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Effects of Low-Dose Glucocorticoid Prophylaxis on Chronic Graft-versus-Host Disease and Graft-versus-Host Disease–Free, Relapse-Free Survival after Haploidentical Transplantation: Long-Term Follow-Up of a Controlled, Randomized Open-Label Trial

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A B S T R A C T

This long-term follow-up study evaluated the effects of corticosteroid prophylaxis on graft-versus-host disease (GVHD)-free, relapse-free survival (GRFS) based on a controlled open-label randomized trial in which 228 allotransplant recipients were categorized as low risk (n = 83, group A) or high risk; patients at high risk were randomly assigned to receive (n = 72, group B) or not receive (n = 73, group C) low-dose methylprednisolone prophylaxis. The cumulative incidences of chronic GVHD, relapse, nonrelapse mortality, leukemia-free survival, overall survival, and GRFS were 60%, 19%, 16%, 68%, 73%, and 46%, respectively, in all cases. Compared with the patients in group C, the cases in group B experienced a lower cumulative incidence of moderate to severe chronic GVHD (42% versus 20%; $P = .010$), herpes zoster infection (28% versus 12%; $P = .010$), pulmonary infections (42% versus 21%; $P = .040$), and osteonecrosis of the femoral head (ONFH; 16% versus 6%; $P = .045$) as well as better GRFS (59% versus 33%; $P = .017$). Factors associated with GRFS included total dose of corticosteroid used in the first 100 days after transplantation (hazard ratio, 1.547; $P = .015$) and platelet recovery (hazard ratio, 1.456; $P = .037$). Our results suggest that low-dose glucocorticoid prophylaxis reduces GVHD and thus reduces the total dose of steroids, which might contribute to lower incidence of infections and ONFH and a superior GRFS, indicating that higher steroid doses are harmful. Reducing the total dose is of course beneficial. (ClinicalTrials.gov number, NCT01607580.)

INTRODUCTION

Allogeneic stem cell transplantation (allo-SCT) is limited by the major lethal complication of acute and/or chronic graft-versus-host disease (GVHD) [1–4]. Corticosteroids are an accepted primary therapy for both acute and chronic GVHD [5,6], which have also been successfully used for prophylaxis of acute GVHD [7–9]. Randomized studies have demonstrated that the addition of corticosteroids resulted in a statistically significant reduction of the cumulative incidence of grades II to IV acute GVHD [7,9–11]. However, corticosteroids are nonspecific immunosuppressive agents that may contribute to high rates of infection and other adverse effects [12–14]. Preliminary results from a randomized, risk stratification–directed GVHD prophylaxis study performed by our group showed that low-dose corticosteroids significantly decreased the incidence and delayed the onset of acute GVHD, grades II to IV, and reduced adverse events [15,16]. These findings suggest that stratification-directed GVHD prophylaxis may benefit patients at high risk of developing GVHD and spare patients at low risk.

Currently, little information is available for the effects of corticosteroid prophylaxis on chronic GVHD, infectious complications, relapse, leukemia-free survival (LFS), and overall survival (OS) because of different time points, doses of corticosteroid application, heterogeneous conditioning regimens, and various terms for transplant outcomes, for example event-free survival, LFS, and relapse-free survival [7,9-11,17,18]. Recently, Ruutu et al. [19] showed that the addition of corticosteroids to cyclosporine (CSA) and methotrexate in GVHD prophylaxis

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resulted in improved long-term survival. However, there are no data concerning the effects of corticosteroid prophylaxis on GVHD-free, relapse-free survival (GRFS) [5–7,10,12,19]. Therefore, in the present study we collected information on the long-term outcome within a homogenously defined population of patients with hematologic diseases who received risk stratification–directed GVHD prophylaxis. We aimed to evaluate whether low-dose glucocorticoid prophylaxis could decrease chronic GVHD, infectious complications, and immune reconstitution, leading to improvements in prognosis, particularly GRFS [20].

METHODS

Study Design

A controlled, randomized, open-label trial was conducted at Peking University Institute of Hematology in Beijing, China to investigate stratification-directed prophylaxis strategy that effectively prevented acute GVHD among patients at high risk for GVHD, without unnecessarily exposing patients at low risk to excessive toxicity from additional immunosuppressive agents (Table 1, Figure 1). The full details of the study design have been published elsewhere [15,21,22]. All included subjects provided signed informed consent. The study protocol was in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of Peking University. This study was registered at http://clinicaltrials.gov/ NCT01607580.

Eligibility and Exclusion Criteria

The study included patients, aged 15 to 60 years, with hematologic neoplasms who were scheduled to receive a haploidentical SCT between May 2012 and November 2013. The exclusion criteria were severe heart, kidney, or liver disease; a prior transplant; and hypersensitivity to rabbit antithymocyte globulin [22,23]. The patient, disease, and transplantation characteristics are summarized in Table 1.

Table 1
Patient and Donor Characteristics

Characteristics	Low-Risk Arm (Group A)	High-Risk Arm	
		Low-Dose Glucocorticoid Prophylaxis Cohort (Group B)	Control Cohort (Group C)
Number of patients	83	72	73
Median age (range), yr	33 (14-58)	26.5 (14-54)	30 (14-56)
Male sex	42 (50.6)	43 (59.7)	40 (54.8)
Diagnosis, n			
AML	32	28	29
ALL	31	25	27
CML	7	8	3
MDS	10	6	12
Others	3	5	2
Disease status	60 (72.3)/23 (27.7)	56 (77.8)/16 (22.2)	55 (75.3)/18 (24.7)
Disease risk index			
Low risk	13 (15.7)	12 (16.7)	8 (11.0)
Intermediate risk	59 (71.1)	47 (65.3)	57 (78.1)
High risk	11 (13.3)	13 (18.1)	8 (11.0)
Conditioning regimen, n			
Regimen 1 (chemotherapy based)	73	67	70
Regimen 2 (total body irradiation based)	10	5	3
HLA-A, B, -DR mismatched grafts, n			
0	0	1	0
1	6	4	2
2	15	17	18
3	62	50	53
Donor–recipient sex matched grafts			
Male–male	21 (25.3)	30 (41.7)	27 (37.0)
Male–female	21 (25.3)	20 (27.8)	23 (31.5)
Female–male	21 (25.3)	11 (15.3)	13 (17.8)
Female–female	20 (24.1)	11 (15.3)	10 (13.7)
Donor–recipient relationship			
Father–child	16 (19.3)	30 (41.7)	27 (37.0)
Mother–child	12 (14.5)	9 (12.5)	7 (9.6)
Sibling–sibling	36 (43.4)	27 (37.5)	21 (28.8)
Child–parent	16 (19.3)	5 (6.9)	14 (19.2)
Other	3 (3.6)	1 (1.4)	4 (5.5)
ABO matched grafts			
Matched	52 (62.7)	45 (62.6)	50 (68.5)
Major mismatch	8 (9.6)	9 (12.5)	8 (11.0)
Minor mismatch	16 (19.3)	12 (16.7)	11 (15.1)
Bidirectional mismatch	7 (8.4)	6 (8.3)	4 (5.5)
Mean cell compositions in allografts (range)			
Infused nuclear cells, 10^8/kg	7.66 (3.36-14.43)	8.32 (4.92-20.29)	7.98 (3.15-15.03)
Infused CD34$^+$ cells, 10^6/kg	2.58 (.85-8.90)	2.62 (.69-10.47)	2.46 (.56-7.41)
Infused lymphocytes, 10^8/kg	2.44 (1.13-30.33)	2.65 (1.05-8.80)	2.75 (1.11-6.64)
Infused CD3$^+$ cells, 10^8/kg	1.69 (.69-3.65)	1.80 (.71-6.66)	1.86 (.77-4.61)
Infused CD4$^+$ cells, 10^8/kg	.90 (.22-2.03)	1.10 (.37-2.97)	1.08 (.44-2.37)
Infused CD8$^+$ cells, 10^8/kg	.73 (.32-1.08)	.62 (.21-2.86)	.59 (.11-2.23)
Infused CD14$^+$ cells, 10^8/kg	1.39 (.33-3.92)	1.49 (.64-4.14)	1.38 (1.08-5.52)
Mean CD4/CD8 ratio in BM grafts (range)	.92 (.56-1.15)*	1.45 (1.17-3.28)	1.79 (1.16-4.73)
DLI after transplant	17 (20.5)	11 (15.3)	11 (15.1)

Values are n (%) unless otherwise defined. AML indicates acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; BM, bone marrow; DLI, donor lymphocyte infusions.

* $P < .01$ compared with low-dose glucocorticoid prophylaxis cohort and high-risk cohort.

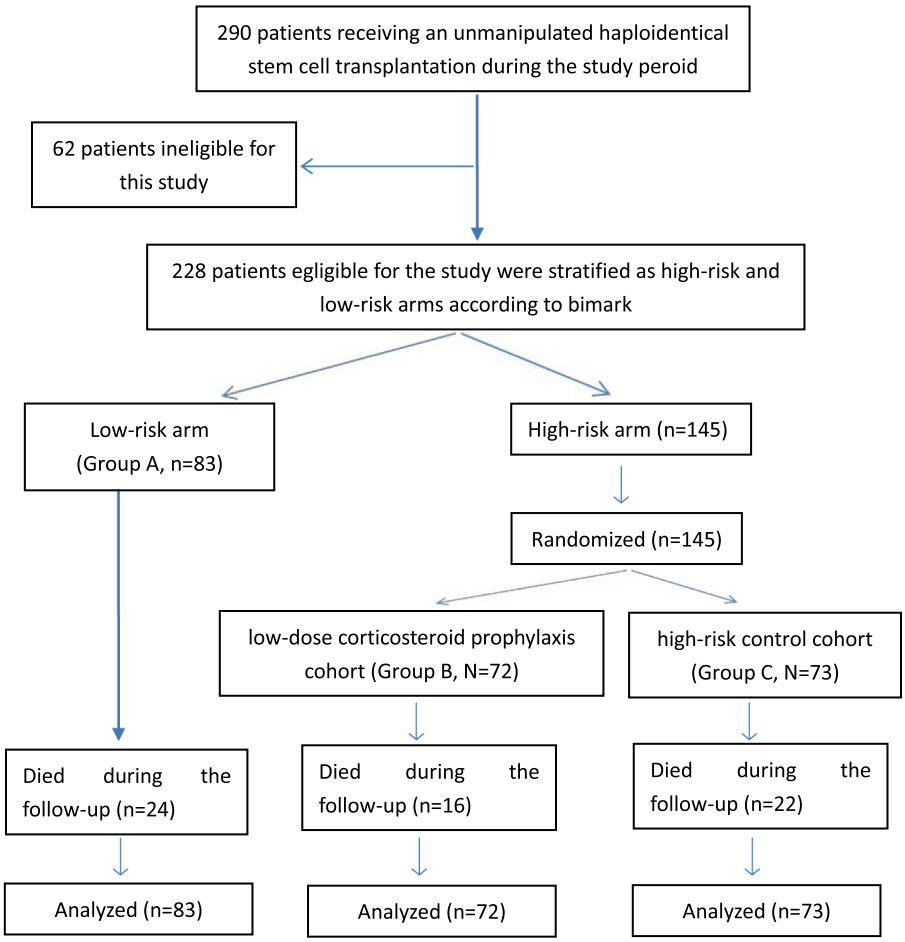

Figure 1. The Consolidated Standards of Reporting Trials diagram.

Randomization and Adjudication of GVHD Diagnosis

The enrolled patients were stratified as high risk or low risk for developing GVHD, based on biomarkers previously identified in haploidentical transplant settings [15,24,25]. Patients were identified as high risk for developing acute GVHD when 1 or both of the following criteria were observed: CD56bright natural killer cells ($>1.9 \times 10^6$/kg) in allogeneic grafts or a high CD4/CD8 ratio ($\geq$1.16) in bone marrow grafts. Patients excluded from the high-risk group were considered at low risk of developing GVHD.

High-risk subjects were randomized to either receive or not receive the investigational intervention. Case report forms included detailed, structured data regarding organ involvement, which were verified by medical record inspection. Based on these data a panel of 5 blinded experts determined whether a subject had acute GVHD and, when present, the grade. Discordances were adjudicated by majority rule [15].

Transplant Procedures

Full details of the study procedures have previously been reported [15,22]. Patients were conditioned with an intensive, chemotherapy-based regimen (regimen 1). Patients with T cell acute lymphoblastic leukemia and incompletely eradicated tumor masses were conditioned with a total body irradiation–based regimen (regimen 2). Regimen 1 consisted of cytarabine, 4 g/m^2 per day i.v. on days −10 and −9 (transplantation was considered day 0); busulfan, 12 mg/kg p.o. or 9.6 mg/kg i.v., administered in 12 doses on days −8, −7, and −6; cyclophosphamide, 1.8 g/m^2 per day i.v., administered on days −5 and −4; simustine (250 mg/m^2), administered on day −3; and rabbit antithymocyte globulin (Sangstat-Genzyme, Cambridge, Massachusetts) 2.5 mg/kg per day i.v., given on days −5, −4, −3, and −2. Regimen 2 consisted of total body irradiation (770 cGy in a single fraction) with particle shielding for the lungs, administered on day −6, and the same cyclophosphamide and antithymocyte globulin treatments as those described for regimen 1 [4,22]. Allogeneic grafts were harvested and infused according to a previous protocol [4,22].

For GVHD prevention all subjects received CSA, mycophenolate mofetil, and methotrexate. CSA was started on day −9 at 2.5 mg/kg/d i.v. After bowel function had normalized, the dosage was changed to 3 to 5 mg/kg/d p.o. Whole-blood CSA concentrations were monitored weekly and adjusted to maintain a trough concentration of 150 to 250 ng/mL. The CSA dose was gradually decreased in subjects without GVHD on day +180 but was continued in patients with acute GVHD above grade I [22]. For risk stratification–directed GVHD prophylaxis subjects in the experimental intervention arm received methylprednisone (MP; .5 mg/kg/d, i.v.) on days +5 to +12. After day +12 the dose was decreased to .25 mg/kg/d i.v. for 10 days, then reduced to .125 mg/kg/d, and finally stopped on day +30 in patients without GVHD. Cytomegalovirus (CMV), Epstein-Barr virus (EBV), and herpes zoster virus (HZV) levels were monitored, and infections were treated as previously described [15,22].

Donor Lymphocyte Infusion

Donor lymphocyte infusions were given as described previously [15,26]. Indications for donor lymphocyte infusion included hematologic leukemia relapse, in which subjects received chemotherapy, followed by donor lymphocyte infusion; evidence via molecular tests of persistent leukemia or a recurrence in subjects without GVHD; graft failure; and CMV or EBV infection unresponsive to antiviral therapy and rituximab.

Definitions and Evaluation

The definition on the occurrence of grades II to IV aGVHD has previously been reported in detail [15]. The following outcomes were examined in the present extension study: chronic GVHD, relapse, NRM, LFS, GRFS, and long-term adverse events, including infections and osteonecrosis of the femoral head (ONFH) [3,15]. GRFS was defined as the time between transplantation and the development of GVHD (grade III to IV acute GVHD or chronic GVHD requiring systemic immunosuppressive treatment), disease recurrence, or death (ie, treatment failure as defined by GRFS) [3,20,22,27]. All outcomes were assessed at the time of last contact.

Immune Reconstitution

Peripheral blood was obtained using heparin anticoagulation tubes and stained without further separation to minimize selective loss shortly after collection, using directly conjugated mAb combinations of FITC, PE, allophycocyanin, and peridimin chlorophyll protein recognizing CD3, CD4, CD8,

CD45RA, and CD45RO. Appropriate isotype controls included FITC-, PE-, allophycocyanin-, and peridimin chlorophyll protein–conjugated IgG1/2a/2b (Becton-Dickinson, Franklin Lakes, New Jersey). Immunophenotype analyses were identified as described previously [28].

Endpoints

The primary study endpoint was the incidence of chronic GVHD and GRFS. Secondary endpoints were the engraftment rate; the incidences of acute GVHD grades III to IV, infection, and chronic GVHD; and the cumulative incidences of NRM, relapse, LFS, and OS.

Statistical Analyses

The 3 groups were compared with the chi-square statistic for categorical variables and the Mann-Whitney test for continuous variables. Cumulative incidence curves were used in a competing risk setting, with relapse treated as a competing event, to calculate NRM probabilities, and with death from any cause as a competing risk for GVHD, engraftment, EBV or CMV reactivation, and relapse. Time to GVHD was defined as the time from transplantation to the onset of GVHD of any grade. Probabilities of LFS and survival were estimated with the Kaplan-Meier method. All variables in Table 1 were included in the univariate analysis; then, only variables with $P < .1$ were included in a Cox proportional hazards model with time-dependent variables. Unless otherwise specified, P values were based on 2-sided hypothesis tests. Alpha was set at .05. Most analyses were performed with SPSS 16.0 (Mathsoft, Seattle, WA).

RESULTS

Transplant Outcomes

The median follow-up for all patients and only surviving cases was 1659 days (range, 25 to 2002) and 1768 days (range, 1435 to 2002), respectively. Thirty-seven of 228 patients (16%) died from nonrelapse causes after transplantation. The cumulative incidences of chronic GVHD, relapse, NRM, LFS, OS, and GRFS were 60% (95% confidence interval [CI], 53% to 67%), 19% (95% CI, 12% to 26%), 16% (95% CI, 11% to 21%), 68% (95% CI, 61% to 75%), 73% (95% CI, 67% to 79%), and 46% (95% CI, 39% to 53%), respectively. Table 2 lists the cumulative incidences of chronic GVHD, relapse, NRM, LFS, OS, and GRFS for subjects in the high-risk arm who received (group B; n = 72) or did not receive (high-risk control cohort; group C, n = 73) low-dose corticosteroid prophylaxis and for subjects in the low-risk arm (n = 83). The total corticosteroid dose used in the first 100 days after transplantation (converted into an equivalent amount of dexamethasone) was 205.41 ± 111.38 mg in group B, which was comparable with that used in group A (229.30 ± 148.91 mg; $P = .256$) but significantly lower than that used in group C (286.54 ± 259.67 mg; P = 0.016).

Low-Dose Glucocorticoid Prophylaxis Reduced the Cumulative Incidence of Moderate to Severe Chronic GVHD

The cumulative, 5-year incidence of overall chronic GVHD in group B (46%; 95% CI, 34% to 58%) was marginally lower than that in group C (65%; 95% CI, 53% to 77%; $P = .095$) and that in group A (67%; 95% CI, 56% to 78%; $P = .084$; Figure 2A). The cumulative, 5-year incidence of moderate to severe GVHD in group B (20%; 95% CI, 10% to 30%) was significantly lower than that in group C (42%; 95% CI, 30% to 54%; $P = .010$) and that in group A (45%; 95% CI, 33% to 57%; $P = .008$; Figure 2B) [29]. Univariate analysis showed that factors associated with moderate to severe chronic GVHD included the onset of grades II to IV acute GVHD ($P = .047$), total dose of corticosteroid used in the first 100 days after transplantation ($P = .075$), total nuclear cells in allografts ($P = .025$), and prophylaxis with low-dose corticosteroid ($P < .0001$). Multivariate analysis showed that GVHD prophylaxis with low-dose corticosteroid was associated with lower incidence of moderate to severe chronic GVHD (Table 3), suggesting that low-dose glucocorticoid prophylaxis could reduce the cumulative incidence of moderate to severe chronic GVHD.

Association of Low-Dose Glucocorticoid Prophylaxis with the Cumulative Incidence of Infections

The cumulative incidences of CMV reactivation, EBV reactivation, and invasive fungal infection 100 days after transplant are shown in Table 3. The cumulative incidences of these complications were similar among the 3 groups. However, the incidence of the HZV infection (12%; 95% CI, 4% to 20%) in group B was significantly lower than that in group C (28%; 95% CI, 17% to 39%; $P = .010$) but similar to that in group A (15%; 95% CI, 7% to 23%; $P = .383$; Figure 2C). Multivariate analysis showed an association between rapid platelet recovery and the cumulative incidence of the HZV infection (hazard ratio [HR], 1.009; 95% CI, 1.002 to 1.017; $P = .017$). The incidence of the pulmonary infections (21%; 95% CI, 11% to 31%) in group B was lower than that in group C (42%; 95% CI, 29% to 55%; $P = .040$) and that in group A (43%; 95% CI, 32% to 54%; $P = .010$; Figure 2D). Multivariate analysis showed that the factors associated with pulmonary infections included rapid neutrophil recovery (HR, 1.099; 95% CI, 1.020 to 1.184; $P = .014$), total dose of corticosteroid used in the first 100 days after transplantation (HR, 2.253; 95% CI, 1.379 to 3.683; $P = .001$), and total $CD8^+$ T cells in the allografts (HR, 1.608; 95% CI, .998 to 2.592; $P = .051$). These

Table 2
Incidence of Adverse Events and Transplant Outcomes for Patients Who Underwent Allo-SCT

Parameter	Low-Risk Arm (Group A, n = 83)	High-Risk Arm	
		Low-Dose Glucocorticoid Prophylaxis Cohort (Group B, n = 72)	Control Cohort (Group C, n = 73)
CMV reactivation after day 100 post-transplantation	21 (11-31)	14 (6-22)	21 (11-31)
EBV reactivation after day 100 post-transplantation	4 (0-8)	2 (0-5)	4 (0-9)
HZV infection after day 100 post-transplantation	15 (7-23)	12 (4-20)*	28 (17-39)
HC after day 100 post-transplantation	5 (0-10)	3 (0-7)	2 (0-6)
Pulmonary infection after day 100 post-transplantation	43 (32-54)	21 (11-31)*,†	42 (29-55)
IFI after day 100 post-transplantation	3 (0-7)	3 (0-8)	7 (1-13)
Five-year cumulative incidence of ONFH	7 (1-13)	6 (0-12)*	16 (6-26)
Five-year cumulative incidence of relapse	27 (11-43)	11 (3-19)	27 (11-43)
Five-year cumulative incidence of NRM	16 (8-24)	17 (8-26)	16 (7-25)
Five-year probability of LFS	61 (46-75)	75 (65-85)	61 (46-75)
Three-year probability of OS	71 (61-81)	77 (67-87)	70 (59-81)

Values are percents, with 95% CIs in parentheses. A log-rank test was performed to compare the differences in the parameters among the 3 groups. HC indicates hemorrhagic cystitis; IFI, invasive fungal infection.

* $P < .05$ compared with those of group C.

† $P < .05$ compared with those of group A.

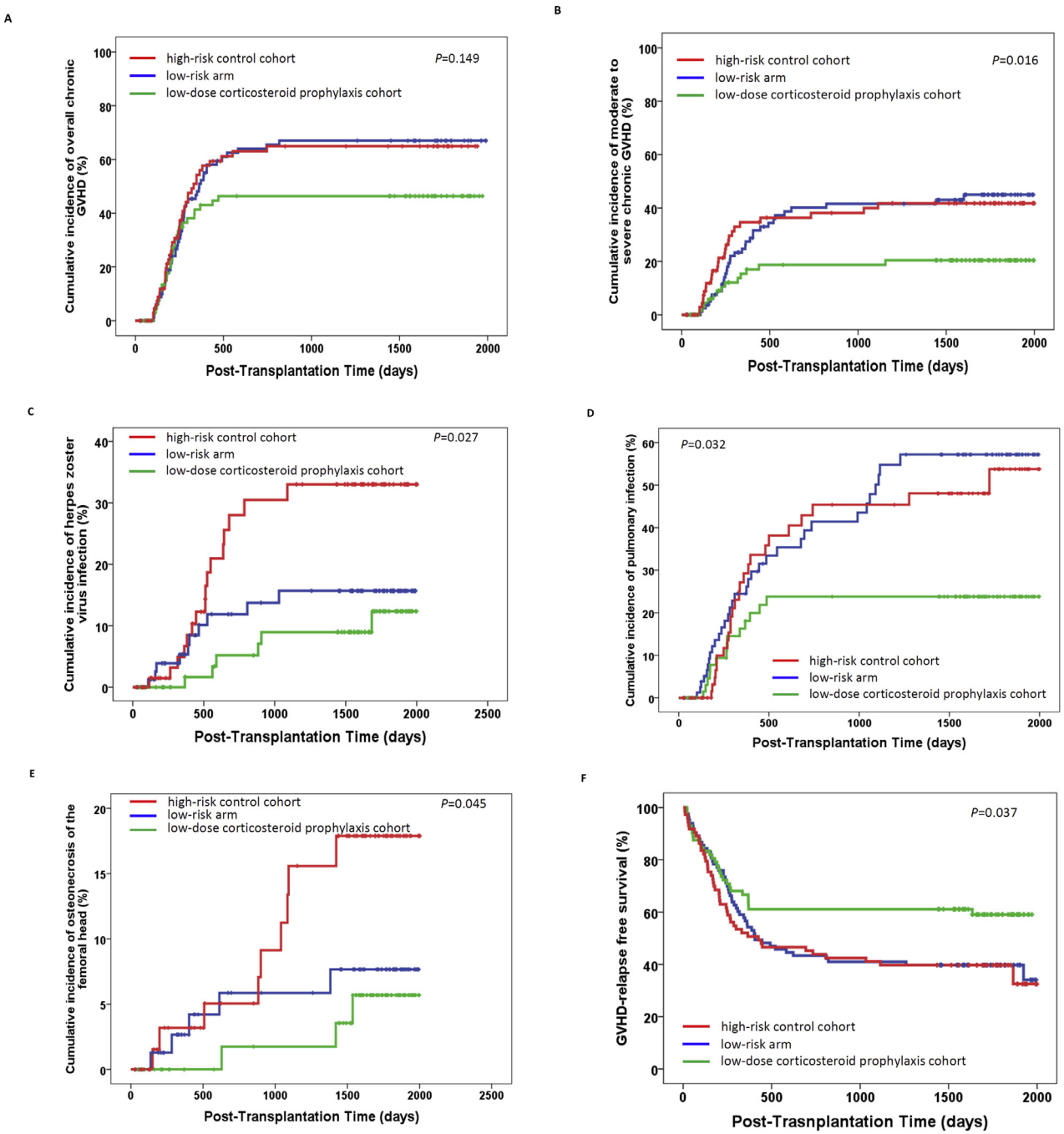

Figure 2. Outcomes of allo-SCT in 3 cohorts after a median follow-up of 1768 days. (A) Overall chronic GVHD, (B) moderate to severe chronic GVHD, (C) HZV infection, (D) pulmonary infection, (E) ONFH, and (F) GRFS. Patient allocations: group A, low-risk arm (n = 83); group B, high-risk arm treated with low-dose corticosteroid prophylaxis (n = 72); and group C, high-risk control cohort (n = 73).

results suggest that low-dose steroids reduce GVHD and thus reduce the total dose of steroids in the first 100 days, which might contribute to the lower cumulative incidence of infections.

Association of Low-Dose Glucocorticoid Prophylaxis with the Cumulative Incidence of ONFH

Several studies have shown that patients with ONFH demonstrated a lower quality of life. Here, the cumulative incidence of ONFH after long-term follow-up was investigated. The cumulative incidences of ONFH (6%; 95% CI, 0% to 12%) in group B was significantly lower than that in group C (16%; 95% CI, 6% to 26%; $P = .045$) but comparable with that in group A (7%; 95% CI, 1% to 13%; $P = .546$; Figure 2E). Multivariate analysis showed that the factors associated with ONFH included total dose of corticosteroid used in the first 1 year after transplantation (HR, 9.924; 95% CI, 2.118 to 40.790; $P = .003$) and gender (HR, 8.031; 95% CI, 2.301 to 28.026; $P = .001$). The total corticosteroid dose used in the first 365 days after transplantation (converted into an equivalent amount of dexamethasone) was 287 (72 to 1807) mg in group B, which was significantly lower than those used in group A [379 (10 to 3908) mg; $P = .030$] and group C [391 (13 to 1968) mg; $P = .039$]. These results suggest that low-dose steroids reduce GVHD and thus

Table 3
Multivariate Analysis of Low-Dose Glucocorticoid Prophylaxis on Allo-SCT

Covariate	Univariate Analysis			Multivariate Analysis		
	HR	95% CI	*P*-value	HR	95% CI	*P*
Moderate to severe Chronic GVHD						
Grades II to IV GVHD	1.616	1.006-2.596	.047	1.316	1.004-1.714	.046
Total dose of MP used in first 100 days after transplantation	1.524	.985-2.424	.075			
Total NC in allografts	.584	.365-.936	.025			
Glucocorticoid prophylaxis or not						
Group A	.958	.753-1.608	.871	.948	.566-1.587	.838
Group B	.417	.214-.812	.010	.401	.202-.797	.009
Group C		1			1	
HZV infection						
Glucocorticoid prophylaxis or not						
Group A	.503	.228-1.109	.088			
Group B	.319	.125-.815	.017			
Group C		1				
Platelet engraftment	1.009	1.002-1.017	.017	1.009	1.002-1.017	.017
Pulmonary infection						
Grades II to IV GVHD	1.759	1.095-2.827	.020			
Total dose of MP used in first 100 days after transplantation	2.322	1.434-3.758	.001	2.253	1.379-3.683	.001
Total dose of MP used in first 365 days after transplantation	1.828	1.151-2.904	.011			
Total $CD8^+$ cells in allografts	1.555	.973-2.486	.065	1.608	.998-2.592	.051
Neutrophil engraftment	1.078	1.001-1.161	.048	1.099	1.020-1.184	.014
Platelet engraftment	1.007	1.003-1.012	.002			
ONFH						
Gender (female vs. male)	6.183	1.777-21.519	.004	8.718	2.499-30.407	.001
Grades II to IV GVHD	2.543	.981-6.594	.055			
Total lymphocyte in allografts	3.753	1.223-11.520	.021			
Total dose of MP used in first 100 days after transplantation	6.079	2.438-6.958	.079			
Total dose of MP used in first 365 days after transplantation	31.784	4.213-239.766	.001	39.991	5.288-302.540	<.0001
Relapse						
Chronic GVHD	.396	.200-.783	.008	.396	.200-.783	.008
NRM						
Recipient age	1.978	.979-3.997	.057			
Grades II to IV GVHD	2.791	1.368-5.697	.005			
Pulmonary infection	2.800	1.421-5.519	.003	2.539	1.171-5.506	.018
Platelet engraftment	1.803	.903-3.603	.095			
Severe chronic GVHD	6.580	3.044-14.222	<.0001	4.828	2.088-11.164	<.0001
LFS						
Recipient age	1.623	1.004-2.626	.048	1.645	1.017-2.662	.042
Disease status	1.547	.932-2.568	.091			
Platelet engraftment	1.528	.947-2.416	.082			
Chronic GVHD	.462	.285-.750	.002	.458	.282-.744	.002
OS						
Recipient age	1.727	1.036-2.879	.036			
Grades II to IV GVHD	1.702	1.010-2.866	.046			
Pulmonary infection	2.456	1.491-4.047	<.0001	2.404	1.385-4.172	.002
Platelet engraftment	1.795	1.077-2.993	.025			
Severe chronic GVHD	4.012	2.080-7.741	<.0001	2.874	1.432-5.766	.003

All variables were first included in the univariate analysis; only variables with $P < .1$ were included in the Cox proportional hazards model with time-dependent variables. In addition, total number of patients (n = 228), such as subjects in the high-risk arm who received (group B; n = 72) or did not receive (high-risk control cohort; group C, n = 73) low-dose corticosteroid prophylaxis and for subjects in the low-risk arm (group A, n = 83), were included in building up univariable and multivariable analysis models. Group A: low-risk arm; group B: high-risk, low-dose corticosteroid prophylaxis cohort; group C: high-risk control cohort. NC indicates nuclear cells.

reduce the total dose of steroids in the first 1 year, which might contribute to the lower cumulative incidence of ONFH.

Effects of Low-Dose Glucocorticoid Prophylaxis on Immune Recovery

The early immune reconstitution was comparable among all 3 groups [15]. Here, long-term immune recovery was investigated. Compared with those of patients in group B, significant delays in the recovery of $CD4^+$ naïve T cells at day 270, $CD3^+CD4^+$ T cells and $CD4^+$ memory T cells at day 360, and $CD3^+$ T cells and $CD4^+$ naïve T cells at day 720 after transplantation were observed in cases of group A ($P < .05$ for all). The recovery of $CD4^+$ naïve T cells was marginally slower for patients in group C than for patients in group B ($P = .052$). The slow recovery of $CD4^+$ naïve T cells for patients in group C compared with those in group B was also demonstrated at days 180, 270, and 360 after transplantation, although no statistically significant differences were observed (Supplementary Table 1S). These results suggest that low-dose steroids reduce GVHD, which might enhance immune recovery.

Low-Dose Glucocorticoid Prophylaxis Significantly Increased GRFS

The 5-year probabilities of relapse, LFS, and OS were similar among the 3 groups (Table 2 and Table 4). Multivariate analysis showed that factors associated with NRM included pulmonary infection (HR, 2.539; 95% CI, 1.171 to 5.506; $P = .018$) and severe chronic GVHD (HR, 4.828; 95% CI, 2.088 to 11.164; $P < .0001$). A variable associated with relapse was chronic GVHD (HR, .396; 95% CI, .200 to .783; $P = .008$). Factors associated with LFS included recipient age (HR, 1.645; 95% CI, 1.017 to 2.662; $P = .042$) and chronic GVHD (HR, .458; 95% CI, .282 to .744; $P = .002$). Factors associated with OS included pulmonary infections (HR, 2.404; 95% CI, 1.385 to 4.172; $P = .002$) and

Table 4
Primary Cause of Death among Patients Who Underwent Allo-SCT

Cause of Death	Low-Risk Arm (Group A, n = 83)	High-Risk Arm	
		Low-Dose Glucocorticoid Prophylaxis Cohort (Group B, n = 72)	Control Cohort (Group C, n = 73)
Relapse	9 (10.8)	5 (6.9)	11 (15.1)
Infection	11 (13.3)	9 (12.5)	7 (9.6)
Graft failure	1 (1.2)	0 (0)	1 (1.4)
Chronic GVHD	2 (2.4)	0 (0)	0 (0)
VOD	1 (1.2)	0 (0)	0 (0)
Others	0 (0)	2 (2.8)	3 (4.1)

Values are n (%) of deaths among the total number of patients in each of the three cohorts. VOD indicates hepatic veno-occlusive disease.

severe chronic GVHD (HR, 2.874; 95% CI, 1.432 to 5.766; $P = .003$).

The probability of 5-year GRFS in group B (59%; 95% CI, 47% to 71%) was significantly higher than that in group A (34%; 95% CI, 20% to 48%; $P = .036$) and that in group C (33%; 95% CI, 20% to 46%; $P = .017$; Figure 2F). Univariate analysis showed that factors associated with GRFS included platelet engraftment ($P = .027$), total dose of corticosteroid used in the first 100 days after transplantation ($P = .011$), and prophylaxis with low-dose corticosteroid ($P = .041$). Multivariate analysis showed that factors associated with GRFS included total dose of corticosteroid used in the first 100 days after transplantation (HR, 1.547; 95% CI, 1.089 to 2.198; $P = .015$) and platelet recovery (HR, 1.456; 95% CI, 1.022 to 2.075; $P = .037$). These results suggest that low-dose steroids reduce GVHD, leading to better GRFS.

DISCUSSION

In this long-term follow-up study of a controlled, randomized, open-label trial [15], we demonstrated that low-dose glucocorticoid prophylaxis for GVHD significantly decreased the cumulative incidence of moderate to severe chronic GVHD and reduced the total dose of steroids, which might contribute to lower incidence of infections and ONFH, as well as a superior GRFS, as we also observed a significant improvement in GRFS after low-dose glucocorticoid prophylaxis. The prospective and randomized design of the present study laid the foundation for the reliability of the findings and provided new evidence consistent with those of current studies [7,9–11,19], indicating that higher steroid doses are harmful. Reducing the total dose is of course beneficial.

Chronic GVHD is 1 of the major causes of mortality after allo-SCT [5]. Consistent with the report by Ruutu et al. [19], we found that GVHD prophylaxis with low-dose glucocorticoid significantly decreased the cumulative incidences of moderate to severe chronic GVHD. These results suggest that MP prophylaxis may decrease chronic GVHD via reducing the cumulative incidence of acute GVHD [9,15,19,30,31], because the disruption of dendritic cell antigen presentation during acute GVHD may lead to regulatory T cell failure and chronic GVHD [30]. However, Storb et al. [11] and Deeg et al. [7] showed that the use of MP prophylaxis significantly increased the cumulative incidences of chronic GVHD, although acute GVHD was significantly reduced. These results are in contrast to those reported by Ruutu et al. [19] and us in the present study. The higher incidence of chronic GVHD in patients who received MP prophylaxis for acute GVHD might be ascribed to the high incidence of infections, as observed by Storb et al. [11], Sayer et al. [17], and Deeg et al. [7] because cytokine storms, such as IFN-γ, during infections are involved in the pathogenesis of chronic GVHD [32,33]. Ruutu et al. [9,19] and us demonstrated that MP prophylaxis significantly decreased the cumulative incidence of infections. Moreover, the differences in effects of glucocorticoid prophylaxis on chronic GVHD between other studies [7,9,11,17,19] and the present study may also be related to the differences in the total doses of MP used for GVHD prevention, patient populations, and transplant modalities, although we could not provide more reasonable explanations.

A number of studies have shown that reduction in infection rate contributed to the improvement of outcomes after allo-SCT [12,13,34]. Here, we indicated that a low incidence of HZV infection in patients receiving low-dose corticosteroid prophylaxis might be related to the rapid recovery of $CD4^+$ T cells (Supplementary Table 1S), particularly $CD4^+$ naïve T cells, as previous studies have shown that viral reactivations were associated with the kinetics of immune recovery, particularly $CD4^+$ T cells [35–37]. Moreover, low-dose corticosteroid prophylaxis significantly decreased the cumulative incidence of infections, which is in contrast to the reports by Storb et al. [11], Sayer et al. [17], and Deeg et al. [7] Several reasons may account for these differences. First, the total dose of MP in our study (7.5 mg/kg) [15] was lower than those used (>28 mg/kg) by Storb et al. [11] and Deeg et al. [7]. Second, treatment of the higher incidence of chronic GVHD with additional immunosuppression agents, including corticosteroids, may contribute to a higher incidence of infections in the MP prophylaxis group as reported by Storb et al. [11], Sayer et al. [17], and Deeg et al. [7] Although a total dose of MP higher than 28 mg/kg was used for GVHD prophylaxis, Ruutu et al. [9,19] observed a significantly lower incidence of infections in cases with MP prophylaxis compared with those without. The results shown by Ruutu et al. [9,19] and us in the present study suggest that except for the lower total dose of corticosteroid itself, prophylaxis with corticosteroids may decrease infection rates via significantly reducing the incidence of GVHD. Overall, the present results indicate that low-dose glucocorticoid prophylaxis might decrease the incidence of infections via reducing GVHD and the total dose of glucocorticoid used without compromising immune recovery (Supplementary Table 1S), both of which might contribute to decreased infection-related mortality as described by others [13].

The quality of life ofallo-SCT survivors not only was related to moderate to severe chronic GVHD but also could be due to osteoporosis [38–40]. Here, we provide the first demonstration that corticosteroid prophylaxis for GVHD significantly decreased the cumulative incidence of ONFH. Similar to previous reports [41], gender and dose of corticosteroids in the first 365 days after transplantation were independent factors associated with ONFH in the present study. These results suggest that low-dose corticosteroid prophylaxis leads to a reduction in the incidence of ONFH via significantly reducing the total dose of corticosteroid [15]. Both provide a logical explanation that low-dose glucocorticoid prophylaxis could improve quality of life through decreasing the cumulative incidence of

chronic GVHD and ONFH, which adversely affect quality of life [39,40,42].

Concerning the effects of prophylactic corticosteroid administration on survival [7,9–11,17,19], Deeg et al. [7] did not observe a long-term survival advantage for the addition of MP to CSA after a median follow-up of 6 years. In the present study we did not observe advantages, such as LFS and OS, for glucocorticoid prophylaxis. However, the results indicated that low-dose glucocorticoid prophylaxis leads to superior GRFS. Ruutu et al. [19] reported an improved long-term survival after adding corticosteroids to CSA and methotrexate in GVHD prophylaxis, showing significant differences in NRM and OS after 15 years of follow-up between cases receiving MP and those without. The results of Ruutu et al. [19] were not in contrast to those reported by Deeg et al. [7] and us in the present study, because Ruutu et al. [19] reported a significantly improved survival in patients with MP prophylaxis compared with those without 10 years after transplantation. Overall, our results suggest that low-dose glucocorticoid prophylaxis can improve GRFS via decreasing chronic GVHD and glucocorticoid-related adverse effects, although long-term follow-up is ongoing.

The present study had several limitations. First, this is a post hoc analysis and was not designed to demonstrate the effectiveness of corticosteroid prophylaxis on chronic GVHD and other outcomes, such as infection incidence, GRFS, LFS and OS. Second, the study is represented by the haploidentical allograft setting. However, the large number of patients and the long term follow-up make it relevant at least for the haploidentical setting, suggesting the same approach could be tested in the setting of matched unrelated donors.

In conclusion, we provided the first demonstration that GVHD prophylaxis with low-dose corticosteroids not only decreased the cumulative incidence of acute GVHD and chronic GVHD but also reduced the total dose of steroids, which might result in the lower cumulative incidence of infections and ONFH as well as an improved GRFS within a homogenously treated population. The results reported by Ruutu et al. [19] and us in the present study suggest a prospect on the use of corticosteroids for the prevention of acute GVHD, although further studies are needed before routine application in the clinic.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2018.11.020.

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