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

by Marco Mielcarek, Terrence Furlong, Barry E. Storer, Margaret L. Green, George B. McDonald, Paul A. Carpenter, Mary E.D. Flowers, Rainer Storb, Michael Boeckh, and Paul J. Martin

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

Running Title: Lower-dose prednisone for acute GVHD

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Key words: GVHD; immunosuppressive therapy; prednisone; hematological malignancies; hematopoietic cell transplantation

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ABSTRACT

We conducted a phase III study to test the hypothesis that initial therapy with “lower-dose” prednisone is effective and safe for patients with newly diagnosed acute graft-versus-host disease. We hypothesized that a 50% decrease in the initial dose of prednisone for treatment of acute graft-versus-host disease would suffice to control graft-versus-host disease without increasing the incidence of secondary treatment. Patients with Grade IIa-manifestations (upper gastrointestinal symptoms, stool volumes <1.0 L/day, rash involving <50% of the body surface, no hepatic dysfunction; n=102) were randomized to start treatment with prednisone at 1 mg/kg/day or 0.5 mg/kg/day. Those with ≥Grade IIb-manifestations (rash involving ≥50% of the body surface, stool volumes ≥1.0 L/day or hepatic involvement; n=62) were randomized to start treatment with prednisone at 2 mg/kg/day or 1 mg/kg/day. The primary study endpoint, a ≥33% relative reduction of the mean cumulative prednisone dose by day-42 after initial treatment with lower-dose prednisone, was not reached. With a median follow-up of 36 (7–53) months, initial treatment with lower-dose prednisone appeared to be effective for patients presenting with Grade IIa-manifestations since it did not increase the likelihood of requiring secondary immunosuppressive therapy. Further exploratory analyses suggested that for patients presenting with skin-predominant ≥IIb-manifestations, initial treatment with lower-dose prednisone was associated with an increased risk of requiring secondary immunosuppressive therapy (41% vs. 7%, p=0.001). In summary, initial treatment of newly diagnosed acute graft-versus-host disease with lower-dose prednisone is effective. Within the statistical
limitations of the study, results showed no suggestion that use of lower-dose prednisone adversely affected survival.

INTRODUCTION

Successful treatment of malignant diseases by allogeneic hematopoietic cell transplantation (HCT) depends on effective management of acute graft-versus-host disease (GVHD), an inflammatory syndrome initiated by alloreactive donor T cells.\textsuperscript{1-3} Acute GVHD develops in 40–80% of HCT recipients despite prophylaxis with immunosuppressive medications, and systemic glucocorticoids are typically administered as first-line treatment.\textsuperscript{4,5} After systemic glucocorticoids control GVHD-manifestations, treatment can eventually be withdrawn in most patients. Patients with persistent or recurrent GVHD symptoms despite glucocorticoid treatment, however, have increased risks of morbidity and mortality related to uncontrolled GVHD, prolonged glucocorticoid exposure, and infections.\textsuperscript{6,7}

Prospective randomized studies have not shown a demonstrable benefit for treatment of acute GVHD with prednisone at doses higher than 2 mg/kg/day.\textsuperscript{8} Studies aimed at identifying the minimally effective yet safe initial glucocorticoid dose, however, have never been performed. While one school of thought advocates that all patients with newly diagnosed acute GVHD should be treated with a prednisone-equivalent dose of at least 2 mg/kg/day to prevent progression to more severe GVHD, another school of thought asserts that lower doses of glucocorticoids can effectively control most presentations of GVHD without exposing patients to the risks of more intense and prolonged immunosuppression.\textsuperscript{9-11} The latter notion was corroborated in a large retrospective analysis in which initial treatment with lower-dose...
prednisone (1 mg/kg/day) of patients with grades I-II acute GVHD did not compromise disease control or survival, and was associated with a reduced risk of invasive fungal infections. Conclusions regarding patients who presented with more severe GVHD manifestations were limited by the small sample size and the retrospective nature of the study.

In the current prospective, randomized study, we hypothesized that a 50% decrease in the initial dose of prednisone for treatment of acute GVHD, followed by tapering at physician discretion depending on response and side effects, would suffice to control GVHD with a lower cumulative steroid dose across time. We anticipated that lower initial steroid doses would not increase the incidence of secondary treatment. We also anticipated that a lower steroid exposure across time would yield clinical benefit by decreasing steroid-related toxicity.

METHODS

Study design and patients
Patients who received allogeneic HCT at the Fred Hutchinson Cancer Research Center between April 2009 and May 2013 and who, in the opinion of the treating physician, required systemic immunosuppressive therapy for newly diagnosed acute GVHD were eligible for this phase III trial. The study was approved by the Institutional Review Board (IRB) and all participating patients signed IRB-approved consent forms. Patients were excluded if they had hallmarks of chronic GVHD, had developed acute GVHD after donor lymphocyte infusion, had received prior systemic immunosuppressive therapy for acute GVHD, or had contraindications to standard-dose prednisone, such as uncontrolled infections or recent diagnoses of recurrent malignancy. The characteristics of patients, donors and transplant regimens are summarized in
Table 1. Details regarding preparative regimens and post-transplant immunosuppressive regimens are provided as supplemental data (Supplemental Methods).

Stratification and Randomization

Acute GVHD was diagnosed and graded according to established criteria\textsuperscript{1,12} and patients were stratified into cohorts A and B according to severity of GVHD at symptom-onset. Biopsy confirmation of the diagnosis was encouraged but not required. Patients with Grade IIA-manifestations\textsuperscript{11} (cohort A: upper gastrointestinal symptoms of anorexia, nausea, vomiting attributed to acute GVHD, with stool volumes <1 L/day, rash involving <50% of the body surface, and no hepatic dysfunction) were randomized to start therapy with a prednisone-equivalent dose of either 1 or 0.5 mg/kg/day. Those with ≥Grade IIB-manifestations\textsuperscript{11} (cohort B: rash involving ≥50% of the body surface, stool volumes ≥1 L/day or hepatic involvement with total serum bilirubin > 2 mg/dL) were randomized to start treatment with a prednisone-equivalent dose of either 2 or 1 mg/kg/day. Randomization was further stratified according to risk of recurrent malignancy (standard vs. high) and pre-transplant comorbidity (hematopoietic cell transplant comorbidity index, 0-1 vs. ≥2).\textsuperscript{13}

Statistical analysis

Primary endpoint. The primary endpoint of the study was a ≥33% reduction of the mean cumulative prednisone dose by day 42 of treatment among patients initially treated with a prednisone dose of 0.5 mg/kg/day in cohort A and a dose of 1 mg/kg/day in cohort B compared to those given standard-dose prednisone. We estimated a 3:2 enrollment ratio for patients presenting with Grade IIA manifestations (cohort A) versus those presenting with ≥Grade IIB-manifestations (cohort B). Planned enrollment of 150 patients provided 93% and 98% power in the two strata, respectively, to detect a true 33% reduction in mean cumulative day-42 dose, at the one-sided 0.025 level of significance.
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. The cumulative incidences of progression to grades III-IV acute GVHD and secondary systemic therapy for acute GVHD by one year after enrollment were also assessed. Infections, hyperglycemia, hypertension, myopathy and quality of life were compared as indicators of prednisone toxicity. Further details regarding statistical considerations pertaining to the secondary endpoints are provided as supplemental data (Supplemental Methods).

RESULTS

Patients enrolled
Between May 2009 and June 2013, the study enrolled 164 patients. Two patients were randomized within the incorrect stratum and were allocated to doses for which they were not eligible, leaving 162 evaluable patients (cohort A, n=102; cohort B, n=60). Most demographic and transplant characteristics were balanced between patients enrolled in the two prednisone-dose arms in cohort A and cohort B, respectively (Table 1). Clinical characteristics of patients enrolled in the study were compared with those of eligible patients who were not enrolled (Supplemental Table S2) and showed no evidence of bias toward selective enrollment of patients with less severe GVHD.

Primary endpoint: mean cumulative prednisone dose at day 42 of treatment
Cumulative prednisone dose was determined among 150 patients treated with prednisone for 42 days. Twelve patients discontinued prednisone before day 42 because of early departure
from the Center (n=7), death (n=3) or withdrawal from study (n=2). For patients in cohort A treated initially with either 1 or 0.5 mg/kg/day, the mean ±SD cumulative prednisone doses were 27.1 ± 12.7 vs. 22.2 ± 13.7 mg/kg, respectively, at day 42 (18% reduction; p=0.08). For patients in cohort B treated initially with either 2 or 1 mg/kg/day, mean ±SD cumulative prednisone doses were 41.3 ± 12.1 vs. 38.4 ± 14.1 mg/kg (7% reduction; p=0.4) (Figure 2).

Pre-specified secondary “no harm” endpoints

Overall survival, progression to Grade III-IV acute GVHD and secondary systemic immunosuppressive therapy. In comparison between the aggregated standard-dose groups (1 mg/kg/day for cohort A; 2 mg/kg/day for cohort B) and the aggregated lower dose groups (0.5 mg/kg/day for cohort A; 1 mg/kg/day for cohort B), no statistically significant differences were observed in the likelihood of overall survival (77% [95% CI 67-86%] vs. 77% [95% CI 67-86%]; HR 1.02 [95% CI 0.6-1.74%]; p=0.95) (Figure 3A, and B-C) and progression to Grade III-IV acute GVHD (13% [95% CI 7-23%] vs. 6% [95% CI 2-14%]; HR 0.43 [95% CI 0.14-1.33%]).

Among patients in cohort A (n=102), the likelihood of progression to Grade III-IV acute GVHD was 10% among patients who started treatment with a prednisone dose of 0.5 mg/kg/day and 6% among those who started treatment with a dose of 1 mg/kg/day (p=0.39). Among patients in cohort B who presented with Grade IIb acute GVHD (n=53), the likelihood of progression to Grade III-IV acute GVHD was 19% among patients who started treatment with a prednisone dose of 1 mg/kg/day and 7% among those who started treatment with a dose of 2 mg/kg/day (p=0.20).

The risk of requiring secondary systemic immunosuppressive therapy was significantly lower in the aggregated standard-dose groups compared to the aggregated lower dose groups (7% [95% CI 2-13%] vs. 23% [95% CI 14-32%]; HR 0.29 [95% CI 0.12-0.74%]; p=0.009) (Figure 3D). Further analysis showed that 9 of 102 patients (9%) in cohort A required secondary
systemic immunosuppressive therapy. Among patients in cohort A, initial prednisone dose was not associated with the likelihood of requiring secondary systemic immunosuppressive therapy (12% vs. 8%, p=0.44) (Figure 3E). In comparison, 13 of 60 patients (22%) in cohort B required secondary systemic immunosuppressive therapy. Among patients in cohort B, initial treatment with a prednisone of 1 mg/kg/day was associated with an increased risk of requiring secondary systemic immunosuppressive therapy (41% vs. 7%, p=0.001) (Figure 3F). Secondary systemic immunosuppressive therapy in cohort B included mycophenolate mofetil (n=6), ATG (n=3), sirolimus (n=2), alemtuzumab (n=1), or etanercept (n=1).

**Pre-specified measures of prednisone toxicity**
Assessment of invasive infections, control of hyperglycemia and hypertension, degree of myopathy and quality of life at weekly intervals during the first 42 days after starting treatment showed no statistically significant associations with initial prednisone dose (Supplemental Figure S1; Supplemental Table S1).

**Exploratory secondary “no harm” endpoints**
*Non-relapse mortality, recurrent malignancy and chronic GVHD.* In comparison between the aggregated standard-dose groups and the aggregated lower dose groups, no statistically significant differences were observed in the risks of NRM (16% vs. 15%), recurrent malignancy (21% vs. 21%) and chronic GVHD (54% vs. 47%) at 12 months after start of therapy (Figure 4). The risks of NRM (24% vs. 25%) at 12 months were also similar in the two prednisone-dose arms of cohort B (n=60) (Figure 4B-C).

**Additional exploratory analyses**
Among patients in cohort B, an exploratory analysis suggested that skin-predominant GVHD (rash involving >50% of body surface) at onset was associated with a higher likelihood of
requiring secondary immunosuppressive therapy after initial treatment with a prednisone dose of 1 mg/kg/day (53% vs. 18%; p=0.06). In contrast, gut-predominant GVHD at onset (stool volume >1.0 L/24 hours) was not associated with the risk of requiring secondary immunosuppressive therapy in this cohort (43% vs. 39%; p=0.86).

DISCUSSION

Since the clinically most meaningful measure of prednisone-associated morbidity in hematopoietic allograft recipients is unknown, a 33% or greater reduction in mean prednisone exposure by treatment day 42 among patients started on the lower dose was chosen as a surrogate primary endpoint for this study. The secondary endpoints were included to rule out evidence of egregious harm and to estimate the possible effect size of any favorable differences for the low-dose regimen before undertaking an adequately powered follow-up study given the expectation that the current study would show a large reduction in cumulative prednisone exposure. The primary endpoint of the study was not reached. The unexpectedly smaller reductions in prednisone exposure during the first 42 days of treatment between the two arms of each cohort reflected an evolving practice of rapid prednisone withdrawal in responding patients who were initially given the higher doses. Importantly, they did not reflect prednisone dose-escalations related to treatment-failure in patients who started treatment at the lower doses (Figure 2).

The results of our study are nevertheless consistent with results of the prior retrospective study suggesting that newly diagnosed acute GVHD in most patients can be managed effectively and safely by initiating treatment with prednisone at doses below the current standard of care. For patients presenting with Grade IIa acute GVHD (cohort A), initial treatment with prednisone at
0.5 mg/kg/day (instead of 1 mg/kg/day) was safe and effective. For patients presenting with ≥Grade IIb symptoms (cohort B), initial treatment with a prednisone dose of 1 mg/kg/day (instead of 2 mg/kg/day) was associated with an increased likelihood of requiring secondary immunosuppressive therapy. Although the study was not powered to detect mortality differences, results showed no suggestion that use of the lower dose adversely affected survival. No patient enrolled in the study or diagnosed with acute GVHD during the study period had Grade IV symptoms at the time of initial presentation.

Larger differences in day-42 prednisone exposure might have emerged between the 2 arms if the protocol had specified a schedule for tapering the prednisone dose. The option of making dose-adjustments according to perceived clinical response greatly facilitated the willingness of physicians and patients to participate in the study by reducing concerns about over or under-treatment. This flexibility had the advantage of more accurately matching clinical practice, where dose adjustments are made routinely according to response. Hence, our study design emphasized clinical effectiveness over clinical efficacy.

In patients who presented with mild to moderately severe symptoms (cohort A), initial treatment with a prednisone dose of 0.5 mg/kg/day was effective and did not compromise survival. Slightly greater proportions of patients initially treated with a prednisone dose of 0.5 mg/kg/day required secondary immunosuppressive therapy (12% vs. 8%) or progressed to severe GVHD (10% vs. 6%). Although the study was not powered to detect statistically significant differences in secondary “no harm” endpoints, these differences were statistically not significant. It is important to note that patients with gastrointestinal symptoms in this group were concomitantly treated with topical oral glucocorticoids (usually BDP and budesonide). It is therefore unclear whether initial treatment with a prednisone dose of 0.5 mg/kg/day would have been equally effective without concomitant use of oral topical glucocorticoids. Conversely, one could speculate that
oral topical glucocorticoids alone might be sufficient initial therapy for patients who present with upper gastrointestinal symptoms, diarrhea volumes under 1.0 L/day and skin rash involving less than 50% of the body surface. This important question should be addressed in future studies.

Among patients who presented with ≥Grade IIb GVHD (cohort B), initial treatment with the lower prednisone dose of 1 mg/kg/day was associated with an increased likelihood of requiring secondary systemic immunosuppressive therapy. Although survival did not appear to be adversely affected by use of lower-dose prednisone in this group, it is important to emphasize that the study was not powered to detect such differences. As a rule, prednisone doses were increased before starting non-prednisone secondary therapies in patients initially treated with prednisone at 1 mg/kg/day. The increased need for secondary immunosuppressive therapy per se among patients in this cohort who were randomized to a prednisone dose of 1 mg/kg/day should not be interpreted as a failure of the lower-dose treatment approach. One could argue that initial prednisone-sparing had to be compensated with non-prednisone immunosuppression at a later time, which did not have a measurable negative impact on outcome. Since cumulative prednisone use was recorded only until treatment day 42, it is unclear whether extended follow-up might have shown reduced prednisone utilization among patients initially treated with standard-dose prednisone compared to those initially treated with lower-dose prednisone.

Additional exploratory analyses suggested that patients in cohort B who had skin-predominant symptoms were more likely to require secondary systemic immunosuppressive therapy than those who had gut-predominant symptoms. This finding was unexpected and suggests that GVHD in the two target organs might have differential sensitivity to prednisone. Organ-specific susceptibility to prednisone could be related to differential prednisone-sensitivity of resident donor effector cells. Pro-inflammatory human Th\textsubscript{17} cells, for example, are refractory to
glucocorticoids,\textsuperscript{14} although it is unknown whether these cells are more abundant in the skin than in gastrointestinal mucosa in patients with GVHD.

The expectation that prednisone-associated toxicity could be reduced without causing harm was an important motivation for conducting this trial. The lack of differences in toxicity profiles between patients treated with lower-dose prednisone \textit{versus} those treated with higher-dose prednisone can be explained by the absence of major differences in prednisone exposure during the initial 42 days of treatment (prednisone exposure difference, $\leq 18\%$). The rate of prednisone withdrawal was not dictated by the protocol, an approach that facilitated enrollment in the study. Clinical practice at our institution has evolved toward accelerated withdrawal of prednisone in patients with GVHD manifestations responding to initial treatment at higher doses, which diminished differences in cumulative prednisone exposure. Other institutions might not follow a similarly rapid prednisone taper schema. If so, initial GVHD treatment at lower prednisone doses could prevent toxicity more effectively than observed in our trial. Finally, since our study did not include patients presenting with Grade I acute GHVD (rash involving $<50\%$ of the body surface without liver or gut involvement), our findings cannot be extrapolated to these patients. As illustrated by our comparison with patients who were \textit{not} enrolled in the study, factors beyond clinical grade such as progression kinetics or appearance of a rash clearly affect treatment decisions. Overall, the estimated effect sizes and confidence intervals for secondary endpoints in this study were too small to justify further trials designed to demonstrate statistically significant advantages for the lower dose regimen, and the medical considerations are not of sufficient importance to justify further trials designed to demonstrate non-inferiority of the lower dose regimen.

Results of this study and the previous retrospective study have important practice implications. When gastrointestinal manifestations of GVHD are present, our findings apply only when topical
oral glucocorticoids are given in addition to systemic glucocorticoids. For patients who present with Grade Ila acute GVHD manifestations (“upper gut syndrome”), initial treatment with a prednisone-equivalent dose of 0.5 mg/kg/day is safe and effective. For patients who present with ≥Grade IIb manifestations, initial treatment with a prednisone-equivalent dose of 1 mg/kg/day is associated with an increased likelihood of requiring secondary immunosuppressive therapy without an apparent negative impact on survival. Whether the risk of requiring secondary immunosuppressive therapy is confined to patients with skin-predominant disease, as suggested by our exploratory analysis, needs to be confirmed in future studies. Thus, for patients who present with ≥Grade IIb manifestations, initial treatment with a dose of 2 mg/kg/day followed by a rapid taper should be considered, if the objective is to minimize the need for secondary immunosuppressive therapy. In addition, further validation of prognostic factors that could help identify a priori those patients at risk for fatal GVHD would be of great clinical importance.15-17 Whether initial therapy with regimens that are more immunosuppressive than prednisone alone would yield better outcomes in these patients is unknown and deserves further study.
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AUTHORSHIP

Marco Mielcarek designed the study, analyzed and interpreted data, and wrote the manuscript.
Terrence Furlong assisted with data collection and edited manuscript
Barry E. Storer performed statistical analysis
Margaret L. Green performed analysis of infectious outcomes and edited manuscript
George B. McDonald assisted with data interpretation and edited manuscript
Paul Carpenter assisted with data interpretation and edited manuscript
Mary E. Flowers assisted with data interpretation and edited manuscript
Rainer Storb assisted with data interpretation and edited manuscript
Michael Boeckh performed analysis of infectious outcomes and edited manuscript
Paul J. Martin assisted with study design, data interpretation and edited manuscript
Conflict-on-interest disclosure:

The authors of this manuscript have no conflicts of interest to disclose as described by the journal *Haematologica*. 
REFERENCES

Reference List as of 01-29-2015


# Table 1: Patient and transplant characteristics

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<thead>
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<th>Grade of acute GVHD at onset</th>
<th>Cohort A</th>
<th>Cohort B</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>IIa</td>
<td>IIb-IV</td>
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<tr>
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<td>1.0 mg/kg/d</td>
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<tr>
<td>N</td>
<td>49</td>
<td>53</td>
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<tr>
<td>Patient age, median years (range)</td>
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<td>48 (6-70)</td>
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<tr>
<td>Age &lt;20 years, n (%)</td>
<td>9 (18)</td>
<td>9 (17)</td>
</tr>
<tr>
<td>Age &lt;10 years, n (%)</td>
<td>2 (4)</td>
<td>4 (8)</td>
</tr>
<tr>
<td>Patient sex, female (%)</td>
<td>23 (47)</td>
<td>19 (36)</td>
</tr>
<tr>
<td>Donor type, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-identical related</td>
<td>16 (33)</td>
<td>24 (45)</td>
</tr>
<tr>
<td>HLA-matched unrelated</td>
<td>22 (45)</td>
<td>21 (40)</td>
</tr>
<tr>
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<td>8 (15)</td>
</tr>
<tr>
<td>Stem cell source, n</td>
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<td></td>
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<tr>
<td>Peripheral blood</td>
<td>33 (67)</td>
<td>39 (74)</td>
</tr>
<tr>
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<td>11 (21)</td>
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<td>Preparative regimen, n (%)</td>
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<tr>
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<td>Immunosuppression, n (%)</td>
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<tr>
<td>methotrexate‡</td>
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<td>Other</td>
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<td>9 (17)</td>
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<tr>
<td>Risk of recurrent malignancy, n (%)</td>
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<tr>
<td>Standard</td>
<td>31 (63)</td>
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<tr>
<td>High</td>
<td>18 (37)</td>
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**Organ involvement, n (%)**

<table>
<thead>
<tr>
<th></th>
<th>Skin alone</th>
<th>Gut alone</th>
<th>Skin + gut</th>
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<tr>
<td></td>
<td>-</td>
<td>36 (73)</td>
<td>13 (27)</td>
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<td>33 (62)</td>
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<td>8 (27)</td>
<td>7 (23)</td>
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</tr>
<tr>
<td></td>
<td>9 (30)</td>
<td>10 (33)</td>
<td>11 (37)</td>
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**Grade acute GVHD at onset, n (%)**

<table>
<thead>
<tr>
<th>Grade</th>
<th>IIb</th>
<th>III</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>18 (60)</td>
<td>12 (40)</td>
</tr>
<tr>
<td></td>
<td>17 (57)</td>
<td>12 (43)</td>
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**Time interval, median days (range)**

<table>
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<tr>
<th>Event</th>
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<tbody>
<tr>
<td>Transplant to prednisone</td>
<td>29 (10-88)</td>
</tr>
<tr>
<td>Treatment</td>
<td>33 (17-83)</td>
</tr>
<tr>
<td>GVHD to prednisone treatment</td>
<td>2 (0-43)</td>
</tr>
<tr>
<td>Treatment</td>
<td>2 (0-26)</td>
</tr>
<tr>
<td>Transplant to GVHD</td>
<td>23 (8-87)</td>
</tr>
<tr>
<td>Biopsy confirmation of GVHD, n (%)</td>
<td>36 (73)</td>
</tr>
<tr>
<td>BDP and/or budesonide use, n (%)</td>
<td>47 (96)</td>
</tr>
</tbody>
</table>

GVHD designates graft-versus-host disease; HLA, Human Leukocyte Antigen; MMF, mycophenolate mofetil; BDP, beclomethasone dipropionate; TBI, total body irradiation.

*Myeloablative conditioning: busulfan, (≥ 8.0 mg/kg orally [PO] or ≥ 6.4 mg/kg intravenously [IV]), with cyclophosphamide (120 mg/kg IV) (n=33); TBI (≥ 12 Gy) with cyclophosphamide (120 mg/kg IV) (n=22); fludarabine (120 mg/m² IV) with treosulfan (42 g/m² IV) (n=21); other (n=20).

†Reduced-intensity conditioning: TBI (2-4 Gy) and fludarabine (90 mg/m²) (n=48); cyclophosphamide (≥ 100 mg/kg), fludarabine (90 mg/m²) and TBI (2-4 Gy) (n=7); TBI only (2 Gy) (n=3); other (n=8).

‡Cyclosporine or tacrolimus twice daily PO or IV (whole blood target levels, 120-360 ng/mL and 5-15 ng/mL, respectively) from day -1 until day +50. In the absence of GVHD, calcineurin-inhibitors were tapered from day +50 through day +180; methotrexate IV at a dose of 15 mg/m² on day +1 and at 10 mg/m² on days +3, +6 and +11.

§Cyclosporine or tacrolimus twice daily PO or IV (whole blood target levels, 120-360 ng/mL and 5-20 ng/mL, respectively) from day -3 until day +180. In the absence of GVHD, calcineurin-inhibitors were tapered from day +56 through day +180; MMF, 15mg/kg PO twice (related donors) or thrice (unrelated donors) daily, from day 0 to day +27. For recipients of unrelated grafts, MMF prophylaxis was typically extended until 40 to 180 days after HCT.

¶One patient (3%) had gut and liver involvement at presentation.
In cohort A, the proportion of patients without diarrhea was 37% (18 of 49) among those randomized to low-dose prednisone and 40% (21 of 53) among those randomized to standard-dose prednisone.

Longer time intervals between GVHD diagnosis and initiation of treatment reflect patients with indolent manifestations that did not require systemic immunosuppressive therapy at initial diagnosis.
FIGURE LEGENDS

Figure 1: Stratification and randomization. During the enrollment period for the study from April 2009 to May 2013, 737 patients were newly diagnosed with ≥ Grade IIa acute GVHD. One-hundred and sixty-four patients (22%) were enrolled and stratified according to severity of manifestations at symptom-onset. Patients in cohort A with Grade IIa-manifestations (upper gastrointestinal symptoms of anorexia, nausea, vomiting attributed to acute GVHD, with stool volumes < 1 L/day, rash involving < 50% of the body surface, and no hepatic dysfunction) were randomized to start GVHD-therapy with a prednisone-equivalent dose of either 1 or 0.5 mg/kg/day. Those in cohort B with ≥Grade IIb-manifestations (rash involving ≥ 50% of the body surface, stool volumes ≥ 1 L/day or hepatic involvement with total serum bilirubin > 2 mg/dL) were randomized to start treatment with a prednisone-equivalent dose of either 2 or 1 mg/kg/day. Randomization was further stratified on recurrence risks of underlying malignancies (standard vs. high) and pre-transplant comorbidity (hematopoietic cell transplant comorbidity index, 0-1 vs. ≥ 2). Two patients were randomized within the incorrect stratum and were allocated to doses for which they were not eligible, leaving 162 patients (Grade IIa manifestations, n=102; ≥Grade IIb-manifestations, n=60) who initiated prednisone treatment according to study. Twelve patients could not be included in the analysis of the primary endpoint (departure from Center before treatment day 42, n=7; study withdrawal, n=2; death before treatment day 42, n=3).

Figure 2: Primary endpoint: prednisone use according to initial dose and GVHD-Grade at onset of symptoms. (A) Patients who presented with Grade IIa-manifestations (cohort A; n=91); initial prednisone dose, 0.5 mg/kg/day (solid line) or 1 mg/kg/day (dashed line). (B) Patients who presented with ≥Grade IIb-manifestations (cohort B; n=59); initial prednisone dose,
1 mg/kg/day (solid line) or 2 mg/kg/day (dashed line). The graphs show mean prednisone doses per day until day 42 after starting treatment. Only patients who completed 42 days of prednisone treatment were included in the cumulative dose analysis (n=150).

**Figure 3: Pre-specified secondary “no harm” endpoints according to initial treatment with lower-dose or higher-dose prednisone.** (A) Kaplan-Meier estimates of overall survival after initiation of prednisone therapy among all patients, (B) among those in cohort A, and (C) among those in cohort B. (D) Cumulative incidence of non-prednisone secondary systemic immunosuppressive therapy after initiation of prednisone therapy among all patients, (E) among those in cohort A, and (F) among those in cohort B. Secondary systemic immunosuppressive therapy in cohort B included mycophenolate mofetil (n=6), ATG (n=3), sirolimus (n=2), alemtuzumab (n=1), or etanercept (n=1). 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).

**Figure 4: Additional secondary “no harm” endpoints according to initial treatment with lower-dose or higher-dose prednisone.** (A) Cumulative incidence of non-relapse mortality among all patients, (B) among those in cohort A, and (C) among those in cohort B after initiation of prednisone therapy. (D) Cumulative incidence of relapse and (E) chronic GVHD requiring systemic immunosuppressive therapy among all patients after initiation of prednisone therapy. 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).
Figure 1, Mielcarek et al.

Patients with newly diagnosed Grade IIa acute GVHD during study period 4/2009 – 5/2013
N = 737

Not enrolled *
N = 573

Enrolled
(and stratified according to GVHD-severity at symptom-onset)
N = 164

Stratification

Grade IIa
(Cohort A)
N = 104

Randomized to initial daily prednisone dose:

<table>
<thead>
<tr>
<th></th>
<th>0.5 mg/kg</th>
<th>1.0 mg/kg</th>
<th>1.0 mg/kg</th>
<th>2.0 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Randomized, n</td>
<td>51</td>
<td>53</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Received study treatment and evaluable, n</td>
<td>49</td>
<td>53</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Receive study treatment and inevaluable, n</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Reason: incorrect stratification, n</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Grade IIb-VI
(Cohort B)
N = 60

Analyzed for primary endpoint, n
44 47 29 30

Excluded from primary endpoint analysis, n
5 6 1 0

Reasons:
- Early departure from Center, n | 4 | 3 | 0 | 0 |
- Study withdrawal, n | 0 | 2 | 0 | 0 |
- Death before day 42, n | 1 | 1 | 1 | 0 |

*Reasons: Patient preference including unwillingness to stay at transplant center until treatment day 42, attending physician preference, not meeting inclusion criteria.
Figure 3, Mielcarek et al.

A  
Percent Survival  
HR 1.02 (95% CI, 0.6–1.74)  
Months from Initial Therapy

B  
Percent Survival  
Months from Initial Therapy

C  
Percent Survival  
Months from Initial Therapy

D  
Percent Secondary Therapy  
HR 0.29 (95% CI, 0.12–0.74)  
Days from Initial Therapy

E  
Percent Secondary Therapy  
Days from Initial Therapy

F  
Percent Secondary Therapy  
Days from Initial Therapy
Figure 4, Mielcarek et al.
SUPPLEMENTAL MATERIAL (ONLINE ONLY)

FOR

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

Marco Mielcarek,¹,² Terrence Furlong,¹ Barry E. Storer,¹,² Margaret L. Green,²,³ George B. McDonald,¹,² Paul A. Carpenter,¹,² Mary E.D. Flowers,¹,² Rainer Storb,¹,² Michael Boeckh,¹,² and Paul J. Martin¹,²

LIST OF SUPPLEMENTAL ITEMS

1) Supplemental Methods

2) Supplemental Table S1: Cumulative incidence of invasive infections between start of prednisone treatment for GVHD and day 100 after transplant according to initial prednisone dose.

3) Supplemental Table S2: Characteristics of patients enrolled and of those not enrolled in the study.

4) Supplemental Figure S1: Measures of prednisone-associated toxicity among all patients according to initial treatment with lower-dose and higher-dose prednisone.

5) References for Supplemental Material
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/m²/day for 3 consecutive days) and fludarabine (30 mg/m²/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. Reduced-intensity conditioning regimens included low-dose TBI (2–4 Gy) alone or in combination with fludarabine (30 mg/m²/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. Details describing the institutional supportive care have been published previously.

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. 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. 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

<table>
<thead>
<tr>
<th>Organism</th>
<th>Lower-dose prednisone (^a)</th>
<th>Higher-dose prednisone (^a)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients, (n)</td>
<td>79</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>Invasive infections during first 100 days of prednisone treatment (^b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any infection, %</td>
<td>52</td>
<td>53</td>
<td>0.89</td>
</tr>
<tr>
<td>Any invasive bacterial infection (^c), %</td>
<td>22</td>
<td>16</td>
<td>0.34</td>
</tr>
<tr>
<td>Gram-negative bacteremia (^d)</td>
<td>11</td>
<td>4</td>
<td>0.06</td>
</tr>
<tr>
<td>Gram-positive bacteremia (^e,f)</td>
<td>10</td>
<td>11</td>
<td>0.88</td>
</tr>
<tr>
<td>Anaerobic bacteremia (^g)</td>
<td>4</td>
<td>0</td>
<td>0.07</td>
</tr>
<tr>
<td>Pneumonia (^h)</td>
<td>3</td>
<td>2</td>
<td>0.96</td>
</tr>
<tr>
<td>C. difficile associated diarrhea (^l)</td>
<td>5</td>
<td>1</td>
<td>0.16</td>
</tr>
<tr>
<td>Invasive fungal infection (^j) (proven/probable), %</td>
<td>5</td>
<td>5</td>
<td>0.94</td>
</tr>
<tr>
<td>Any viral infection (^k), %</td>
<td>39</td>
<td>47</td>
<td>0.32</td>
</tr>
<tr>
<td>CMV-PCR positivity, any level (^l)</td>
<td>39</td>
<td>43</td>
<td>0.59</td>
</tr>
<tr>
<td>CMV-PCR positivity, &gt;500 copies/mL (^l)</td>
<td>10</td>
<td>11</td>
<td>0.88</td>
</tr>
<tr>
<td>CMV-disease (^m)</td>
<td>4</td>
<td>4</td>
<td>0.95</td>
</tr>
<tr>
<td>Viral lower respiratory tract infection (^n,o)</td>
<td>3</td>
<td>4</td>
<td>0.69</td>
</tr>
</tbody>
</table>

\(^a\) “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.

bFirst episode in each category is shown.

cInvasive bacterial disease was defined as previously published\textsuperscript{11} and included end organ disease and bacteremia.

dIncident episodes of gram-negative bacteremia (n): Lower dose group- 1 \textit{Escherichia coli}, 2 \textit{Klebsiella pneumoniae}, 2 \textit{Klebsiella oxytoca}, 2 \textit{Pseudomonas spp.}, 1 \textit{Serratia marcescens}, 1 \textit{Haemophilus sp.}, 1 \textit{Delftia Acidovorans}; Higher dose group- 1 \textit{Klebsiella pneumoniae}, 1 \textit{Serratia marcescens}, 1 \textit{Pseudomonas spp.}

eBacteremia due to coagulase negative \textit{Staphylococci} was excluded.


gIncident episodes of anaerobic bacteremia (n): Lower dose group- 2 \textit{Bacteroides fragilis}, 1 \textit{Actinomyces odontolyticus}; Higher dose group- none.

hEtiologies of incident bacterial pneumonias isolated from BAL (n): Lower dose group- 1 \textit{Pseudomonas aeruginosa}, 1 \textit{Haemophilus sp.}; Higher dose group 1 \textit{Stenotrophomonas maltophilia}, 1 \textit{Staphylococcus aureus}.

i\textit{C. difficile} infections only recorded during the first 42 days of therapy.

jInvasive fungal disease was defined according to international guidelines (n):\textsuperscript{12} Lower dose group- 4 fungal pneumonia- 1 \textit{Malassezia restricta}, 3 \textit{Aspergillus spp.} and 0 fungemia; Higher dose group- 3 fungal pneumonia- all \textit{Aspergillus spp.} and 1 episode of fungemia due to \textit{Candida glabrata}. 
Viral infections include CMV reactivation, CMV disease, or viral lower respiratory tract infections.

Plasma DNA PCR was performed weekly.\textsuperscript{13}

CMV disease was defined according to standard guidelines.\textsuperscript{14}

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.

Pathogens identified as causing LRTI were: \textit{Lower dose group}- 1 Rhinovirus, 1 Parainfluenza virus Type 2; \textit{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

<table>
<thead>
<tr>
<th></th>
<th>Random sample of eligible patients not enrolled in the study</th>
<th>Enrolled in protocol 2327</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade IIa</td>
<td>Grade IIb-IV</td>
</tr>
<tr>
<td>Number of patients, ( n )</td>
<td>94</td>
<td>57</td>
</tr>
<tr>
<td>Proportion of total, %</td>
<td>62</td>
<td>38</td>
</tr>
<tr>
<td>Age, median (range)</td>
<td>47 (0-73)</td>
<td>46 (0-69)</td>
</tr>
<tr>
<td>Patient sex, female (%)</td>
<td>34 (36)</td>
<td>21 (37)</td>
</tr>
<tr>
<td>Donor type, ( n ) (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-identical related</td>
<td>36 (38)</td>
<td>5 (9)</td>
</tr>
<tr>
<td>HLA-matched unrelated</td>
<td>41 (44)</td>
<td>36 (63)</td>
</tr>
<tr>
<td>HLA-mismatched</td>
<td>17 (18)</td>
<td>16 (28)</td>
</tr>
<tr>
<td>Nonmyeloablative regimen, ( n ) (%)</td>
<td>28 (30)</td>
<td>18 (32)</td>
</tr>
<tr>
<td>GVHD organ involvement*, ( n ) (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin alone</td>
<td>3 (3)</td>
<td>36 (63)</td>
</tr>
<tr>
<td>Gut alone</td>
<td>65 (69)</td>
<td>6 (11)</td>
</tr>
<tr>
<td>Skin + gut</td>
<td>25 (27)</td>
<td>13 (23)</td>
</tr>
<tr>
<td>Neither</td>
<td>1 (1)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Grade III at onset, ( n ) (%)</td>
<td>--</td>
<td>6 (11)</td>
</tr>
<tr>
<td>HCT to therapy, median (range)</td>
<td>35 (13-118)</td>
<td>23 (7-171)</td>
</tr>
<tr>
<td>GVHD to therapy, median (range)</td>
<td>2 (0-55)</td>
<td>3 (0-82)</td>
</tr>
<tr>
<td>Received BDP, ( n ) (%)</td>
<td>81 (90)</td>
<td>33 (58)</td>
</tr>
</tbody>
</table>

*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. 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).
REFERENCES FOR SUPPLEMENTAL MATERIAL


