival (OS).** Cumulative incidence of **(A)** NRM and **(B)** relapse/progression. Kaplan-Meier survival curves of **(C)** PFS and **(D)** OS.

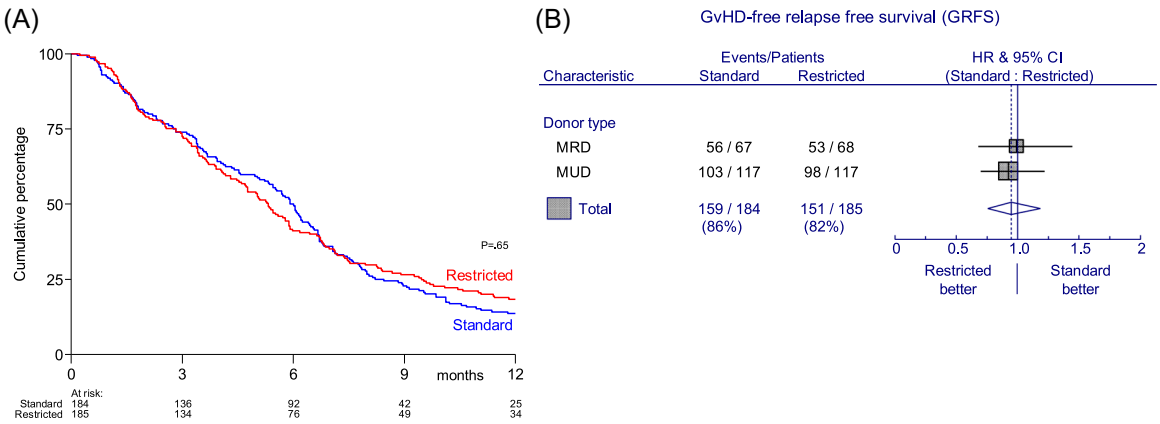

FIGURE 4 **GVHD-free, relapse-free survival (GRFS).** **(A)** Kaplan–Meier curve of GRFS and **(B)** forest plot of GRFS by donor type. AlloSCT, allogeneic stem cell transplantation; CI, confidence interval; HR, hazard ratio; MRD, matched related donor; MUD, matched unrelated donor. **(B)** for MRD, HR = 1.00, 95% CI = 0.69–1.46; for MUD, HR = 0.92, 95% CI: 0.70–1.21; test for interaction, $p = 0.77$. For total, HR = 0.95, 95% CI = 0.76–1.19.

the development of acute GVHD.[24] Strikingly, no significant association was observed between the incidence of chronic GVHD and donor type. Moreover, GRFS was independent of donor type, being similar to MRD and MUD. Although being a risk factor for chronic GVHD, it has been shown that the use of unrelated donors has a greater impact on the risk of acute GVHD rather than on chronic GVHD.[24] However, with the improvement in matching strategies, including the possibility for ultra-high resolution HLA typing, it is now possible to identify 10 out of 10 HLA-matched unrelated donors with a reduced risk of acute GVHD.[25]

Independent of the duration, conventional IS with CsA and MMF/MPA after NMA-matched alloHSCT is still associated with a considerable rate of chronic GVHD and, in particular, a relatively low GRFS. So, there is definitely room for improvement and a need for

exploring additional or even alternative lymphodepleting strategies instead of adapting the currently used standard immunosuppressive regimens. Especially in the last decade, several new drugs and new approaches have been explored as reviewed by Holtan et al.[5] Earlier, ATG has been shown to reduce the risk of chronic GVHD following MAC.[26–28] As a result, the European guideline recommends the use of ATG following MAC.[29] Large prospective randomized trials assessing the efficacy of ATG in the NMA setting, however, are lacking.

Both sirolimus and tacrolimus have been studied, either in addition to standard CsA/MMF or instead of CsA. Although a reduction in acute GVHD was observed, the effect of both substances on the incidence of chronic GVHD was disappointing.[30,31] PT-Cy, as pioneered by Luznik et al., might be an alternative lymphodepleting strategy to prevent GVHD.[32] Our recently published prospective randomized trial comparing conventional IS to PT-Cy following NMA-matched alloHSCT showed a significant improvement in GRFS due to a significant reduction in severe acute and chronic GVHD without significantly affecting the relapse rate.[12] Two other randomized controlled studies comparing conventional IS to PT-Cy following NMA-matched alloHSCT showed comparable results.[33,34] Another argument in favor of the application of PT-Cy as GVHD prophylaxis is the potential for implementation of early posttransplant chemo- and immunotherapy, as relapse after transplantation remains a major concern even in the presence of GVHD. As shown in our study, the relapse rate was almost 30% in both groups of patients despite a cumulative incidence of extensive chronic GVHD of no less than 50%. As a result, GRFS as a measure of cure without ongoing morbidity was relatively low in both groups of patients. By protecting against GVHD while preserving the GVL effect, PT-Cy might not only increase the GRFS but also the QoL of patients after alloHSCT.

How PT-Cy relates to prophylaxis with ATG remains an important question and is currently addressed in prospective randomized trials.

Other approaches that are particularly promising include Ixazomib, which was recently granted market authorization by the food and drug administration[35] and approaches that focus particularly on prevention of gut GVHD.[5]

Of note, the conditioning regimen used in this cohort of patients was not uniform. Overall, 13% of patients received MAC (13% vs. 14%) and 87% of patients received a NMA schedule. As only a minority of patients received a myeloablative regimen, translation of our findings to MAC should be performed with caution.

In conclusion, the results of the present study show that time-restricted combined use of MPA and CsA has no impact on transplant-associated alloreactivity in terms of GVHD and relapse as compared to the standard immunosuppressive regimen. Although one might argue that early discontinuation of IS after NMA-matched alloHSCT is feasible, the high incidence of chronic GVHD and low GRFS calls for a change in the strategy for GVHD prevention. PT-Cy might be an attractive candidate not only for its impressive effect on the incidence of acute and chronic GVHD and subsequent GRFS but also for its potential to implement posttransplant chemo- and immunotherapy early after transplant to prevent relapse.

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AUTHOR CONTRIBUTIONS

Annoek E. C. Broers: Design; acquisition; interpretation; drafting the manuscript; **Bronno van der Holt**: Design; analysis; interpretation, drafting and critically reviewing the manuscript; **Ellen Meijer**: Design; acquisition; interpretation; critically reviewing the manuscript; **Cornelis N. de Jong**: Acquisition; interpretation; drafting and critically reviewing the manuscript. **Erfan Nur**: Acquisition; critically reviewing the manuscript. **Geerte L. van Sluis**: Acquisition; critically reviewing the manuscript. **Goda Choi**: Acquisition; critically reviewing the manuscript. **Michel van Gelder**: Acquisition; critically reviewing the manuscript. **Johan A. Maertens**: Design; acquisition; critically reviewing the manuscript. **Jürgen Kuball**: Design; critically reviewing the manuscript. **Dries Deeren**: acquisition; critically reviewing the manuscript. **Heleen A. Visser-Wisselaar**: Data management; critically reviewing the manuscript. **Lamberdina A. H. M. Meulendijks**: Data management; critically reviewing the manuscript. **Jan J. Cornelissen**: Design; acquisition; interpretation; critically reviewing the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found in the online version of this article.

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