Effectiveness and safety of lower dose prednisone for initial treatment of acute graft-versus-host disease: a randomized controlled trial

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SUPPLEMENTAL MATERIAL (ONLINE ONLY)

FOR

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Supplemental Methods

Preparative regimens and post-transplant immunosuppressive regimens

Myeloablative conditioning regimens included targeted oral busulfan (4 mg/kg/day for 4 consecutive days) and intravenous cyclophosphamide (60 mg/kg/day for 2 consecutive days) (n=33); cyclophosphamide (60 mg/kg/day for 2 consecutive days) followed by fractionated total body irradiation (TBI; ≥12 Gy) (n=22); intravenous treosulfan (14 g/m^2/day for 3 consecutive days) and fludarabine (30 mg/m^2/day for 5 consecutive days) followed by single-fraction TBI (2 Gy) (n=21); and other regimens (n=20) (**Table 1**). Patients treated with these conditioning regimens were given a calcineurin inhibitor (cyclosporine or tacrolimus) in combination with methotrexate after the transplant.[1] Reduced-intensity conditioning regimens included low-dose TBI (2–4 Gy) alone or in combination with fludarabine (30 mg/m^2/day for 3 consecutive days) (n=51) and other regimens (n=15). Patients treated with these conditioning regimens were given a calcineurin inhibitor in combination with mycophenolate mofetil or methotrexate after the transplant.[2,3] Details describing the institutional supportive care have been published previously.[4-8]

Management of immunosuppressive medications

Medications for initial treatment of GVHD included the prednisone dose as dictated by randomization plus oral beclomethasone dipropionate emulsion (BDP; 4 mg/day) and enteric-coated budesonide (6 mg/day) in patients with gastrointestinal GVHD.[9] Medications administered for GVHD-prophylaxis were continued as tolerated. Prednisone doses were tapered as manifestations of GVHD resolved and the rate of taper was not prescribed by the protocol. The protocol allowed the attending physician to increase the dose of prednisone in patients who had an insufficient response or GVHD-progression after 48 hours of treatment with prednisone at doses <2 mg/kg/day. Secondary immunosuppressive therapy was defined as any

intervention intended to control GVHD through oral or parenteral administration of any systemic medication not given previously. An increase in the dose of prednisone or the resumption of treatment with prednisone after previous discontinuation was not considered secondary therapy. Decisions regarding the timing and choice of secondary therapy were made at the discretion of the attending physician.

Supplemental statistical considerations

Secondary endpoints. Given concern that a lower initial dose of prednisone (0.5 mg/kg/day in cohort A and 1 mg/kg/day in cohort B) may lead to worse outcomes, overall mortality ("harm") at 1 year after the initiation of therapy was evaluated in both cohorts. A 7.5% absolute reduction in overall survival in the lower-dose arm of each cohort compared to the standard-dose arm was considered the allowable "no harm" threshold. With true overall mortality rates of 35% and 50% in the higher and lower initial dose arms, for example, the probability of exceeding this limit (power) with 75 patients per arm was 83%. If the true overall mortality rates were both 35%, the probability of exceeding the 7.5% limit (type I error) was 18%. Progression to grades III-IV acute GVHD and secondary systemic therapy for acute GVHD by one year after enrollment were assessed as cumulative incidence. Infections, hyperglycemia, hypertension, myopathy and quality of life were compared as indicators of prednisone toxicity.

Statistical methods. Overall survival was estimated by the Kaplan-Meier method. The cumulative incidence of other time-to-event endpoints (relapse, non-relapse mortality [NRM], chronic GVHD, initiation of secondary therapy, and progression to grade III-IV acute GVHD) was estimated by standard methods accounting for competing risks.[10] Comparison of cumulative prednisone dose between dose-groups was by 2-sample t-test. Comparison of all

time-to-event endpoints between dose-groups was based on hazard-ratio analysis using Cox regression. All p-values are 2-sided.

Data safety monitoring. A Data Safety Monitoring Board (DSMB) reviewed study results every 6 months. The option of stopping the trial early for safety was based primarily on the “no harm” endpoint. The DSMB was charged with recommending early study closure if it appeared reasonably certain that the “no harm” criterion could not be satisfied.

Supplemental Table S1: Incidence of invasive infections during the first 100 days of prednisone treatment for newly diagnosed acute GVHD according to initial prednisone dose

	Lower-dose prednisone[a]	Higher-dose prednisone[a]	
Number of patients, *n*	79	83	
Organism	**Invasive infections during first 100 days of prednisone treatment[b]**		**P**
Any infection, %	52	53	0.89
Any invasive bacterial infection[c], %	22	16	0.34
Gram-negative bacteremia[d]	11	4	0·06
Gram-positive bacteremia[e,f]	10	11	0·88
Anaerobic bacteremia[g]	4	0	0.07
Pneumonia[h]	3	2	0.96
C. difficile associated diarrhea[i]	5	1	0,16
Invasive fungal infection[j] (proven/probable), %	5	5	0·94
Any viral infection[k], %	39	47	0.32
CMV-PCR positivity, any level[l]	39	43	0·59
CMV- PCR positivity, >500 copies/mL[l]	10	11	0·88
CMV-disease[m]	4	4	0·95
Viral lower respiratory tract infection[n,o]	3	4	0.69

[a] "Lower dose prednisone" designates all patients in cohorts A and B who were randomized to treatment with an initial prednisone dose of 0·5 mg/kg/day and 1 mg/kg/day, respectively.

"Higher dose prednisone" designates all patients in cohorts A and B who were randomized to treatment with an initial prednisone dose of 1 mg/kg/day and 2 mg/kg/day, respectively. Analyses were also performed separately in each cohort and were not significantly different.

[b]First episode in each category is shown.

[c]Invasive bacterial disease was defined as previously published[11] and included end organ disease and bacteremia.

[d]Incident episodes of gram-negative bacteremia (n): *Lower dose group*- 1 *Escherichia coli*, 2 *Klebsiella pneumoniae*, 2 *Klebsiella oxytoca*, 2 *Pseudomonas spp*., 1 *Serratia marcescens*, 1 *Haemophilus sp.*, 1 *Delftia Acidovorans*; *Higher dose group*- 1 *Klebsiella pneumoniae*, 1 *Serratia marcescens*, 1 *Pseudomonas spp*.

[e]Bacteremia due to coagulase negative *Staphylococci* was excluded.

[f]Incident episodes of gram-positive bacteremia (n): *Lower dose group*- 3 alpha hemolytic *Streptococcal spp*, 2 *Enterococcus faecalis*, 2 *Staphylococcus aureus*, 1 *Rothia mucilaginosa*, 1 *Corynebacterium aurimucosum*; *Higher dose group*- 1 *Viridans Streptococcus*, 1 *Weisella sp*, 1 Non-hemolytic *Streptococcus*, 2 *Staphylococcus aureus*, 3 *Enterococcus faecium*, 1 *Corynebacterium jeikieum*.

[g]Incident episodes of anaerobic bacteremia (n): *Lower dose group*- 2 *Bacteroides fragilis*, 1 *Actinomyces odontolyticus*; *Higher dose group*- none.

[h]Etiologies of incident bacterial pneumonias isolated from BAL (n): *Lower dose group*- 1 Pseudomonas aeruginosa, 1 Haemophilus sp.; *Higher dose group* 1 *Stenotrophomonas maltophilia*, 1 *Staphylococcus aureus*.

[i]*C. difficile* infections only recorded during the first 42 days of therapy.

[j]Invasive fungal disease was defined according to international guidelines (n):[12] *Lower dose group*- 4 fungal pneumonia- 1 *Malassezia restricta*, 3 *Aspergillus spp.* and 0 fungemia; *Higher dose group*- 3 fungal pneumonia- all *Aspergillus spp.* and 1 episode of fungemia due to *Candida glabrata.*

[k] Viral infections include CMV reactivation, CMV disease, or viral lower respiratory tract infections.

[l]Plasma DNA PCR was performed weekly.[13]

[m]CMV disease was defined according to standard guidelines.[14]

[n]Lower respiratory tract infection is defined by the detection by PCR of a respiratory virus in bronchoalveolar lavage specimens in a patient with respiratory symptoms and abnormal chest imaging.

[o]Pathogens identified as causing LRTI were: *Lower dose group*- 1 Rhinovirus, 1 Parainfluenza virus Type 2; *Higher dose group*- 2 Rhinovirus, 1 Parainfluenza virus Type 3.

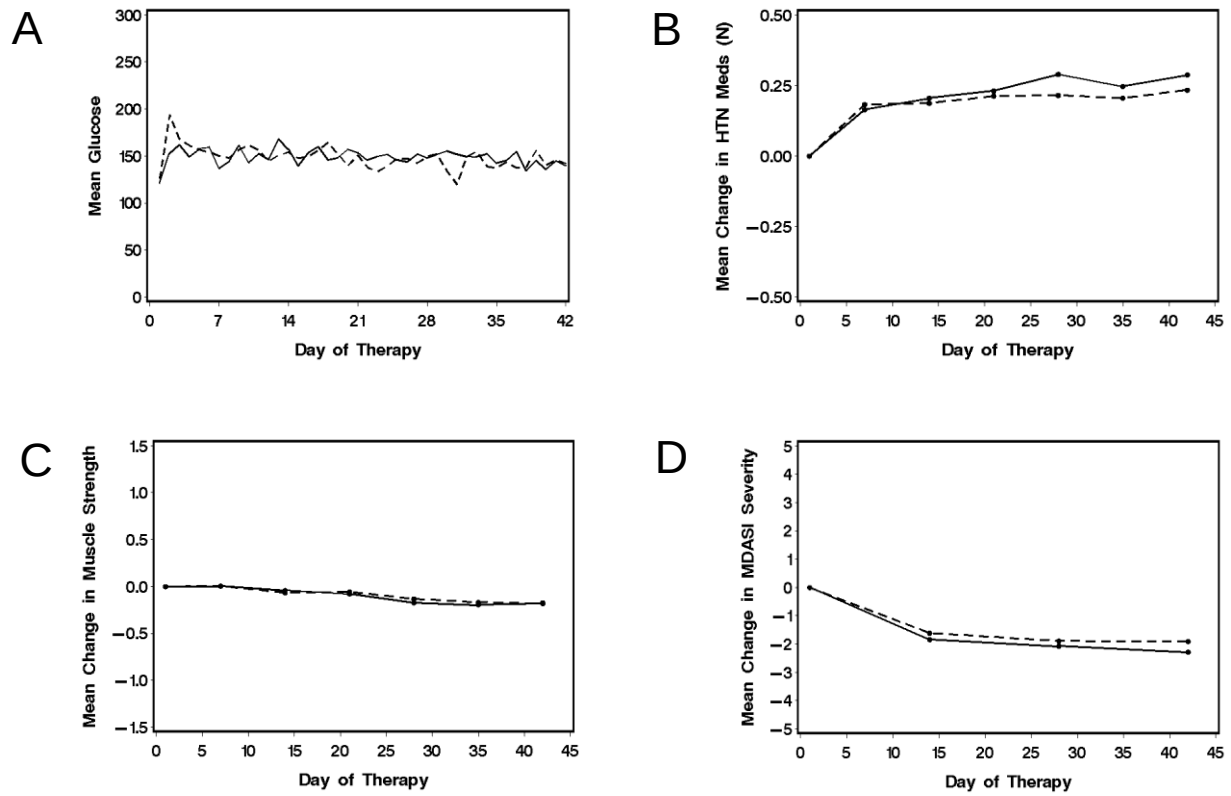
Supplemental Table S2: Characteristics of patients enrolled and of those *not* enrolled in the study*

	Random sample of eligible patients not enrolled in the study		Enrolled in protocol 2327	
	Grade IIa	**Grade IIb-IV**	**Grade IIa**	**Grade IIb-IV**
Number of patients, *n*	94	57	102	60
Proportion of total, *%*	62	38	63	37
Age, *median (range)*	47 (0-73)	46 (0-69)	49 (6-75)	48 (1-74)
Patient sex, *female (%)*	34 (36)	21 (37)	42 (41)	20 (33)
Donor type, *n (%)*				
HLA-identical related	36 (38)	5 (9)	40 (39)	10 (17)
HLA-matched unrelated	41 (44)	36 (63)	43 (42)	32 (53)
HLA-mismatched	17 (18)	16 (28)	19 (19)	18 (30)
Nonmyeloablative regimen, *n (%)*	28 (30)	18 (32)	40 (39)	26 (43)
GVHD organ involvement[#], *n (%)*				
Skin alone	3 (3)	36 (63)	0 (0)	17 (28)
Gut alone	65 (69)	6 (11)	69 (68)	17 (28)
Skin + gut	25 (27)	13 (23)	33 (32)	25 (42)
Neither	1 (1)	2 (4)	0 (0)	1 (2)
Grade III at onset, *n (%)*	--	6 (11)	--	24 (40)
HCT to therapy, *median (range)*	35 (13-118)	23 (7-171)	31 (10-88)	26 (12-91)
GVHD to therapy, *median (range)*	2 (0-55)	3 (0-82)	2 (0-43)	3 (0-29)
Received BDP, *n (%)*	81 (90)	33 (58)	99 (97)	18 (47)

*During the enrollment period (4/09 – 5/13), 573 patients with newly diagnosed ≥Grade IIa acute GVHD were not enrolled in the study. To address the question of possible selection bias with respect to study enrollment, we retrieved a random sample of 200 patients who were newly

diagnosed with acute GVHD during the study period and who were *not* enrolled. Of those, 26 never received systemic therapy, and an additional 22 patients were treated with prednisone for Grade I acute GVHD at onset. The table compares key clinical characteristics of patients enrolled (n=162) with the random sample of patients who were eligible for the study but were *not* enrolled (n=151).

[#]At onset



Supplemental Figure S1: Measures of prednisone-associated toxicity among all patients according to initial treatment with lower-dose and higher-dose prednisone. Measures of prednisone toxicity were assessed at baseline and then weekly until 42 days after starting prednisone treatment. (**A**) Mean blood glucose concentrations. (**B**) Mean change in number of anti-hypertensive medications. (**C**) Mean change in muscle strength by manual testing, which assessed the degree of resistance against pressure applied by tester on a 5-point scale. Testing included upper and lower extremities, shoulder (deltoid muscle at 90 degrees), and hip and knee in a sitting position. (**D**) Mean change in quality of life assessed by MD Anderson Cancer Center Symptom Inventory.[15] Results showed no statistically significant reduction for these toxicity measures in patients treated at lower initial prednisone doses. The results were not changed after sub-group analyses of cohort A and cohort B (not shown). **Solid line:** Patients who started treatment with *lower*-dose prednisone (0.5 mg/kg/day or 1 mg/kg/day for those in

cohorts A and B, respectively). **Dashed line:** Patients who started treatment with *higher*-dose prednisone (1 mg/kg/day or 2 mg/kg/day for those in cohorts A and B, respectively).

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