Prophylactic rituximab after allogeneic transplantation decreases B-cell alloimmunity with low chronic GVHD incidence

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B cells are involved in the pathogenesis of chronic GVHD (cGVHD). We hypothesized that prophylactic anti–B-cell therapy delivered 2 months after transplantation would decrease alloimmune donor B-cell immunity and possibly the incidence of cGVHD. Therefore, in the present study, patients with high-risk chronic lymphocytic leukemia (n = 22) and mantle-cell lymphoma (n = 13) received a total lymphoid irradiation of 80 cGy for 10 days and antithymocyte globulin 1.5 mg/kg/d for 5 days. Rituximab (375 mg/m²) was infused weekly on days 56, 63, 70, and 77 after transplantation. The incidence of acute GVHD was 6%. The cumulative incidence of cGVHD was 20%. Nonrelapse mortality was 3%. Rituximab treatment after allogeneic transplantation significantly reduced B-cell alloimmune immunity, with complete prevention of alloreactive H-Y Ab development in male patients with female donors (P = .01). Overall survival and freedom from progression at 4 years for chronic lymphocytic leukemia patients were 73% and 47%, respectively; for mantle-cell lymphoma patients, they were 69% and 53%, respectively. This study is registered at www.clinicaltrials.gov as NCT00186628. (Blood. 2012;119(25): 6145-6154)

Introduction

Chronic GVHD (cGVHD) remains a significant cause of late morbidity and mortality after allogeneic hematopoietic cell transplantation (alloHCT). However, strategies to prevent cGVHD have been largely disappointing.1-4 Although traditionally thought to be mediated by alloreactive T lymphocytes,5-6 increasing evidence supports a role for B cells in the pathogenesis of cGVHD.7 Autoantibody and alloantibody associations with cGVHD have also been reported.8 Specifically, alloreactive Abs against H-Y antigens9-10 and coordinated B- and T-cell responses11 were found to be strongly associated with the occurrence of cGVHD in sex-mismatched alloSCT. A murine study demonstrated that alloimmune Abs deposit in cGVHD-affected tissues, and cGVHD was prevented when the donor graft was genetically prevented from secreting IgG.12 Other evidence comes from studies showing dysregulated B-cell reconstitution13 and increased B-cell activating factor levels in cGVHD patients.14 B cells collected from cGVHD patients were more responsive to TLR-9 signaling and exhibited increased CD86 expression.15 Furthermore, established steroid-refractory cGVHD patients have reduced numbers of naive B cells and increased activated CD27+ B cells,13,14 further supporting a role for B cells in cGVHD pathogenesis. Clinically, anti–B-cell–directed therapy with rituximab has been shown to be an effective treatment for established cGVHD, with several studies reporting clinical response rates of 40%-70% in steroid-refractory cases.16-20 Evidence for the potential use of rituximab as cGVHD prophylaxis comes from clinical observations that rituximab added to fludarabine and cyclophosphamide conditioning resulted in a low rate of cGVHD in 10 chronic lymphocytic leukemia (CLL) patients.21 Others have shown a decrease in acute GVHD (aGVHD) and/or cGVHD in patients with B-cell malignancies treated with rituximab within 6 months after alloHCT.22-23

These findings suggest that rituximab depletion of donor B cells after alloHCT may reduce cGVHD. We hypothesized that prophylactic anti–B-cell therapy with rituximab after alloHCT would deplete adoptively transferred alloreactive donor B cells and thus decrease cGVHD. The present study investigated the effect of rituximab treatment infused 2 months after alloHCT, focusing on safety, feasibility, B-cell immune reconstitution, and overall clinical outcomes. Cognizant that alloimmune B-cell responses may also have antitumor benefits, the present study piloted in vivo B-cell–depletion strategy in patients with CD20-expressing B-cell malignancies. We modified our institution’s total lymphoid irradiation–antithymocyte globulin (TLI-ATG) alloHCT regimen to study in vivo B-cell depletion after rituximab treatment, and show decreased alloimmune H-Y Ab development with promising low chronic GVHD incidence.

Methods

Patient selection

Between July 15, 2005 and November 30, 2007, 35 patients with high-risk CLL (n = 22) and mantle cell lymphoma (MCL; n = 13) were enrolled in the protocol, which was approved by the Stanford institutional review board (Table 1). High-risk CLL eligibility included: (1) FISH with 17p deletion or 11q deletion, (2) unmutated heavy chain immunoglobulin (VH-IgG; < 2%...
PBMCs were thawed and washed. Cells were stained with a cocktail of TLI-ATG patients who had never received rituximab. Cryopreserved in preparation. For comparison, we measured B-cell recovery in B-cell phenotype in chronic graft-versus-host disease patients; manuscript J. Wilhelmy, J.R., P. Wadia, K. Heydari, W. Xiao, M. Mindrinos, D.B.M.; rituximab will be reported separately (B.S., A. C. Logan, B.N., S.A., B-cell reconstitution was assessed in donors and patients pre-rituximab and at day 90, day 180, and 1 year.

ATG: Anti-thymoglobulin 1.5mg/kg x 5 days

TLI: Total lymphoid irradiation 80cGy x 10 days

Analysis of donor chimerism and disease responses

Chimerism analyses were performed on whole blood and PBMCs separated into CD3, CD19, CD15, and CD56 populations using Dynal-coated immunomagnetic beads. Donor engraftment used DNA genotyping of simple sequence-length polymorphic markers that encode short tandem repeats, as described previously.33 Chimerism analyses were performed at 30, 56, and 90 days after transplantation. Full donor chimerism was defined as ≥ 95% donor peripheral blood CD3+ T cells.

Rituximab quantitation

Rituximab levels were measured by ELISA10 on pretransplantation and day 56 serum samples.

GVHD grading and therapy

Diagnosis and scoring of aGVHD was based on standard criteria,31 and cGVHD was based on National Institutes of Health consensus guidelines.32 Scoring was performed by a designated GVHD team member. Initial therapy for aGVHD and cGVHD was prednisone 1 mg/kg/d.

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H-Y Ab assays

Plasma samples from the 10 male patients with female grafts treated on the trial were tested by ELISA for Abs to H-Y antigens.10-11 Samples were diluted 1:50 and quantified for H-Y--specific IgG by ELISA with absorption at 550-450 nm in optical density units. An optical density of 0.1 was established as the cutoff value for positive Ab reactivity for all antigens.10-11 For comparison, plasma samples were similarly tested for H-Y Abs from 25 male patients with female donors undergoing concurrent TLI-ATG alloHCT who had never received rituximab and who had a 1-year posttransplantation blood sample collected.

Statistical analysis

Overall survival and freedom from progression were estimated by the Kaplan-Meier method.38 Cumulative incidence estimates were calculated for aGVHD and cGVHD, relapse, and nonrelapse mortality. Death and relapse were treated as competing events in analyses of GVHD. Factors considered in the univariate analyses of relapse/progression and cGVHD included pretransplantation rituximab level, graft CD34 dose, graft CD3 and CD19 composition, absolute CD19 B-cell count at day 56, donor T-cell (CD3) chimerism at 30 and 90 days, MCL versus CLL, matched related donor versus unrelated donor, and prior aGVHD. All P values were derived from log-rank statistics.
Table 1. CLL patient characteristics (n = 22)

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<th>CLL VH-IgG</th>
<th>No. of prior regimens</th>
<th>Mos from prior R</th>
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R indicates rituximab; LN, lymph node; RD, matched related donor; URD, unrelated donor; MM, mismatch; NA, not available; ND, not determined; and PD, progressive disease.

*Defined as failure to achieve a PR or CR to at least 1 fludarabine-containing regimen, disease progression while on fludarabine treatment, or disease progression within 6 months of the last dose of fludarabine.

†Unmutated VH-IgG CLL clone (CLL VH-IgG sequence varies by < 2% from germline).
Table 2. MCL patient characteristics (n = 13)

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</table>

R indicates rituximab; RD, matched related donor; URD, unrelated donor; MM, mismatch; NA, not available; and PET, positron emission tomography.

Results

Patient characteristics

The high-risk features of the 22 CLL patients are described in Table 1. Fifteen of 22 patients (68%) were fludarabine refractory, and 18 of 20 (90%) had an unmutated VH-IgG. Eight of 16 CLL patients (50%) had a 17p deletion and 5 of 16 (31%) had an 11q deletion. Only 4 CLL patients were in clinical remission before transplantation. The MCL group characteristics are shown in Table 2.

Hematopoietic recovery

The median CD34-cell dose for the 35 patients on study was 7.5 x 10⁶/kg (range, 2.3-19.2) and the median CD3-cell dose was 2.7 x 10⁶/kg (range, 1.6-6.1). All patients had hematopoietic recovery except for 1 patient with primary graft failure who had autologous recovery 30 days after transplantation and remains alive with CLL. Ten patients (7 with CLL and 3 with MCL) had neutropenia (< 500/μL) before graft infusion (day 0). Twenty-five patients (71%) never reached a platelet nadir below 20 000/μL.

B-cell reconstitution and rituximab infusion

Our prior studies showed that donor B-cell engraftment by donor chimerism analysis occurred approximately 60 days after TLI-ATG transplantation. Therefore, in the present study, rituximab infusion on days 56, 63, 70, and 77 was timed to coincide with this donor B-cell recovery. We quantified blood CD19⁺ B cells present before rituximab infusion on day 56, and confirmed rituximab-mediated B-cell depletion at 90 and 180 days after alloHCT by FACS analysis. CD19⁺CD5⁻ cells were reported to exclude persistent CD5⁺ malignant B cells from donor B-cell reconstitution. Figure 2A shows that whereas normal donors have 175-500 CD19⁺CD5⁻ cells/μL of blood, CLL and MCL patients had significantly fewer absolute donor B cells at day 56 (median, 7 cells/μL), with 13 patients having no detectable CD19⁺CD5⁻ B cells. In comparison, the B-cell recovery at day 56 in 19 TLI-ATG patients who had never received rituximab (supplemental Table 1, online) was significantly higher (median, 70 CD19⁺CD5⁻ cells/μL; P < .01). We presumed that this reduced donor B-cell recovery at day 56 and the similarly low number of B cells observed before alloHCT in the CLL/MCL group resulted from extensive rituximab therapy before transplantation that persisted in the patient’s blood after transplantation, depleting donor B cells. To test this directly, pretransplantation and day-56 serum samples were measured for rituximab by ELISA. All patients who received rituximab within 6 months of transplantation had detectable drug before transplantation. The patients with detectable rituximab before transplantation had few day-56 donor B cells than those who had no detectable rituximab at the time of transplantation (Figure 2B). Alemtuzumab treatment before transplantation also appeared to impair day-56 donor B-cell engraftment (supplemental Figure 1).

CLL decreased after day-56 rituximab

Immunophenotyping detected persistent CLL 56 days after transplantation in most CLL patients; 10 of 16 (63%) patients had > 10 CD19⁺CD5⁺CD23⁻ cells/μL in the peripheral blood (Figure 2C). However, after the completion of 4 weekly rituximab doses, only 6 of 20 (30%) CLL patients had any detectable CLL cells by flow cytometry, suggesting that rituximab provided an
antitumor benefit. There was negligible MCL detected by flow cytometry 56 days after HCT or on day 90 after rituximab (Figure 2C).

Prophylactic rituximab infusion 2 months after transplantation is associated with low cGVHD

Grade II-IV aGVHD was observed in 2 of 35 patients (6%; Table 3). The 2 affected patients had grade 2 skin involvement that resolved with a short course of prednisone. cGVHD developed in 7 of the 35 patients, including the 2 patients with prior aGVHD (Table 3). The cumulative incidence of cGVHD at 4 years was 20% (95% confidence interval [CI], 634%; Figure 3).

The median time to onset of cGVHD was 200 days (range, 146-413). Using National Institutes of Health consensus guidelines for scoring cGVHD severity,12 2 patients had mild, 4 had moderate, and 1 had severe cGVHD. All 5 surviving patients were successfully tapered off of prednisone and their disease remains quiescent. Two patients (Stanford patient number [SPN] 3723 and SPN 3879) died from complications of infection after cGVHD, 1 with H1N1 infection.

Rituximab prophylaxis prevents allogeneic Ab development

We hypothesized that prophylactic rituximab infusion after allo-HCT would deplete alloreactive B cells and alloantibodies. To measure B-cell alloimmunity, we evaluated H-Y Ab development in male HCT patients with female donors (F→M HCT). The frequency and intensity of Ab development to 5 H-Y antigens are shown by heat-map presentation (Figure 4). None of the 10 F→M HCT patients who received prophylactic rituximab in the present
study developed H-Y Abs and none developed cGVHD. As a comparison, 25 F → M HCT patients who underwent the same TLI-ATG conditioning during this time period without posttransplantation rituximab were evaluated, and 14 of the 25 (56%) developed H-Y Abs, with 13 of the 25 (52%) patients developing cGVHD. Supplemental Table 2 shows the concordance of H-Y Ab development in association with cGVHD (P < .005), which is consistent with what we have reported previously.11 Whereas rituximab infusion 2 months after F → M HCT prevented H-Y Ab development (P = .01), rituximab infusion before F → M HCT did not. Twelve of the 25 comparison patients had received rituximab within 6 months before alloHCT; 5 of 12 (42%) developed H-Y Abs and 3 of 5 developed cGVHD. The results of the present study confirm that H-Y Ab develops in association with cGVHD after TLI-ATG conditioning, and rituximab infusion after this RIC regimen has achieved our goal of reducing B-cell alloimmunity with no H-Y Ab development. We believe, but have not yet proven, that reduced B-cell alloimmunity may decrease cGVHD incidence.

Allogeneic HCT with posttransplantation rituximab maintains disease control

The median clinical follow-up time for our patients is 4 years. For the CLL patients, the 4-year overall survival was 73% (95% CI, 57%-94%) and 4-year freedom from progression was 47% (95% CI, 30%-75%). For the MCL patients, the 4-year overall survival was 69% (95% CI, 48%-99%) and 4-year freedom from progression was 53% (95% CI, 31%-89%; Figure 5A-B). We further assessed CLL disease by measuring MRD using allelic-specific oligonucleotide quantitative PCR assays in 19 of the 22 patients who had measurable clones. Ten of 19 (53%) of the VH-IgG–unmutated CLL patients achieved MRD negativity by 1 year after HCT. Of the 10 patients who were MRD− at 1 year after transplantation, 8 (80%) are still alive and in remission.

Twelve patients (11 CLL and 1 MCL) received DLI after disease progression. Six of the CLL patients achieved MRD negativity and remain in clinical remission. The 1 MCL patient who has received 3 DLIs is alive with persistent mixed CD3 chimerism and no GVHD. Ten trial patients have died: 7 from relapse, 2 from infection and cGVHD, and 1 from DLI-related GVHD.

Predictors for cGVHD, disease progression, and overall survival: univariate analysis

Graft CD34 cell dose, CD3 and CD19 cell composition, absolute CD19 B-cell count at day 56, donor T-cell (CD3) chimerism at days 30 and 90, disease type (MCL vs CLL), matched related versus unrelated donor, prior aGVHD, and pretransplantation rituximab level were explored in univariate analysis. Although none of the variables was significant for the development of cGVHD, relapse/progression, or overall survival, there was a trend toward an association of relapse with CD3 chimerism at day 90 after transplantation (P = .07) and a trend toward more cGVHD (P = .056) in patients with no detectable rituximab at the time of transplantation.

Rituximab infusion after transplantation is well tolerated with a low infection rate

Nonrelapse mortality at day 100 and 1 year was 0% and 3%, respectively. No rituximab-related infusional toxicities occurred. However, rituximab-related neutropenia (defined as any absolute neutrophil count < 500/µL detected after the day-56 rituximab infusion) developed in 14 (8 CLL and 6 MCL) of the 35 patients (40%). Supplemental Figure 2 illustrates when neutropenia was detected in patients on the trial. Fourteen patients were neutropenic 1 or more times after the day-56 rituximab infusion, but 10 of these 14 were neutropenic before day-56 rituximab as well. Rituximab-related neutropenia was treated with G-CSF 5 µg/kg/d for an average of 5 doses under the discretion of the treating physician. In general, the neutropenia resolved without infectious complications. Only 1 patient was hospitalized with infection and neutropenia after day-56 rituximab. The average duration of neutropenia was 2 weeks to 2 months. One patient (SPN 3489) had persistent neutropenia and required intermittent G-CSF support for 1.5 years until resolution. This patient’s MCL disease remains in remission with normal blood counts 5 years after HCT. Univariate analysis of rituximab-related neutropenia was not significant for survival outcome.

Documented infections

CMV reactivation occurred in 12 of 21 patients at risk (57%) and is detailed in supplemental Figure 3. The median time to CMV reactivation was 10 days after transplantation (range, −4 to 83), which was before rituximab infusion, suggesting that TLI-ATG conditioning alone leads to early CMV reactivation, as has been found previously.39 Only 1 patient (SPN 3975) developed pulmonary CMV disease which resolved with intravenous ganciclovir and immunoglobulin. Overall, 15 of the 35 patients (43%) had severe grade 3 infectious complications in the first year after transplantation, none of which were fatal. Among the 15 patients,
there were 5 bacterial, 9 viral, and 5 fungal infections, with 3 patients having a combination. IgG concentrations remained unchanged during the first year.

Discussion

In this first prospective study of rituximab as cGVHD prophylaxis after alloSCT, we show that rituximab infusion 56, 63, 70, and 77 days after alloHCT is well tolerated and reduces B-cell allogeneic immunity. The results of the present study confirmed that H-Y Abs develop in association with cGVHD after TLI-ATG conditioning, and rituximab infusion after this RIC regimen has achieved our goal of reducing B-cell alloimmunity with no H-Y Ab development. We believe, but have not yet proven, that reduced B-cell alloimmunity may decrease cGVHD incidence. Our institution’s standard TLI-ATG RIC regimen has already reported a low incidence of aGVHD of 2%-10%,25-26 and the results of the present study support this finding. The addition of rituximab to the TLI-ATG regimen was associated with a low cGVHD cumulative incidence of 20% (95% CI, 6%-34%). We recognize that our group has previously reported a low cGVHD cumulative incidence of 27% for TLI-ATG without rituximab,26 however, it should be noted that 60% of the patients reported on previously had a diagnosis of non-Hodgkin lymphoma and had received prior rituximab. The reduction of allogeneic Ab against H-Y antigens in the F3M HCT patients was correlated with the clinical reduction in cGVHD, providing biologic support for rituximab prophylaxis decreasing B-cell alloimmunity with low cGVHD incidence.

Investigators at the Dana-Farber Cancer Institute are studying an alternative rituximab prophylaxis dose schedule infusing 375 mg/m² at 3, 6, 9, and 12 months.40 The 1-year cumulative incidence of cGVHD was 40% compared with 65% for their historical controls, adding support to the hypothesis that B-cell depletion with rituximab may be effective in preventing or controlling cGVHD.40 Reduction of extensive cGVHD has also been observed in patients treated with rituximab within 6 months before reduced intensity transplantation in a retrospective series.23 Our prospective study took this observation a step further by quantifying rituximab levels before transplantation. Patients with no detectable rituximab at the time of transplantation appeared to have a trend toward more cGVHD ($P = .056$); however, these results must be interpreted with caution because of the small numbers of patients.

The design of our study developed from our hypothesis that prophylactic anti-B-cell therapy delivered 2 months after transplantation would decrease allogeneic donor B-cell immunity, and possibly the incidence of cGVHD. In normal B-cell development, CD20, the target of rituximab, is first expressed after heavy- and light-chain gene rearrangement, and CD20 is no longer expressed on the majority of mature plasma cells. Therefore, B-cell depletion 2 months after alloSCT was expected to: (1) deplete donor-derived alloreactive B cells, (2) permit reconstitution of newly generated tolerant B cells derived from donor hematopoietic stem cells, and (3) maintain CD20⁻ plasma cells and immunoglobulin levels to protect against infection. Using H-Y Ab development as a biomarker for alloreactivity, we demonstrated directly that rituximab infusion 2 months after alloSCT depleted B cells that are alloreactive for the recipient. None of the F→M HCT patients receiving rituximab 2 months after transplantation developed H-Y Abs or cGVHD. In contrast, 14 of 25 (56%) F→M HCT patients who never received rituximab after alloHCT did develop H-Y Ab in
strong association with cGVHD (P < .005), further supporting our hypothesis that alloreactive B cells play a central role in cGVHD pathogenesis. The prevention of H-Y Ab development and cGVHD is in agreement with a recent murine study showing that donor B-cell alloantibody deposition and germinal center B-cell infiltration affected cGVHD liver and lung tissue. Further, Srinivasan et al demonstrated that BM grafts obtained from mice genetically incapable of somatic hypermutation or undergoing IgG isotype switching significantly decreases cGVHD development. Consistent with this murine study, our low cGVHD incidence may result from rituximab prophylaxis 2 months after alloHCT decreasing/preventing alloimmune Ab development.

A recent suggestion that alloimmune Abs are pathogenic for cGVHD development, the prevention of alloimmune Ab development may be a worthwhile pharmacodynamic goal for cGVHD prevention.

Figure 4. Rituximab prophylaxis prevents H-Y allogeneic Ab development. Blood IgGs against 5 H-Y antigens were determined by ELISA in 25 F → M HCT patients who never received rituximab after TLI-ATG (left panel) and 10 study patients treated with rituximab on days 56, 63, 70, and 77. These heat maps show that no alloreactive H-Y Abs developed in study patients receiving rituximab 2 months after TLI-ATG alloHCT, whereas 1 or more H-Y Abs developed in 56% (14 of 25) of patients receiving TLI-ATG without posttransplantation rituximab. Considering all patients who survived 9 months, rituximab prophylaxis prevents H-Y Ab development (P = .01).

Figure 5. Overall survival after rituximab prophylaxis exceeds 70%. For the CLL patients, the 4-year overall survival was 73% (95% CI, 57%-94%) and freedom from progression was 47% (95% CI, 30%-75%). For the MCL patients, the 4-year overall survival was 69% (95% CI, 48%-99%) and freedom from progression was 53% (95% CI, 31%-89%).
Extensive BM immunophenotyping studies of patients in the present study confirmed that rituximab treatment depleted adoptively transferred immunoglobulin-expressing mature donor B cells. After rituximab prophylaxis, B cells developed from donor hematopoietic stem cells and lymphoid progenitor cells by 1 year after transplantation (B.S., manuscript in preparation). Long-lived CD20⁺ plasma cells were unaffected by rituximab in the TLI-ATG transplantation, thereby explaining the relatively unchanged IgG concentrations in our study patients.

Our study of prophylactic rituximab was restricted to patients with CD20⁺ malignancies, so any loss of potential graft-versus-tumor (GVT) effects by allogeneic B-cell depletion would be offset by the direct antitumor effects of the mAb. We recognize that donor B-cell reconstitution could be affected by a patient’s pretransplantation rituximab therapy. Compared with the patients with myeloid malignancies undergoing the same TLI-ATG conditioning but never receiving rituximab, the CLL and MCL patients reconstituted fewer CD19⁺ B cells at day 56 after HCT (Figure 2A). Therefore, future studies of posttransplantation rituximab in patients with non–B-cell malignancies will be required to further elucidate the potential impact of prophylactic anti–B cell–directed therapy on cGVHD incidence and severity.

The survival and freedom from progression at 4 years for related and unrelated donors in high-risk CLL patients with RIC is comparable to other published studies from Sorror et al⁴¹ and Khouri et al,²¹ albeit with low transplantation-related risk. Moreover, MRD durability was achieved with associated GVT responses in 53% of patients, with low incidence and severity of cGVHD. Only 4 of the 10 MRD⁻ patients developed cGVHD, and all surviving patients have been tapered off of immune-suppression medications. The present results compare favorably with those of the CLL3X trial performed by the German CLL Study Group.⁴² With MCL, the patients were more varied between CR and PR status, but overall survival and freedom from progression were comparable to prior reports.⁴³ The nonrelapse mortality of only 3% at 1 year and the low incidence of aGVHD (6%) and cGVHD (20%) compare favorably.

Neutropenia after rituximab infusion was observed in 40% of our patients and should be a caution when using rituximab after transplantation. Neutropenia has been reported in other rituximab studies, including some from our own institution.⁴⁴-⁴⁶ Often, neutropenia will resolve with short-course G-CSF support without serious infectious complications. Wherein infections were generally not increased in our cohort, one important consideration in using posttransplantation rituximab is the impaired ability to mount a humoral immune response against neoantigens such as H1N1, as was observed in 1 of the fatalities among the cGVHD patients in the present study. Long-term clinical follow-up and immunologic reconstitution studies are critical to fully evaluate rituximab prophylaxis after alloHCT.

Because this trial used a nonmyeloablative regimen, mixed chimerism was expected, and provision was made for patients with progressive disease after transplantation to receive dose-escalated DLI. Thirteen patients received DLI for disease progression and 8 of the patients are alive. Mixed chimerism at day 90 had a trend of association with an increased relapse rate in univariate analysis (P = .07). Rituximab was not felt to have an effect on mixed chimerism in this study because the rate was comparable to that seen in our standard TLI-ATG regimen.²⁶ There was also the observation that 53% of the CLL patients achieved CR to the level of molecular remission from PR without DLI, which must be attributed to potent GVT effects, even with the minimal-intensity conditioning of TLI-ATG.

In conclusion, the results of the present study show that rituximab prophylaxis after RIC transplantation is feasible and associated with a low cGVHD incidence while maintaining disease control. Furthermore, posttransplantation rituximab depletes alloreactive B cells, as shown by H-Y Ab testing. In this first prophylactic study of rituximab after alloHCT, we provide important insights into donor B-cell depletion by applying rituximab pharmacokinetic measurements, B-cell phenotyping, and allogeneic Ab assessment of 35 patients receiving day 56, 63, 70, and 77 rituximab. The optimal timing of rituximab prophylaxis may be suggested by comparing clinical outcomes using 3 dosing schedules: (1) rituximab-included conditioning,²¹ (2) rituximab depletion of alloreactive B cells 2 months after alloHCT, or (3) sustained and repeated B-cell depletion infusing rituximab every 3 months through 1 year after alloHCT.⁴⁰ Correlative laboratory studies of rituximab pharmacokinetics, donor B-cell reconstitution, and both allogeneic and protective anti-infection Abs will aid the design of a well-powered and informative randomized trial to test the efficacy and safety of rituximab prophylaxis for cGVHD prevention.

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Authorship

Contribution: S.A., B.S., and D.B.M. designed the experiments, analyzed the data, and wrote the manuscript; B.N. analyzed the data; G.L.C. and J.R. performed the experiments; C.D.J. and J.L.Z. contributed reagents/ materials/analysis tools; and all authors revised the manuscript.

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References


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