

Accelerated Allogeneic Haemopoietic Engraftment after Preceding Rituximab-Therapy

William H. Krüger*, Thomas Kiefer, Christian Lotze, Fabian P. Thomsen, Carsten Hirt, Frank Schüler, Christoph Busemann and Gottfried Dölken

Department of Internal Medicine C – Haematology and Oncology, Stem Cell Transplantation, Ernst-Moritz-Arndt-University, Greifswald, Germany

Abstract: The chimeric monoclonal anti-CD20 antibody rituximab and the graft-versus-leukaemia/lymphoma effect after allogeneic stem cell transplantation have improved therapy of lymphatic malignancies during the last decade. In addition, successful therapy of chronic graft-versus-host disease after stem cell transplantation with rituximab has been published. Other investigators correlated prolonged neutropenia after autologous stem cell transplantation with prior rituximab-therapy.

To address the question, if rituximab pre-treatment prior to transplantation diminishes the graft versus lymphoma effect afterwards and can prolong engraftment of allogeneic haemopoiesis, we retrospectively analysed data from 34 patients allografted for lymphoid malignancies. 19 patients had received at least one course of rituximab prior to allogeneic stem cell transplantation and 15 did not. Both groups matched very good.

Patients with rituximab pre-treatment engrafted with 1 leukocyte/nl significantly faster than patients without prior rituximab therapy (12 days [median, range 1-32] vs. 18 days [median, range 12-31], $p < 0,005$). Engraftment of platelets (PLT) occurred as well faster after rituximab pre-treatment. The differences were significant for all three steps [**20PLT/nl**: 11,5 days (median, range 1-28) vs. 27 days (median, range 11-54) $p < 0,001$; **50PLT/nl**: 16,5 days (median, range 11-37) vs. 42 days (median, range 22-70) $p < 0,001$; **100PLT/nl**: 22,5 days (median, range 13-52) vs. 60 days (median, range 25-117) $p < 0,005$]. The mechanism leading to faster engraftment remains unclear so far. Preceding therapy with rituximab had no influence on event-free survival, overall survival, or manifestation freedom from graft-versus-host disease (GVHD) after allogeneic stem cell transplantation. Patients with GVHD had a significantly better overall survival ($p = 0,0001$) comparing to patients without.

In conclusion, this investigation gives no hint for a suppression of GvH and GvL effects by rituximab administration prior to allogeneic stem cell transplantation. In addition, in contrast to results published for autologous stem cell transplantation, no detrimental effects on establishment of graft haemopoiesis after allogeneic stem cell transplantation by preceding rituximab therapy were found.

Key Words: Allogeneic stem cell transplantation, Haemopoietic engraftment, Rituximab, Graft-versus-host-disease, Graft-versus-leukaemia effect.

INTRODUCTION

The introduction of the chimeric monoclonal anti-CD20 antibody rituximab has become a landmark in therapy of B-cell derived non-Hodgkin's lymphoma [1, 2]. However, a considerable percentage of patients may relapse even after combined immunochemotherapy with or without subsequent consolidation by high-dose therapy and autologous stem cell reinfusion. Allogeneic stem cell transplantation is a potentially curative salvage therapy for these patients [3-5]. Myeloablative conditioning regimen used in earlier approaches were associated with significant morbidity and mortality, especially in elderly or heavily pre-treated patients [6]. Allogeneic stem cell transplantation using reduced intensity

(RIC) conditioning protocols is a promising and potentially curative approach for patients after failure of conventional therapy since the results of transplantation for lymphoma have improved dramatically over the last decade [4, 6, 7].

Substantial contribution of graft-versus-leukaemia/lymphoma effect to eradication of minimal residual disease after allogeneic haemopoietic cell transplantation has been described by a variety of investigators. A considerable percentage of patients require donor lymphocyte infusions (DLI) after transplantation for definite eradication of the malignant clone, however, this approach bears an increased risk of acute or chronic graft-versus-host disease [4, 8-10].

Some concern is raised by publications describing activity of rituximab against chronic graft-versus-host disease [11-13]. Here the question must be addressed, if pre-treatment with rituximab can diminish GvHD and graft-versus-lymphoma effect after allografting. This situation can not be easily compared with successful rituximab-therapy of mini-

*Address correspondence to this author at the Medizinische Klinik C (Hämatologie und Onkologie, Transplantationszentrum), Ernst-Moritz-Arndt-Universität Greifswald, Ferdinand-Sauerbruch-Straße, 17475 Greifswald, Germany; Tel: +49-3834-86-22007/22006; Fax: +49-3834-86-22012; E-mail: william.krueger@uni-greifswald.de

mal residual disease after allogeneic SCT, since here an engraftment and partial expansion of transplanted lymphopoiesis took place [14].

In addition, rituximab has been suspected to have detrimental effects on leukocyte engraftment after autologous stem cell transplantation [15, 16]. Since rapid leukocyte engraftment is essential to circumvent serious infectious complications, this is another critical point of rituximab pre-treatment of candidates for allogeneic stem cell transplantation.

To address both questions we have retrospectively analysed our data of allogeneic transplantation of patients with lymphoid malignancies and compared data from patients with a history of rituximab therapy with data from patients not exposed to the antibody.

PATIENTS AND METHODS

Patients

The data from thirty-four consecutive patients allografted for high-risk lymphoid malignancies were included in this investigation (Table 1). Twenty-seven patients were male (79,4%) and seven patients (20,6%) were female. The median age at the time of stem cell transplantation was 46 years (range 22-67). The underlying diagnoses were high-grade non-Hodgkin's lymphoma (n=14, 41,2%), acute lymphoblastic leukaemia (n=8, 23,5%), chronic lymphocytic leukaemia (n=6, 17,7%), mantle cell lymphoma (n=3, 8,8%), multiple myeloma (n=2, 5,9%), and variant hairy cell leukaemia (n=1, 2,9%). The vast majority of lymphomas was B-cell derived (n=30, 88,2%)

All patients were at highest risk for relapse and had no curative option by conventional chemotherapy or autologous stem cell transplantation. At the time of allogeneic transplantation, five patients were in first (14,7%), nine (26,5%) in second, and two (5,9%) in third or higher complete remission. Six (17,7%) patients each had either a partial remission or progressive or relapsing disease. The patients were heavily pre-treated with a median of seven cycles chemotherapy (range 2-37) of three (median, range 1-10) different regimens. Additionally, 12 patients (35,3%) had a history of prior myeloablative therapy followed by autologous (n=9) or allogeneic (n=3) haemopoietic cell transplantation.

History of Rituximab Pre-Treatment

Nineteen (55,9%) patients had a history of pre-treatment with rituximab. At median 4 (range 1-10) doses of 375mg/m² of the chimeric antibody were given. The median cumulative dose was 1500mg/m² with a range from 375mg/m² to 3750mg/m². The median interval from the last rituximab infusion to allogeneic transplantation was 5,9 weeks (range 0,1-212,9). Since Edwards *et al.* published a complete B-cell depletion by rituximab for more than 25 weeks without upcoming recovery and Leandro *et al.* described B-cell depletion for more than four years in a patient with lupus erythematoses after Rituximab-treatment, we decided not to exclude the single patient with the interval of 212,9 weeks from analysis [17, 18]. Patients with and without a history of rituximab-pre-treatment matched very well without significant differences in all relevant parameters (Table 1).

Transplantation and Supportive Care

The thirty-four patients were allografted 16 months (median, range 1-124) after initial diagnosis from related (n=13) and unrelated (n=21) donors. The donors were fully matched with the exception of one major-mismatch in a related transplantation. Twenty-five (73,5%) of the patients and 17 (50,0%) of the stem cell donors had prior exposition to human cytomegalovirus as indicated by IgG. Three male patients were grafted from a female donor.

Conditioning regimens were myeloablative in 8 patients (23,5%) and toxicity-reduced in 26 patients (76,5%). Myeloablative regimen were Busulfan₁₆/Cyclophosphamid₁₂₀ (n=7, 20,6%) and TBI_{12Gy}/Cyclophosphamid₁₂₀ (n=1, 2,9%). For toxicity reduced conditioning were Treosulfan/Fludarabin (n=17, 50,0%), Busulfan/Fludarabin (n=5, 14,7%), and TBI(2Gy)/Fludarabin (n=2, 5,9%) chosen [19-21]. All patients were grafted with non-manipulated G-CSF mobilised peripheral blood stem cells. Given was a median dose of 5,7*10⁶ (range 0,9-13,9) CD34⁺ cells per kg bodyweight. Antithymocyte globuline was given when no HLA-identical sibling donor was available for stem cell donation.

Patients with and without prior rituximab-exposition matched excellent with exception of the constellation of CMV-negative donors for CMV-positive patients, which occurred significantly more in the rituximab pre-treated group (Table 1).

Haemopoiesis was stimulated with G-CSF until leukocyte-engraftment after transplantation. Antimicrobial prophylaxis with quinolones, antimycotics and virustatics as well as Pneumocystis jrovecii prophylaxis followed standard procedures [22]. GVHD prophylaxis was carried out with methotrexate and cyclosporine-A following standard protocols. Additional mycophenolate mofetil was given after conditioning with 2Gy TBI [21]. Acute and chronic GVHD were treated with steroids according to standard protocols [23, 24]. Donor-lymphocyte transfusions (median 3, range 1-5) were given to six (17,7%) patients for molecular or clinical relapse.

Assesment of Outcome

Leukocyte engraftment was defined as the first of 3 consecutive days that the absolute leukocyte count exceeded 1,0 cells per nl of blood and steps of platelet count recovery were defined as the first day that the platelet count exceeded 20, 50 and 100 platelets per nl of blood, respectively, independent of platelet transfusions. Acute and chronic GVHD were graded following the published criteria [25-27].

Survival was calculated using the log-rank test and the Kaplan-Meyer analysis. Event-free survival (EFS) and overall survival (OS) are defined as intervals from day of transplantation until lost of follow-up or relapse not treated successfully with donor-lymphocyte transfusions (EFS) or death (OS).

Data Collection and Statistics

Data were collected and analysed using the softwares MS-Excel (Microsoft, Munich, Germany) and GraphPad Prism (GraphPad Software, San Diego, CA, USA). Statistical tests are mentioned where appropriate.

Table 1. Patients Characteristics. Given are Either the Number (n) and Percentage or Median and Range

Parameter	Rituximab (n=19)	No Rituximab (n=15)	p-Value	Test
Gender male	16 (84,2%)	11 (73,3%)	0,36	Fisher's exact p
Age	42,2 (23,2-65,2)	46,4 (22,1-67,3)	0,48	U-test (Mann-Whitney)
High-grade malignancy	12 (63,2%)	10 (66,7%)	0,56	Fisher's exact p
Remission state at TX	CR 8 PR 7 RD/PD 4	CR 8 PR 6 RD/PD 1	0,49	Chi-square
Preceding CTX regimens	3 (2-10)	3 (1-5)	0,29	U-test (Mann-Whitney)
Preceding CTX cycles	8 (4-37)	7 (2-21)	0,43	U-test (Mann-Whitney)
Female donor → male patient	9 (47,4%)	2 (13,3%)	0,04	Fisher's exact p
CMV-positive (patient)	15 (79,0%)	10 (66,7%)	0,34	Fisher's exact p
CMV-positive (donor)	9 (47,4%)	8 (53,3%)	0,50	Fisher's exact p
CMV ^{neg} -donor → CMV ^{pos} -patient	3 (13,8%)	0	0,16	Fisher's exact p
Donor-type mud	11(57,9%)	10 (66,7%)	0,44	Fisher's exact p
Use of ATG in conditioning	11 (57,9%)	12 (80,0%)	0,16	Fisher's exact p
RIC	13 (68,4%)	13 (86,7%)	0,20	Fisher's exact p
2 nd TX	8 (42,1%)	3 (20,0%)	0,16	Fisher's exact p
CD34 ⁺ -cells (10 ⁶ /kg)	5,3 (1,6-13,9)	6,4 (0,9-11,4)	0,67	U-test (Mann-Whitney)
Time from primary diagnosis to transplantation (months)	24,1 (2,9-123,5)	14,3 (1,0-67,0)	0,66	U-test (Mann-Whitney)

Abbreviations: ALL: acute lymphoblastic leukaemia, CLL: chronic lymphocytic leukaemia, HCL: hairy-cell leukaemia, MM: multiple myeloma, NHL: non-Hodgkin's lymphoma, CR: complete remission, PD: progressive disease, PR: partial remission, REL: relapse, CTX: chemotherapy, ATG: anti-thymocyte globulin, CMV: cytomegalovirus, RIC: reduced-intensity conditioning, TX: transplantation.

RESULTS

Engraftment

Thirty-two patients (94,1%) engrafted with 1 leukocyte/nl 15 days (median, range 1-32 days) after transplantation. Patients with rituximab pre-treatment reached 1 leukocyte/nl significantly faster than patients without prior rituximab therapy (11,5 days [median, range 1-32] vs. 18 days [median, range 12-31, p<0,005, Mann-Whitney U-test) (Table 2).

Engraftment of platelets (PLT) with 20/nl was reached by 29 patients (85,3%) 17 days (median, range 1-54 days) after transplantation; 50 platelets/nl and 100 platelets/nl were reached by 23 patients (67,7%) after 25 days (median, range 11-70) and 31 days (median, range 13-117) after transplantation. Engraftment of platelets occurred as well faster after rituximab pre-treatment. The differences were significant for all three steps (**20PLT**: 11,5 days (median, range 1-28) vs. 27 days (median, range 11-54) p<0,001; **50PLT**: 16,5 days (median, range 11-37) vs. 42 days (median, range 22-70)

Table 2. Engraftment of Leukocytes (WBC) and Platelets (PLT). Given is the First of Three Consecutive Days after Stem Cell Transplantation that the Absolute Leukocyte Count Exceeded 1,0 Cells Per nl of Blood and Steps of Platelet Count Recovery were Defined as the First Day that the Platelet Count Exceeded 20, 50 and 100 Platelets Per nl of Blood, Respectively, Independent of Platelet Transfusions. P-Values were Calculated Using the Mann-Whitney U-Test

Parameter	Rituximab (n=19)	No Rituximab (n=15)	P
WBC>1/nl (day)	12 (1-32)	18 (12-31)	<0,005
PLT>20/nl (day)	11,5 (1-28)	27 (11-54)	<0,001
PLT>50/nl (day)	16,5 (11-37)	42 (22-70)	<0,001
PLT>100/nl (day)	22,5 (13-52)	60 (25-117)	<0,005

$p=0,001$; **100PLT**: 22,5 days (median, range 13-52) vs. 60 days (median, range 25-117) $p<0,005$; Mann-Whitney U-test) (Table 2). Scatter diagrams for time to engraftment of leucocytes and platelets in relation to the grafted $CD34^+$ -cell dose are shown in Fig. (1). The $CD34^+$ -cell dose was identical in both collectives with a median dose of $5,3$ (range: $1,6-13,9$) $\cdot 10^6$ $CD34^+$ -cells/kg in the patients with exposition to rituximab and $6,4$ (range: $0,9-11,4$) $\cdot 10^6$ $CD34^+$ -cells/kg in rituximab-naïve patients. In addition, Fig. (1) shows that the differences can not be put down on the stem cell dose.

The risk to fail engraftment with leukocytes or platelets at any step was slightly but non-significant increased for patients who did not receive rituximab prior to allogeneic stem cell transplantation (data not shown).

Graft-Versus-Host Disease

The overall-incidence of acute graft versus host disease was 52,9% (n=18) in all patients. Differences between patients with and without rituximab pre-treatment were not seen (11/19 [57,9%] vs. 7/15 [46,7%]). Overall, nine patients

(26,5%) suffered from acute liver GVHD, 17 patients (50,0%) from acute GVHD of the skin and 14 patients (41,2%) from acute GVHD of the gastrointestinal tract. The median stages of acute GVHD for liver, skin and gut were 0 (range 0-3), 0,5 (0-4) and 0 (0-4), respectively. Three patients (8,8%) developed grade IV acute GVHD. Grade III and II acute GVHD was seen in seven (20,6%) cases each and one (2,9%) patient suffered from grade I GVHD, respectively. Obvious differences between both groups of patients were not observed.

The overall incidence of chronic GVHD was 38,2% (13/34) after an interval of 6,8 (median, range 2,2-20,0) months. Eight (23,5%) suffered from extensive disease and five (14,7%) from limited stage chronic GVHD. Within sub-groups, cGVHD-incidence was 26,7% (4/15) without and 47,4% (9/19) after prior rituximab-exposition. There was a trend that chronic graft versus host disease did manifest earlier in patients not exposed to rituximab, however, without statistical significance (median 5,1, range 3,0-9,5 months vs. 9,9, range 2,2-20,0 months, n.s.).

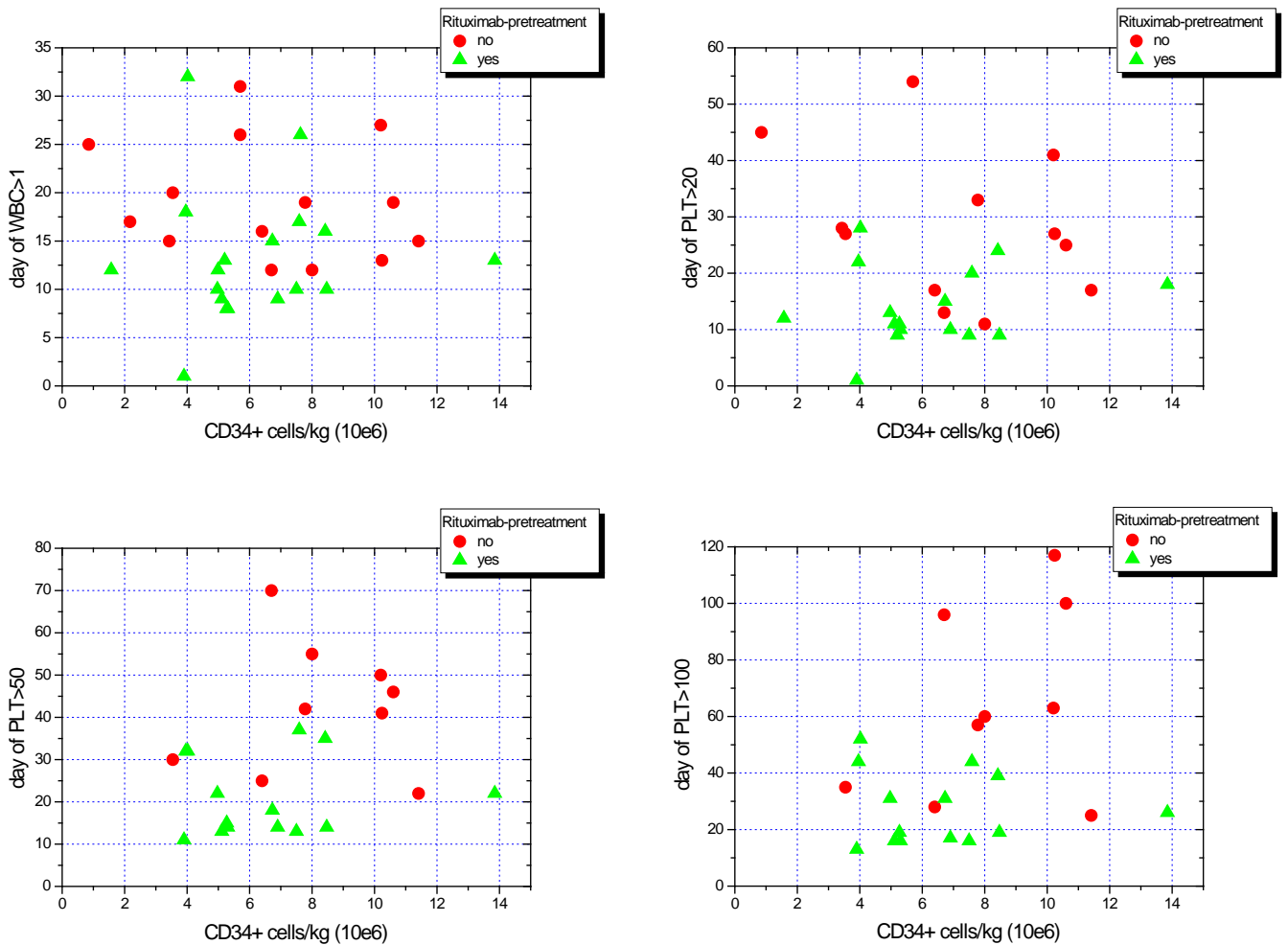


Fig. (1). Scatter-diagrams of engraftment of white blood cells (WBC) with a count of 1 cell/nl and of platelets with 20, 50 and 100 per nl in relation to the transplanted $CD34^+$ cell dose per kilogram bodyweight of the recipient (x-axis). The y-axis shows the first day of three consecutive days after transplantation on which the cell count was reached independently from transfusions.

To exclude any bias from other events such as transplant-related mortality or mortality due to relapse, the interval freedom from GVHD was calculated using log-rank test and Kaplan-Meyer curves. The endpoints were manifestation of GVHD, the day of last follow-up or death. The curves for freedom from any GVHD are shown Fig. (2). The median interval 'freedom from any GVHD' was 2,2 months without and 1,1 months after rituximab pre-treatment. The curves from separate analyses for acute and chronic GVHD are also strictly in parallel (data not shown).

Survival and Outcome

Thirteen (38,2%) of the patients are alive after a median follow-up was 8,8 months with a range from 0,1 to 72,9 months. Twenty-one (61,8%) patients have died from relapse (n=6, 17,7%), acute GVHD (n=2, 5,9%), chronic GVHD (n=3, 15,8%), infection (n=5, 14,7%), EBV-lymphoproliferative disease (n=2, 5,9%) multi-organ failure, secondary cancer and bleeding (each n=1, 2,9%). Pre-treatment with rituximab had neither impact on event-free or overall sur-

vival nor on manifestation of GVHD after stem cell transplantation (Fig. 2). However, overall survival was significantly improved by manifestation of acute GVHD (p=0,02, log-rank test), chronic GVHD (p<0,01, log-rank test) or any form of GVHD (p=0,001, log-rank test). The median overall survival was 2,3 months (range 0,1-33,8 months) without GVHD comparing to 14,5 months (median, range 0,6-72,9 months) in patients with acute, chronic or both forms of GVHD (Fig. 2).

DISCUSSION

The surprising result of the present retrospective investigation is the significant faster engraftment of white blood cells and platelets in patients treated with rituximab prior to stem cell transplantation compared to engraftment in rituximab-naïve patients. Despite the small sample size of n=34, the differences are consistently of high significance always with p<0,005. There were no differences in the patient characteristics predicting a slower engraftment in rituximab-naïve patients. These patients were more frequently condi-

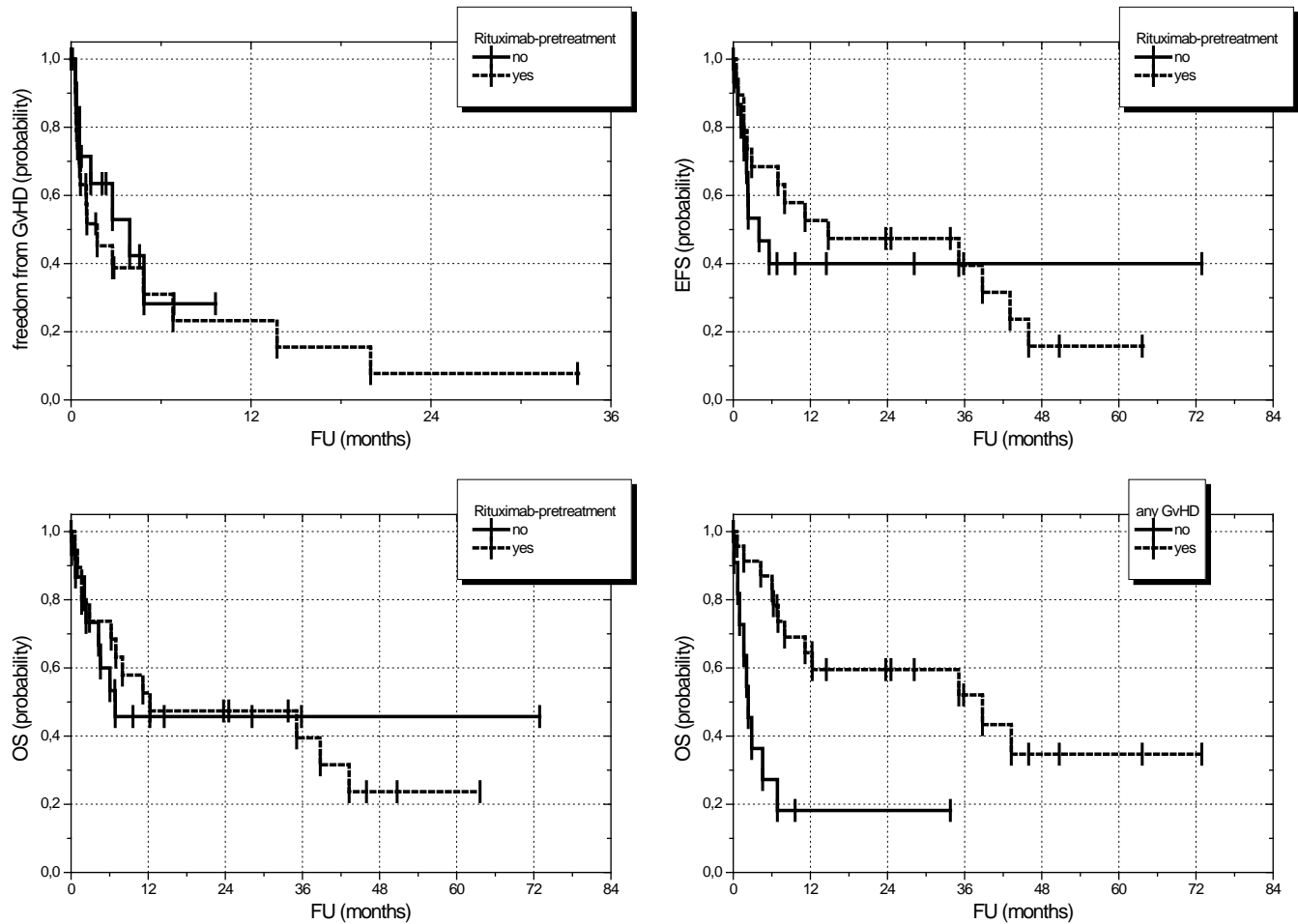


Fig. (2). Kaplan-Meier plots for patients with and without rituximab-pretreatment, follow-up time is shown in months. Shown are the curves for freedom-from-GvHD (top-left) (n.s.), event-free survival (EFS, top-right) (n.s.), and overall survival (OS, bottom-left) (n.s.). The diagram at the bottom right shows overall survival curves for patients with manifestation of acute and/or chronic graft versus host disease (GvHD) comparing to patients without any GvHD (p=0,001).

tioned with reduced intensity regimen, have been grafted with a slightly higher CD34⁺-cell dose and were slightly less heavily pre-treated as the rituximab-positive patients [28]. In addition, they were less frequently CMV-seropositive than the patients from the rituximab-collective [29]. However, all these differences were statistically non-significant. The observed results suggest inhibition of allogeneic leukocyte and platelet engraftment by CD20-antigen positive cell populations, either host or donor derived, with facilitation of engraftment after in-vivo depletion of these cells by Rituximab. No comparable results have been published so far.

Rituximab has been used with success for therapy of antibody-mediated thrombocytopenia and haemolysis after allogeneic transplantation and in other situations [30-32]. In addition, the successful treatment of chronic GVHD with rituximab as reported by Rathanatarathorn and Cutler suggests interaction of CD20⁺ cells with cellular immune response [11-13].

Interaction of rituximab with engraftment has been described after high-dose therapy and autologous stem cell transplantation, however, there is some inconsistency in these results. Benekli *et al.* described a lower yield of CD34⁺-cell in stem cell harvests and delay of leukocyte engraftment after rituximab pre-treatment. Hoerr *et al.* described delayed platelet engraftment but no detrimental effects on stem cell mobilisation and Buckstein *et al.* and Hosing *et al.* observed also no affection of stem cell mobilisation [33-35]. Cairoli *et al.* and Lemieux *et al.* have observed late onset cytopenia after rituximab-treatment after autologous stem cell transplantation [15, 16]. Few data have been published for allogeneic transplantation so far. Khouri *et al.* have included rituximab into dose- reduced conditioning for chronic lymphocytic leukaemia and administered additional doses after SCT for elimination of minimal disease [14]. However, comparison of engraftment was not subject of their analysis. From the present and other investigations it can be concluded, that CD20⁺ B-cells play a role in engraftment after stem cell transplantation and further clinical and basic research for clarification of the mechanisms is necessary.

The present investigation gives no evidence for suppression of acute or chronic GVHD by rituximab-treatment prior to allogeneic stem cell transplantation since the curves for freedom from GVHD are strictly in parallel. This is even true for manifestation of acute and chronic GVHD alone. In addition, rituximab-therapy prior to allogeneic stem cell transplantation did not influence overall survival after SCT (Fig. 2). It is very difficult to explain these observations in context with those of effective GVHD-treatment by rituximab when infused after SCT, especially since the research on immune recovery after allogeneic SCT has focused on T-cells so far [36]. Possible mechanisms might be such as differences in the amount of circulating anti-CD20 antibodies, the relation between host and donor derived B-cells, and the extent of upcoming immune tolerance. The observation by Hedge *et al.*, that rituximab-treatment of fludarabine-associated immune-thrombopenia in CLL patients is effective, suggests an important role of T-lymphocytes in regulation of B-cell-(antibody)-mediated cytopenia [37].

In conclusion, there we did not found any concern against rituximab-therapy prior to planned allogeneic stem cell

transplantation since it has no detrimental effects on allogeneic engraftment, it does not diminish manifestation of graft-versus-host disease and there is even no evidence for negative effects on graft-versus-lymphoma effects. The manifestation of graft-versus-host disease remains highly significant associated with a superior outcome compared to patients without GVHD as described for allogeneic transplantation of patients with other entities [8, 38]. The observed acceleration of allogeneic engraftment in this retrospective analysis should be confirmed in a prospective trial.

ACKNOWLEDGEMENTS

We wish to thank the nurses for their excellent patient care.

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Received: July 19, 2008

Revised: November 27, 2008

Accepted: December 11, 2008

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