Evaluation of a CD5-Specific Immunotoxin for Treatment of Acute Graft-Versus-Host Disease After Allogeneic Marrow Transplantation


Acute graft-versus-host disease (GVHD) is most often treated with high dose glucocorticoids, but less than half of patients have durable overall improvement. Previous phase I and phase II studies suggested that treatment with a CD5-specific immunotoxin (XomaZyme-CD5 Plus) could ameliorate symptoms of GVHD. In a randomized, double-blind trial, we compared XomaZyme-CD5 Plus and glucocorticoids versus placebo and glucocorticoids as initial therapy for 243 patients who developed acute GVHD after allogeneic marrow transplantation. The study drug (XomaZyme CD5-Plus or an identical appearing placebo) was administered at a dose of 0.1 mg/kg body weight on each of 14 consecutive days. All patients were treated concomitantly with a standard regimen of methylprednisolone. At the time of entry on study, 94% of patients had a rash, 56% had hyperbilirubinemia, 61% had diarrhea, and 84% had nausea and vomiting. At 3, 4, and 5 weeks after starting treatment, symptom severity was less in the CD5 group than in the placebo group.

At 4 weeks, 40% of patients assigned to the CD5 group had complete response compared with 25% of those assigned to the control group (P = .019). At 6 weeks, 44% of patients assigned to the CD5 group had complete response as compared with 38% in the placebo group (P = .36). Clinical extensive chronic GVHD developed in 65% of patients in the CD5 group compared with 72% in the control group (P = .35). Survival at 1 year after treatment was 49% in the CD5 group and 45% in the control group (P = .88). Side effects required close monitoring and appropriate adjustment of treatment. The combined administration of a CD5-specific immunotoxin and glucocorticoids controls GVHD manifestations more effectively than treatment with glucocorticoids alone during the first 5 weeks after starting treatment. Use of this immunotoxin does not result in any long-term clinical benefit for patients with acute GVHD.

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Materials and Methods

Enrollment. Patients were eligible to participate in this study when they developed acute GVHD manifested by (1) a characteristic rash involving over 50% of the body surface; (2) rash of any extent together with visceral involvement as indicated by increasing serum bilirubin >2.0 mg/dL, persistent nausea and vomiting, or diarrhea >500 mWd (>30 mL/kg in children); or by (3) visceral involvement in the absence of rash but confirmed by biopsy. Patients were excluded if they had received T-cell-depleted marrow or more than one marrow transplant or had any prior treatment for acute GVHD. Also excluded were patients who had acute life-threatening illness other than GVHD. Randomization was carried out after obtaining written informed consent using forms approved by the Institutional Review Board. Assignment to one of the two treatment arms was made by computerized biased coin adaptive randomization in order to ensure that the two treatment arms were balanced with respect to risk factors known or suspected to influence the response to treatment.

Treatment. All patients were administered methylprednisolone at a dose of 1.0 mg/kg twice daily for 18 days. When tolerated, oral prednisone was substituted for intravenous methylprednisolone with appropriate adjustment in the dose. Beginning on the 19th day, the dose of prednisone was decreased by 0.2 mg/kg body weight every 5 days in patients who had no clinical evidence of active GVHD. No tapering of glucocorticoid doses was allowed until symptoms of acute GVHD had resolved. The taper schedule was suspended whenever symptoms of acute GVHD recurred and was resumed when symptoms resolved.

The study drug (XomaZyme CD5-Plus or an identical appearing...
placebo) was supplied by Xoma Corp (Berkley, CA) and was adminis-
tered daily as a 1 hour intravenous infusion at a dose of 0.1 mg/ kg body weight. Administration of the study drug was continued for a total of 14 doses regardless of clinical response or secondary therapy; it was suspended per protocol whenever there was evidence that it might be causing moderate or severe organ toxicity, hypoal-
buminemia, or renal impairment. Resumption of treatment with the study drug was allowed if toxicity improved within 3 days. Other-
wise, it was discontinued.

Medications originally administered for GVHD prophylaxis were continued throughout treatment of GVHD unless there was toxicity or contraindication. When renal toxicity occurred, priority was given to continuing the administration of cyclosporine prophylaxis rather than the study drug. Protocol guidelines for starting secondary treat-
ment of acute GVHD included (1) worsening of symptoms for at least 3 days; (2) unimproving grades III-IV GVHD persisting for at least 1 week despite treatment; or (3) unimproving grade II GVHD persisting for at least 2 weeks despite treatment (see ref 18 for grading of GVHD).

Symptom severity. Measurements of rash, serum bilirubin, aver-
age daily stool volume (3 day measurements for inpatients with diar-
aea), and serum creatinine were recorded weekly through week 6 after starting treatment for GVHD and at the time of departure from Seattle. Additional weekly notations recorded the presence of visible blood in the stool or symptoms of nausea, vomiting, or abdomi-
dral cramping. All abnormalities were recorded whether caused by GVHD or by other complications.

Grading of clinical symptom severity was patterned after the sys-
tem described by Glucksberg et al.18 For all organs, stage 0 indicates normal function. Skin severity was categorized according to the extent of rash and presence of bullae: stage 1, <25% of the skin surface area; stage 2, 26% to 50%; stage 3, >50%; stage 4, bulla formation. Liver severity was categorized according to the serum total bilirubin concentration: stage 1, 2.9 mg/dL; stage 2, 3 to 5.9 mg/dL; stage 3, 6 to 14.9 mg/dL; stage 4, >15 mg/dL. Gut severity was categorized according to a scoring system for symptoms of diarrhea, cramps, and visible blood in the stool. Symptoms were evaluated on the day of enrollment (day 1) and the preceeding days, and on days 6-8, 13-15, 20-22, 27-29, 34-36, and 41-43. Scores of 1, 2, and 3 were assigned, respectively, for diarrhea with average daily volumes <1,000 mL, 1,000-1,499 mL, and >1,500 mL (<556 mL/m² body surface area, 556-833 mL/m², and >833 mL/m² in children with body surface area <1.0 m²). A score of 2 was assigned when abdominal cramps were present and also when visible blood was present in the stool. Overall stage 1 gut severity was assigned for a total symptom score of 1 and for patients whose symptoms were limited to nausea and vomiting. Overall stages 2, 3, and 4 were assigned, respectively, for total gut symptom scores of 2, 3-4, and 5-7. Diarrhea volumes containing admixed urine were not included in calculating the 3-day average. If urinary mixing was present on all 3 days, a score of 1 was assigned when the average daily volume was <1,000 mL (<556 mL/m² in children), and no evaluation of gut severity could be made when the average daily volume was >1,000 mL (>556 mL/m² in children).

Grade I overall symptom severity was defined as stage 1-2 skin severity with stage 0 liver and gut severity. Grade II overall symptom severity was defined as stage 3 skin severity or stage 1 liver or gut severity. Grade III overall symptom severity was defined as stage 2-3 liver or gut severity. Grade IV overall symptom severity was assigned for patients with stage 4 symptom severity in the skin, gut, or liver.

Assessment of response. Before the study blind was opened, outcome after treatment was evaluated by two methods, each with a different approach for incorporating the confounding effects of complications other than GVHD. The first method assigned response categories solely according to changes in symptom severity without considering whether abnormalities were caused by GVHD or by other complications. Skin disease was considered improved when there was resolution of rash or decrease in the involved surface area by ≥25 percentage points. Progressive skin disease was defined as an increase in the involved surface area by ≥25 percentage points.

Liver disease was considered improved when there was a decrease in serum bilirubin to <2 mg/dL for patients with baseline values of 2 to 4 mg/dL, a decrease of ≥2 mg/dL for patients with baseline values of 4 to 8 mg/dL, or a ≥25% decrease in serum bilirubin for patients with baseline values ≥8 mg/dL. Progressive liver disease was defined as an increase in serum bilirubin by ≥2 mg/dL for patients with baseline values <8 mg/dL or ≥25% increase in serum bilirubin for patients with baseline values ≥8 mg/dL. Because an increase or decrease in serum bilirubin can reflect altered renal function, improvement and progression of liver disease were not scored when the direct serum bilirubin and serum creatinine both increased or decreased such that the direct bilirubin:creatinine ratio was changed by <25%.10,11 Likewise, liver disease was considered non-
evaluable when a "stable" bilirubin was accompanied by an increase or decrease in serum creatinine such that the direct bilirubin:creati-
nine ratio was changed by >25%. In this situation, an improvement or deterioration in hepatic function could have been masked by an opposite change in renal function.

Gut disease was considered improved when there was resolution of diarrhea or decrease in the 3-day average stool volume by ≥500 mL/day with clearing of cramps and bleeding if present. Clearing of cramps and bleeding was considered as evidence of improvement in patients without diarrhea but not in patients with persistent diarr-
hea. Gut disease was also considered improved when nausea and vomiting resolved in patients who had these symptoms as the only gut abnormalities. Progressive gut disease was defined as an increase in the 3-day average stool volume by ≥500 mL/day or the development of new cramps or bleeding. Stool volumes were not considered when urinary mixing was present. Factors such as the platelet count and the amount of oral intake and anti-diarrhea medications were not taken into account regarding their effect on stool blood and diarrhea volume.

For all organs, assessment of treatment response was made en-
tirely by clinical criteria regardless of biopsy or autopsy findings. Overall complete response (CR) was defined as the absence of symp-
toms referable to GVHD in all organs. Partial response (PR) was defined as an improvement in at least one organ without deterioration in others.

In the second method for assessment of response, an expert re-
viewer evaluated results at 4 and 6 weeks after treatment in light of clinically relevant findings other than GVHD. Patients with abnor-
malities involving the skin, liver, or gut at 4 or 6 weeks could none-
thless be assigned as having a CR by this "global assessment" when the abnormalities were restricted to a single organ and were not present in preceding or subsequent evaluations, and a cause other than GVHD could be implicated.

Toxicity. For purposes of toxicity evaluation, renal impairment was defined as a serum creatinine concentration >0.6 mg/dL (53 µmol/L) for patients with baseline values <0.3 mg/dL (27 µmol/L), an increase to twice the baseline value for patients with serum creatinine concentration of 0.3 to 1 mg/dL (27-88 µmol/L) at the beginning of treatment, or a serum creatinine concentration ≥2 mg/ dL (177 µmol/L) for patients with baseline values >1 mg/dL (88 µmol/L). Methods for measurement of the IgG response to immuno-
toxin have been described previously.20 A positive response was defined as a 10-fold increase above the baseline titer as determined by the enzyme immunoassay.

Analysis. The prespecified primary endpoint for the study was a comparison of the proportion of patients assigned to each group
who were alive with CR at 6 weeks after starting treatment. Secondary endpoints were comparisons of CR at 4 weeks, global assessment of CR at 4 and 6 weeks, CR or PR at 4 and 6 weeks, time to treatment failure defined as secondary treatment for acute GVHD or death from any cause, all-cause mortality, death with infection, clinical extensive chronic GVHD, and renal impairment.

Before comparing treatments, the donor category (HLA-identical relative versus unrelated donor or HLA-mismatched relative), GVHD prophylaxis (methotrexate plus cyclosporine versus other), onset day of GVHD and baseline serum total bilirubin concentration were tested for association with the primary endpoint of complete response at 6 weeks. Only the baseline serum bilirubin concentration showed a significant (inverse) association with this endpoint. Symptom severity scores were compared at each time point with the Wilcoxon rank sum test and across time points by using rank analysis of covariance with adjustment for baseline serum bilirubin level. Response rates were analyzed by logistic regression with adjustment for baseline serum bilirubin level. Survival and times to secondary therapy, treatment failure, and chronic GVHD were analyzed by the log rank test. The frequencies of renal impairment and death with infection were compared with the Chi-square test.

RESULTS

Patients and symptom severity. Demographic characteristics and initial symptom severity were similar among the 129 patients assigned to receive CD5-Plus and the 114 patients assigned to receive placebo (Table 1 and Fig 1). The proportion of evaluable patients relatively free of symptoms (ie, those with grades 0 or 1 symptom severity) in the CD5 group increased from 1% at the beginning of treatment to 15%, 20%, 30%, 35%, 35%, and 44% during the 6 successive weeks after treatment (Fig 1). The proportion of evaluable patients in the placebo group increased from 0 at the beginning of treatment to 1%, 2%, 6%, 13%, 15%, 16%, and 25% during the same time interval. At 3, 4, and 5 weeks after starting treatment, symptom severity was less in the CD5 group than in the placebo group. At 6 weeks, symptom severity was again similar in the two groups. Symptom severity across the entire 6-week study period was lower in the CD5 group than in the placebo group ($P = .033$).

Response categories. At 4 weeks, 51 (40%) of the 127 evaluable patients assigned to the CD5 group were alive with a CR compared with 28 (25%) of the 114 in the control group ($P = .019$) (Fig 2). Two patients in the CD5 group left Seattle before day 29 and could not be formally evaluated. One of the two had no clinical evidence of acute GVHD at the time of departure. At 6 weeks, 54 (44%) of the 123 evaluable patients assigned to the CD5 group were alive with a CR, compared with 43 (38%) of 114 in the control group ($P = .36$). Six patients in the CD5 group could not be evaluated at this time, one because of incomplete data, and five because they had already left Seattle. Five of the six had no clinical evidence of acute GVHD at the time of the last complete assessment. The proportions of patients with CR or PR remained relatively constant throughout the period between 2 and 6 weeks after starting treatment (Fig 2).

Global assessment. Preexisting or new complications affecting the skin, liver, or gut persisted or developed during treatment for GVHD in 74% of patients. Skin complications other than GVHD were identified in 7% of patients, liver complications in 60%, and gastrointestinal complications in 32%, with no significant differences between the CD5 group and the placebo group (data not shown).

After accounting for complications other than GVHD, the proportion of patients with CR at 4 weeks after treatment was higher in the CD5 group than in the placebo group (Fig 3). A similar trend was evident at 6 weeks. Among the 71 evaluable patients in the CD5 group who did not have CR at 4 weeks, 20 (28%) had CR at 6 weeks. Among 81 patients in the control group who did not have CR at 4 weeks, 18 (22%) had CR at 6 weeks. Ten (18%) of the 57 patients in the CD5 group who had CR at 4 weeks were no longer in CR at 6 weeks. Three had a major recurrence of GVHD.
Fig 1. Symptom severity distributions before and after treatment with XomaZyme-CD5 Plus or placebo together with high dose glucocorticoids. Column heights indicate the proportions of evaluable patients with stage 0 ( ), I ( ), II ( ), or IV ( ) symptom severity and the proportions of patients who had died ( ) after treatment with CD5-Plus (left side of each pair) or placebo (right side of each pair). Proportions through week 6 are based on the number of evaluable patients in each group, including those who had died. At each time point, it was not possible to determine symptom severity for certain patients. Most often this occurred because the stool volume could not be measured in patients with diarrhea as the deciding factor in determining symptom severity. Departure evaluations were generally performed at 80 days (range, 62 to 136 days) after transplantation. Only the departure evaluations performed after the sixth week of treatment for GVHD are included in the analysis. Because the time interval from starting treatment for GVHD to the departure evaluation was not uniform (range, 44 to 104 days), no statistical analysis is given for the comparison of symptom severity distributions in the two groups at the departure evaluation, and proportions for the departure evaluation do not include patients who had died.

Fig 2. CR or PR after treatment with XomaZyme-CD5 Plus or placebo together with high dose glucocorticoids. The filled part of each column and the lower percentage indicate the proportion of patients with CR, whereas the open part indicates the proportion with PR. The upper percentage indicates the proportion with CR or PR. Percentages are based on the number of evaluable patients in each group, including those who had died. Reasons for inability to assess all patients are described in Results. See Fig 1 legend for explanation of the departure evaluation.

Fig 3. Global assessment of CR after treatment with XomaZyme-CD5 Plus or placebo together with high dose glucocorticoids. Percentages are based on the number of evaluable patients in each group, including those who had died.
edema and weight gain, and weakness or increased serum CPK levels. One patient treated with CD5-Plus had severe rhabdomyolysis complicated by acute tubular necrosis.

Certain adverse events were notable in the CD5 group. Marked weight gain with severe edema sometimes accompanied by dyspnea occurred in 17% of patients in the CD5 group, compared with 9% in the placebo group. Overall, 10% of patients in the CD5 group had muscle weakness, compared with 5% in the placebo group. One patient in the CD5 group developed an Epstein-Barr virus-related lymphoproliferative syndrome. Human antibody against murine immunoglobulin was detected in 15 (16%) of 93 patients tested in the CD5 group, compared with none of 93 in the placebo group. Renal impairment occurred in 48% of patients in the CD5 group compared with 40% in the placebo group (P = .25). The incidence and severity of hypoaalbuminemia were comparable in the two groups (data not shown). In most cases, side effects other than myopathy were transient and resolved after treatment with immunotoxin was completed.

No significant differences between groups were found in the incidence of infections categorized by organism and site (data not shown). The proportions of patients with absolute neutrophil counts less than 0.5 × 10^9 per liter and absolute lymphocyte counts in the blood during successive weeks after treatment were similar in the CD5 group and in the placebo group (data not shown).

**DISCUSSION**

This report shows that treatment with a CD5-specific immunotoxin is effective in alleviating the symptoms of acute GVHD when added to a conventional regimen of high dose glucocorticoids. Differences between the CD5 group and the placebo group were most apparent between 3 to 5 weeks after starting treatment when there was approximately a 15 percentage point advantage in the proportion of patients with complete resolution of symptoms in the CD5 group. The proportions of patients who had CR or PR were similar in the two groups, indicating that treatment with the immunotoxin did not increase the number of patients with overall improvement.

The optimal timing for assessment of response was not known at the time the study was designed. Results of the
present study underscore the desirability of assessing the entire clinical course after treatment for GVHD because the time course of improvement after treatment for GVHD was different in the CDS group and in the placebo group. The initial rate of improvement was much faster in the CDS group than in the placebo group, but this difference did not persist after the fifth week. No consistent pattern of organ response was found to explain the delayed improvement among patients in the placebo group.

Inflammatory cytokines including interferon-γ and tumor necrosis factor-α play a prominent role in causing tissue injury during GVHD.22 Glucocorticoids inhibit transcription of cytokine genes by interfering with NF-κB and AP-1 promoter mechanisms in activated cells.23-25 Among other actions, glucocorticoids inhibit some effects of interferon-γ on macrophages26,27 and prevent the release of tumor necrosis factor when macrophages are activated.28 In addition, glucocorticoids impair the production of interleukin-2 (IL-2) by activated T cells29 and inhibit clonal expansion of T cells.30 Treatment with glucocorticoids might also eliminate activated T cells,31 but symptoms of GVHD recur if glucocorticoid doses are tapered too rapidly,12 suggesting that not all activated T cells are sensitive to glucocorticoids.

The use of a T-cell–specific immunotoxin for treatment of GVHD was based on the supposition that the T cells responsible for initiating the disease would be eliminated by this treatment. Even though CD5-specific antibodies are rapidly internalized after binding to T cells, most CD5-specific ricin A chain immunoconjugates are not highly cytotoxic in the absence of potentiators such as ammonium chloride or chloroquine which retard delivery of endosomes to lysosomes where proteolytic degradation occurs.13-32 Previous studies have shown that treatment with CD5-Plus can cause transient reductions in the number of circulating T cells, although the mechanisms for this effect have not been fully elucidated.33-35 Reasons for the absence of lymphopenia induced by treatment with CD5-Plus in the present study remain unclear. Lymphopenia associated with GVHD and glucocorticoid treatment could have obscured effects of the immunotoxin on the number of circulating lymphocytes.

For the most part, the side effects associated with the use of CD5-Plus were manageable. Allergic reactions resolved when treatment was discontinued after the first dose. Weight gain, edema, and dyspnea could be managed in most patients by treatment with diuretics. After the initial case of rhabdomyolysis, serum CPK levels were monitored frequently in all patients, and treatment was discontinued in patients with a pattern of increasing values above the upper limit of normal. Even with this precaution, it is possible that treatment with CD5-Plus might exacerbate myopathy caused by glucocorticoids. The incidence of infection was not increased by treatment with the immunotoxin, consistent with observations that CD5-Plus did not cause either neutropenia or lymphopenia.

Treatment of GVHD with high dose glucocorticoids causes considerable morbidity. Hypertension, hyperglycemia, and infections occur in nearly all patients. In the present study, we made no effort to accelerate the withdrawal of glucocorticoids in patients who had a CR after treatment.
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