



ARTICLE OPEN



Rituximab added to conditioning regimen significantly improves erythroid engraftment in major incompatible ABO-group hematopoietic stem cell transplantation

Maria Chiara Finazzi^{1,2} , Alessandra Weber², Chiara Pavoni², Anna Grassi², Maria Caterina Micò², Alessandra Algarotti², Federico Lussana^{1,2}  and Alessandro Rambaldi^{1,2}

© The Author(s) 2024

ABO-group major incompatibility hematopoietic stem cell transplantation (HSCT) increases the risk of delayed red cell engraftment and other immunological complications. In this study, we evaluated the efficacy of pre-transplant infusion of rituximab in patients with ABO-incompatibility in improving red blood cell engraftment after HSCT, measured by time to reach transfusion independence. We performed a retrospective, single-center study including 131 consecutive patients transplanted with major or bidirectional ABO-incompatible grafts between 1st January 2013 and 31st December 2019. Fifty-one patients received an infusion of rituximab during the conditioning regimen, while 80 patients did not receive any additional preventive treatment. Time to transfusion independence was significantly reduced for patients treated with rituximab (1 month, 95% CI, 0.5–2) compared with the control group (3.2 months, 95% CI 1.5–3.2, $p = 0.02$). By multivariable analysis, rituximab use was associated with a faster red blood cell (RBC) engraftment (RR 1.88, 95% CI 1.17–3.03, $p = 0.009$), while a pre-transplant anti-donor isohemagglutinins titer $>1:128$ was associated with delayed transfusion independence (RR 0.61, 95% CI 0.37–0.99, $p = 0.05$). Although limited by the retrospective nature of the study, the results of this analysis suggest that rituximab added to conditioning regimens is feasible, safe, and able to improve post-transplant red blood cell engraftment.

Bone Marrow Transplantation; <https://doi.org/10.1038/s41409-024-02247-w>

INTRODUCTION

Approximately 40–50% of all allogeneic hematopoietic stem cell transplantation (HSCT) are performed with some degree of ABO blood group incompatibility between the donor and the recipient [1]. Three types of ABO mismatches have been described: major, minor, and bidirectional [2]. Although the transplantation of an ABO-mismatched graft is feasible, this condition is at risk for delayed hemolysis, pure red cell aplasia (PRCA) [3] delayed red blood cell (RBC) engraftment [4, 5].

Globally, the clinical outcome of ABO-incompatible HSCT is generally considered inferior to ABO-compatible HSCT. ABO incompatibility may have an impact on platelet and neutrophil engraftment. Since ABO blood group antigens are also expressed on platelets, previous studies reported a significant delay in platelet engraftment [6–8]. Even if AB antigens are not directly expressed by neutrophils, delayed engraftment of neutrophils was also previously reported by other groups [6, 9] speculating that anti-donor isohemagglutinin may bind to A/B antigens adsorbed on the surface of neutrophils or their precursors. ABO incompatibility has been associated with graft failure [6, 10], even if others did not confirm this data [11]. Furthermore, since AB antigens are also expressed in non-hematopoietic tissues, ABO major incompatibility may impact GVHD incidence. Some studies reported an increased incidence of acute graft versus host disease (GVHD)

[5, 12–14], even if results are conflicting [5]. Lastly, ABO incompatibility may have a detrimental effect on non-relapse mortality and overall survival [5, 15], especially during the first 100 days after transplant [16].

The prevention of immune complications after an ABO-incompatible transplant relies mainly on the possibility of reducing the isohemagglutinins titer in the recipient before transplant by apheresis; [17] however, this approach is not standardized and there is no consensus about it [2].

Rituximab is an anti-CD20 chimeric IgG1 monoclonal antibody. It has been used in the treatment of post-transplant PRCA to reduce isohemagglutinin titers in the host with some success [18]. Given its effect in reducing the antibody production by B lymphocytes, we hypothesized that pre-transplant infusion of rituximab in patients with ABO-incompatibility between the host and the donor may improve the RBC engraftment.

In this study, we retrospectively analyzed the outcome of 131 patients transplanted in our center with major or bidirectional ABO-incompatible grafts between 1st January 2013 and 31st December 2019. Of these, 51 patients received rituximab during the conditioning regimen, while 80 did not receive additional treatment. We compared these two cohorts of patients to detect differences in time to reach RBC transfusion independence.

¹Department of Oncology and Onco-Hematology, Università degli Studi di Milano, Milano, Italy. ²Hematology and Bone Marrow Transplant Unit, ASST Papa Giovanni XXIII, Bergamo, Italy. ✉email: mfinazzi@asst-pg23.it

Received: 8 November 2023 Revised: 7 February 2024 Accepted: 9 February 2024

Published online: 24 February 2024

Table 1. Patients' characteristics.

Characteristics	Rituximab, N = 51	No Rituximab, N = 80	P
Age, median (range)	52 (18–70)	52 (20–69)	0.8354
Sex, n (%)			0.1927
F	25 (49)	30 (37.5)	
M	26 (51)	50 (62.5)	
Disease, n (%)			0.8310
AA	2 (3.9)	2 (2.5)	
ALL	5 (9.8)	11 (13.8)	
AML	25 (49)	38 (47.5)	
CML	3 (5.9)	2 (2.5)	
MDS	4 (7.8)	10 (12.5)	
MM	1 (2)	3 (3.8)	
Myelofibrosis	6 (11.8)	5 (6.2)	
CLL / NHL	5 (9.8)	9 (11.2)	
Disease status, n (%)			0.9592
1°RC	25 (49)	40 (50)	
2°RC	6 (11.8)	6 (7.5)	
>2°RC	1 (2)	3 (3.8)	
active disease	14 (27.5)	21 (26.2)	
RP / stable disease / clinical improvement	3 (5.9)	6 (7.5)	
First CP / never treated	2 (3.9)	4 (5)	
N previous lines, median (range)	1 (0–4)	1 (0–6)	0.6275
0	4 (7.8)	7 (8.8)	
1	30 (58.8)	42 (52.5)	
2	11 (21.6)	21 (26.2)	
>2	6 (11.7)	10 (12.6)	
IgG (mg/dl), median (range)	812 (168–1640)	954.5 (323–1370)	0.1624
Serum Gamma globulins (g/dl), median (range)	0.8 (0.2–1.5)	0.9 (0.3–1.4)	0.0612
Conditioning, n (%)			0.5904
Immunosuppressive	1 (2)	3 (3.8)	
Myeloablative	27 (52.9)	37 (46.2)	
Reduced intensity	22 (43.1)	40 (50)	
Donor, n (%)			0.9915
Cord blood	1 (2)	3 (3.8)	
Haploidentical	2 (3.9)	4 (5)	
Ident. sibling	5 (9.8)	8 (10)	
Matched unrelated	26 (51)	41 (51.2)	
Mismatched unrelated	17 (33.3)	24 (30)	
Source, n (%)			0.4734
Bone marrow	2 (3.9)	8 (10)	
Cord blood	1 (2)	3 (3.8)	
Peripheral blood	48 (94.1)	69 (86.3)	
CMV serological status patient/donor			0.5110
Positive/Positive	24 (47.1)	36 (45)	
Positive/negative	21 (41.2)	32 (40)	

Table 1. continued

Characteristics	Rituximab, N = 51	No Rituximab, N = 80	P
Negative/positive	3 (5.9)	2 (2.5)	
Negative/Negative	3 (5.9)	10 (12.5)	
ATG, n (%)	41 (80.4)	71 (88.7)	0.1853
N CD34+ infused x 10 ⁶ /kg, median (range)	4.7 (0.1–6.8)	4.6 (0.1–8.1)	0.8212
Total nucleated cells infused x 10 ⁶ /kg, median (range)	6.6 (0.3– 14.6)	6.5 (0.3– 28.4)	0.8247
Anti-donor isohemagglutinins titer	Rituximab, N = 48	No Rituximab, N = 59	P
Titer, median (IQR)	1:128 (1:32– 1:512)	1:64 (1:16– 1:256)	0.1920
<=1:128	29 (60.4)	39 (66.1)	
>1:128	19 (39.6)	20 (33.9)	

METHODS

Patients

We performed a single-center retrospective analysis on 131 consecutive patients who received major or bidirectional ABO-incompatible allogeneic HSCT at the Hematology and Bone Transplant Unit, ASST Papa Giovanni XXIII, Bergamo between 1st January 2013 and 31st December 2019. All patients included in this study signed an informed consent to allow the scientific use of their transplant data to the EBMT (European Bone Marrow Transplantation) database [IRB00003888].

ABO incompatibility prophylaxis

From September 2016 to December 2019, patients with major ABO incompatibility were evaluated for rituximab infusion before transplant. Of the 66 patients transplanted with ABO mismatch after this date, 51 patients received rituximab during the conditioning regimen, while 15 patients did not receive any additional treatment. The decision to omit rituximab in these 15 patients was due to a low anti-donor isohemagglutinins titer before transplant (<1:64). These patients were included in the control group. Similarly, the decision to administer rituximab to 51 patients was made on a combination of factors, including the presence of a high anti-donor isohemagglutinins titer (median titer 1:128) and clinical factors such as the combination of donor-patient blood groups, the disease status at transplant and the clinical infection history. Forty-eight patients received a single administration of rituximab 375 mg/m² on day -7 before transplant while 3 patients received two separate infusions of rituximab 375 mg/m² due to a very high anti-donor isohemagglutinin titer (375 mg/m² at day -7 and -1 before transplant).

The control group (80 patients) was made by all consecutive patients with ABO incompatibility transplanted between January 2013 and August 2016 (65 patients) and includes a small series of 15 patients transplanted between September 2016 and July 2019 who did not receive rituximab because of a low anti-donor isohemagglutinins titer before transplant.

Engraftment definitions, transfusion policy, GVHD, infection prophylaxis and intravenous immunoglobulin infusion policy are available in the Supplementary Material.

Statistical analysis

Categorical characteristics at allogeneic HSCT were reported with absolute and percentage frequencies and compared, between rituximab or no rituximab treatment, with the Chi-squared test or Fisher's exact test. Continuous variables were presented as median with range or interquartile range (IQR) and compared using the Mann-Whitney *U* test. Overall survival (OS) and Disease-free survival (DFS) were estimated with the Kaplan-Meier method and compared using the log-rank test with 95% confidence intervals (CIs). Cumulative incidence of relapse (CIR), transplant-related mortality (TRM), and relapse mortality (RM) were estimated using the

cumulative incidence function, considering death as a competing event for CIR, relapse as a competing event for TRM and transplant-related death as competing event for RM, using Gray's test to assess differences between groups. The median time to RBC transfusion independence, neutrophil engraftment, and platelet engraftment was estimated with the cumulative incidence function, considering death as a competing event. To calculate time to transfusion independence patients were also censored at the time of eventual relapse, sepsis, or grade IV acute GVHD. Univariate and multivariable analyses on transfusion independence were performed by fitting Fine-Gray sub-distribution hazard models. The proportional hazard assumption was verified for all estimated models. A hazard ratio with 95% confidence intervals was reported. In this context, the thresholds used to categorize the number of CD34+ infused and the number of total nucleated cells infused were identified through receiver operating characteristic analysis, maximizing Youden's index. Other outcomes were compared between rituximab or no rituximab treatment with chi-squared test or Mann-Whitney U test, as opportune. *P* values were two-sided, with a 5% significance level. Statistics were performed with R software, version 3.5.0.

RESULTS

Study population

The main characteristics of the study population are listed in Table 1. The median follow-up was 16 months for the rituximab group and 25 months for the control group. The ABO-match combinations between patients and donors are listed in Supplementary Table 1.

Red blood cell transfusions and engraftment

The median number of RBC units transfused after transplant was 6 (range 0–145) and 8 (range 0–150) respectively in the rituximab and the control group (*p* = 0.22). RBC transfusion independence at 6 months was reached respectively by 41 (80%) and 48 (60%) patients in the rituximab and the control group (*p* = 0.03) (Fig. 1). In addition, the median time to transfusion independence was significantly reduced for patients in the rituximab group (1 month, 95% CI, 0.5–2), compared to the control group (3.2 months, 95% CI 1.5–3.2, *p* = 0.02). The median time to neutrophil engraftment was not clinically different among the two groups (15 days (95% CI, 14–16) and 14 days (95% CI, 13–15) respectively, *p* = 0.03) (Fig. 2a). The median time to platelet engraftment was 15 days (95% CI 14–18) and 13 days (95% CI, 13–16) respectively for the rituximab and the control group (Fig. 2b, *p* = 0.07).

PRCA was observed in 1 (2%) patient in the rituximab group and 5 (6%) patients in the control group (*p* = 0.49). None of the

patients experienced immune hemolytic anemia after transplant. Primary graft failure was observed uniquely in one patient in the control group. A secondary graft failure was documented in 2 patients in the rituximab group and 2 patients in the control group. Two patients in the rituximab group and 3 patients in the control group experienced poor graft function.

Post-transplant immune reconstitution and infections

At day 90 and 180 after transplant, the median number of peripheral blood CD19 + B cells was significantly inferior for patients in the rituximab group (0/mcl, range 0–42 at day+90 and 1/mcl, range 0–420 at day +180) compared to the control group (27/mcl, range 0–1369 at day +90 and 47/mcl, range 0–689 at day +180). This difference was no longer evident at day +360 (112/mcl, range 0–647 in the rituximab group and 112/mcl, range 0–774 in the control group, *p* = 0.89) (Fig. 3). The number of peripheral blood CD4+ cells was similar in the two groups at any time point (Table 2). The median value of serum gamma globulin at day +90 and +180 was significantly lower in the rituximab group (0.6 g/dl versus 0.8 g/dl at day +90, *p* = 0.0002 and 0.4 g/dl

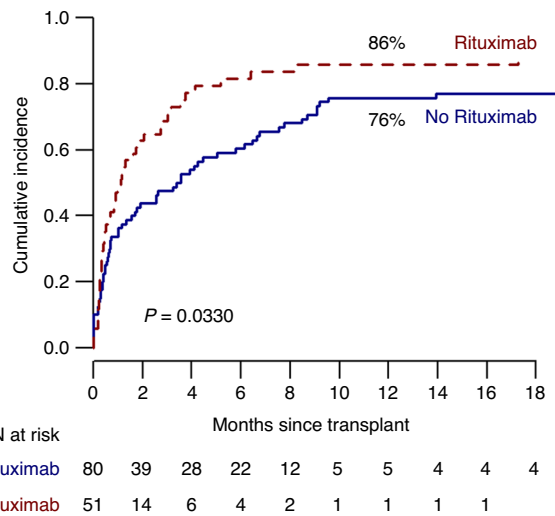


Fig. 1 Time to red blood cell transfusion independence. Time to red blood cell transfusion independence after transplant in patients treated with (dotted line) or without (continuous line) rituximab.

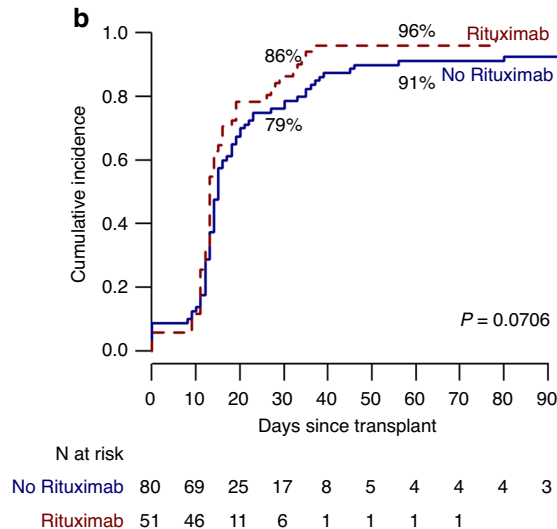
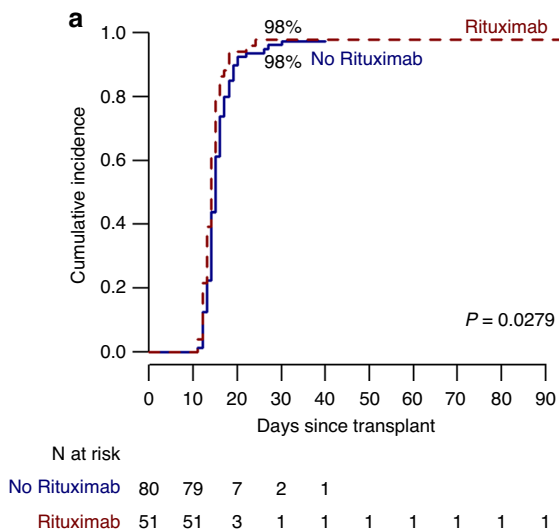


Fig. 2 Neutrophil and platelet engraftment. Time to neutrophil (a) and platelet (b) engraftment in patients treated with (dotted line) or without (continuous line) rituximab.

versus 0.7 g/dl at day +180, $p < 0.0001$), while at day +360 the gamma globulin value was similar in the two groups (0.5 g/dl in the rituximab group and 0.7 g/dl in the control group, Table 2).

Intravenous immunoglobulin (IVIG) infusion after transplant was necessary for 32 (63%) patients in the rituximab group and 39 (49%) patients in the control group ($p = 0.16$). The median number of post-transplant IVIG infusions received by patients was 1.5 (1–12) and 2 (1–22) respectively in the rituximab and the control group ($p = 0.25$).

Post-transplant CMV reactivation was detected in 27 (53%) patients in the rituximab group and 15 (19%) patients in the control group ($p < 0.001$); however, none of the patients in the rituximab group developed CMV disease, while only one patient in the control group developed CMV disease.

Epstein-Barr virus reactivation was detected respectively in 1 (2%) and 7 (9%) of patients in the rituximab and the control group ($p = 0.15$). None of the patients in both groups developed post-transplant lymphoproliferative disorder (PTLD).

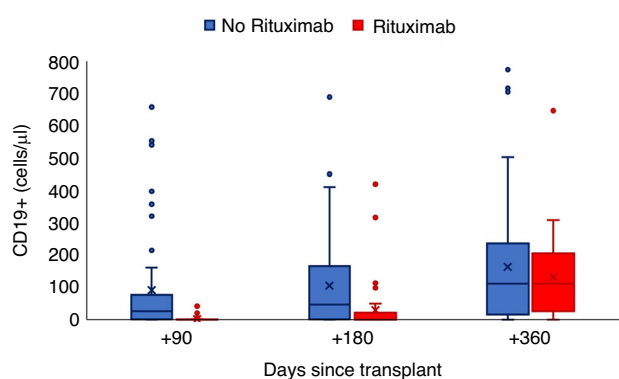


Fig. 3 Post-transplant B cell reconstitution. The number of CD19+ B cells/mcl determined at +90 days, +180 days and +360 days after transplant.

Episodes of infections of any grade (including bacterial, fungal, or viral infections) occurring within the first 180 days after transplant were recorded in 22 patients (43%) in the rituximab group and 36 patients (45%) in the control group.

Transplant-related complications

Grade II–IV acute GVHD developed in 15 (29.4%) patients in the rituximab group and 23 (28.8%) patients in the control group ($p = 0.93$), while chronic GVHD was observed in 18 (35%) and 29 (36%) patients respectively in the rituximab and the control group ($p = 0.91$). Moderate to severe chronic GVHD was recorded in 12 (24%) and 19 (24%) patients respectively in the rituximab and the control group. Deaths and causes of death are listed in Supplementary Table 2.

Transplant outcomes

There was no difference in OS for patients treated with or without rituximab (respectively, 68 and 61%), ($p = 0.41$) (Fig. 4a). The estimated 2-year Disease Free Survival (DFS) was 55% in the rituximab group and 50% in the control group ($p = 0.47$) (Fig. 4b). The estimated 2-year Relapse Incidence was 30% in the rituximab group and 36% in the control group ($p = 0.48$) (Fig. 4c). Finally, the estimated 2-year Transplant Related Mortality was 15% in the rituximab group and 13% in the control group ($p = 0.79$) (Fig. 4d).

Factors influencing transfusion independence

On univariable analysis (Table 3), factors positively associated with the reaching of transfusion independence were the use of rituximab (RR 1.5, 95% CI 1.01–2.23 $p = 0.045$) and having received at transplant a total number of CD34⁺ cells $> 3.7 \times 10^6$ /kg (RR 1.58, 95% CI 1.02–2.45, $p = 0.039$), while a disease status at transplant other than complete remission was a risk factor for delayed transfusion independence (RR 0.54, 95% CI 0.37–0.81, $p = 0.002$ for active disease; RR 0.27, 95% CI 0.09–0.77, $p = 0.015$ for chronic phase). Univariable analysis of the impact of anti-donor isohemagglutinins titer demonstrated that patients with a pre-transplant higher titer ($> 1:128$) were at higher risk for delayed

Table 2. Post-transplant peripheral blood CD19⁺ cells, CD4⁺ cells and gamma globulin recovery.

	Rituximab, N = 51	No Rituximab, N = 80	P
Day + 90			
Total lymphocytes	645 (219–4169)	862 (77–4269)	0.4563
CD19/mcl	0 (0–42)	27 (0–1369)	<0.0001
CD19%	0 (0–9.6)	2.5 (0–107.6)	<0.0001
CD4/mcl	110 (4–971)	119 (3–1011)	0.6247
CD4%	16 (1.3–60.3)	12.5 (2.1–47.1)	0.0958
Gamma_globulin (g/dl)	0.6 (0.3–1.4)	0.8 (0.1–2.1)	0.0002
Day + 180			
Total lymphocytes	1258 (157–3488)	1130 (91.3–6060)	0.5237
CD19/mcl	1 (0–420)	47 (0–689)	0.0001
CD19%	0.1 (0–23.2)	4.1 (0–65.9)	<.0001
CD4/mcl	172 (33–696)	145 (5–3486)	0.3279
CD4%	16.1 (5.6–55)	13.7 (3.2–103.1)	0.5072
Gamma_globulin (g/dl)	0.4 (0.1–1.1)	0.7 (0.1–2)	<.0001
Day + 360			
Total lymphocytes	1838 (320–4027)	1557 (201–8528)	0.2293
CD19/mcl	112 (0–647)	112 (0–774)	0.8929
CD19%	5.9 (0–21.1)	8 (0–46.7)	0.6765
CD4/mcl	315.5 (87–885)	215 (35–2046)	0.0787
CD4%	18.2 (6.8–41.4)	16.6 (5.9–57.5)	0.2075
Gamma_globulin (g/dl)	0.5 (0.1–1.6)	0.7 (0.1–5.3)	0.0809

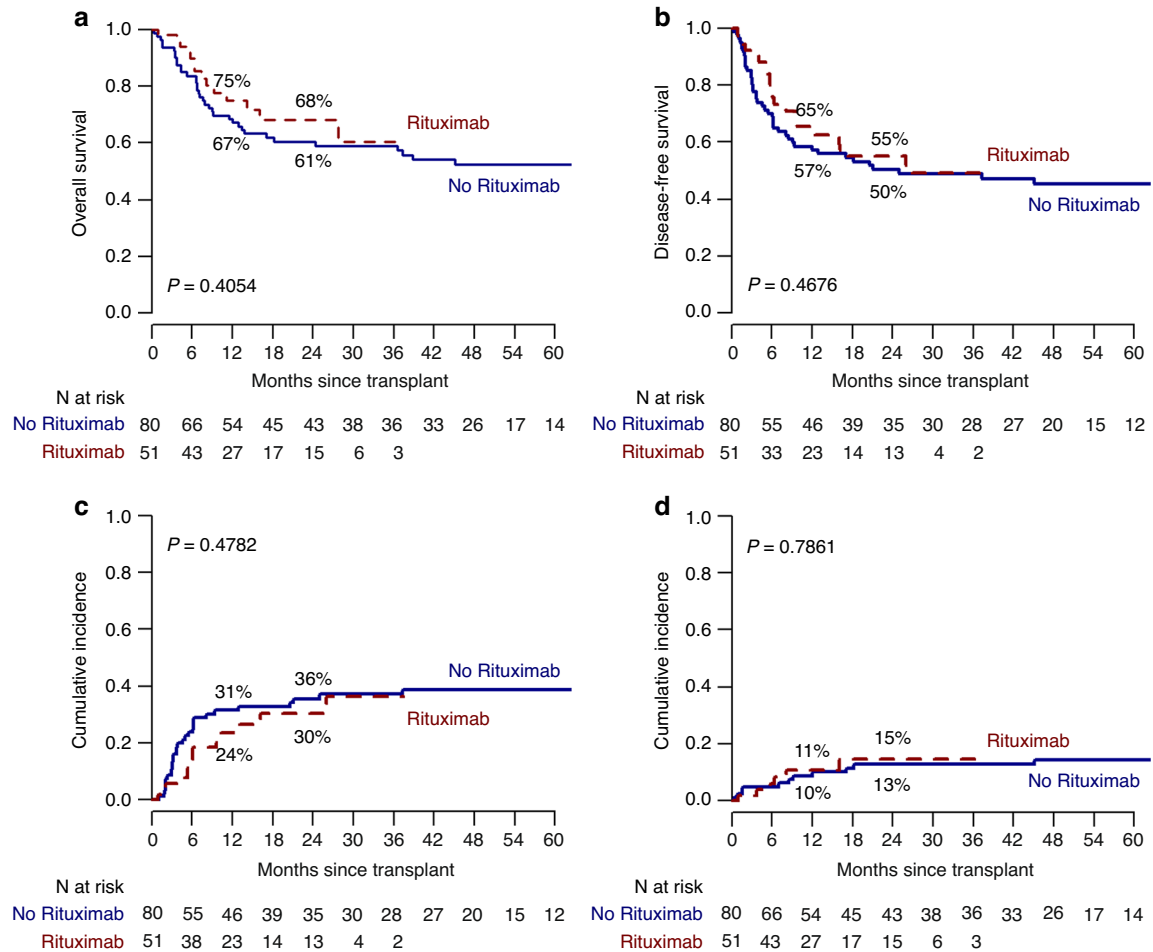


Fig. 4 Clinical outcomes according to rituximab added to the conditioning regimen. Overall survival (a), disease-free survival (b), cumulative incidence of relapse (c), and transplant-related mortality (d). No rituximab (continuous line), rituximab (dotted line).

transfusion independence (HR 0.64, 95% CI 0.43–0.95, $p = 0.029$). We also separately analyzed the impact of anti-donor isohemagglutinins titer in the two study groups and this association was confirmed only in the rituximab group (HR 0.45, 95% CI 0.24–0.86, $p = 0.016$), but not in the control group (HR 0.78, 95% CI 0.46–1.31, $p = 0.35$). By multivariable analysis, rituximab use, disease status at transplant, the total number of CD34+ cells infused at transplant and anti-donor isohemagglutinins titer maintained their prognostic significance on transfusion independence (Table 3).

DISCUSSION

In this retrospective, single-center study we originally tested the hypothesis that pre-transplant administration of rituximab in patients with major or bidirectional ABO incompatibility improves RBC engraftment. Adding rituximab to conditioning resulted in a statistically significant reduction of the time to achieve transfusion independence after transplant compared to a historical cohort of patients who did not receive any preventive strategy.

Rituximab is currently used in ABO-incompatible solid organ transplantation as part of desensitization methods [19, 20]. In the setting of hematopoietic stem cell transplantation, current guidelines for the prevention of delayed RBC engraftment and PRCA include the following measures: to reduce graft RBC to ensure an RBC volume <20 mL, pre-transplant plasma exchange or immunoadsorption [15]. The use of plasma exchange alone for the reduction of isohemagglutinins seems to have only a temporarily limited effect since the rebound of anti-donor isohemagglutinins

titer is described [15]. Pre-transplant immunoadsorption may be performed with an infusion of donor-type fresh frozen plasma positive in A or B antigens to neutralize respective isohemagglutinins [21, 22] or with donor-type RBC transfusion [23, 24]. Although effective, in vivo immunoadsorption requires high expertise in transfusion medicine, and the transfusion reactions reported after this procedure may limit the feasibility of this method.

In our center, plasma exchange or in vivo immunoadsorption do not represent standard procedures in ABO major incompatibility transplants. Only 3 over 55 patients were treated with plasma exchange in the rituximab group, and this prevents us from speculating on a comparison between rituximab and plasma exchange in reducing the time to RBC engraftment.

Another option to treat delayed RBC engraftment after allogeneic stem cell transplantation is Daratumumab, a human monoclonal antibody targeting CD38. In a previous study [25], one patient allografted for myelodysplastic syndrome who developed PRCA and resulted refractory to many treatments was successfully treated with daratumumab. Although intriguing, this result was limited to a single patient, and data about the long-term effects of post-transplant daratumumab are lacking. Furthermore, we tried to explore a prophylactic strategy to prevent the development of delayed RBC engraftment rather than treating the complication itself.

Previous studies reported that, in the context of an ABO incompatible transplant, the anti-donor isohemagglutinin titer was associated with post-transplant transfusion need [17, 26]. Our

Table 3. Univariable and multivariable analysis of factors influencing time to reach transfusion-independence.

Factors	Univariate		Multivariable	
	HR (95%CI)	P	HR (95%CI)	P
Rituximab				
No	1		1	
Yes	1.5 (1.01–2.23)	0.045	1.88 (1.17–3.03)	0.009
Age				
<55	1			
≥55	0.84 (0.57–1.23)	0.36		
Sex				
F	1			
M	1.24 (0.84–1.83)	0.28		
Disease				
AML + MDS + CML	1		1	
ALL + CLL + NHL + MM	1.84 (1.18–2.88)	0.0075	3.06 (1.85–5.06)	<0.0001
Myelofibrosis+AA	0.62 (0.35–1.09)	0.0970	0.56 (0.23–1.37)	0.20
Disease status				
RC	1		1	
Active disease	0.54 (0.37–0.81)	0.0024	0.49 (0.30–0.79)	0.003
Chronic phase	0.27 (0.09–0.77)	0.015	0.28 (0.09–0.90)	0.03
Conditioning				
Myeloablative	1			
Immunosuppressive	0.37 (0.11–1.27)	0.11		
Reduced intensity	0.75 (0.52–1.1)	0.14		
Donor				
Ident. sibling	1		1	
Cord blood	2.22 (1.3–3.78)	0.0033	4.99 (1.93–12.88)	0.0009
Haploidentical	0.91 (0.29–2.86)	0.87	2.79 (0.81–9.64)	0.11
Matched unrelated	1.24 (0.73–2.12)	0.42	1.74 (0.81–3.74)	0.16
Mismatched unrelated	1.68 (0.97–2.93)	0.066	1.17 (0.49–2.79)	0.72
ATG				
No	1			
Yes	1.28 (0.72–2.27)	0.41		
N CD34+ infused				
<3.7	1		1	
>3.7	1.58 (1.02–2.45)	0.039	1.88 (1.01–3.48)	0.05
Total nucleated cells infused				
<4	1			
>4	1.53 (0.86–2.73)	0.15		
Anti-donor isohemagglutinins titer				
≤1:128	1		1	
>1:128	0.64 (0.43–0.95)	0.029	0.61 (0.37–0.99)	0.05

study confirms this observation since there was a positive correlation between a higher titer (>1:128) and delayed RBC engraftment by univariable and multivariable analysis. We also analyzed this correlation separately in the two groups, and we observed this correlation only in the rituximab group but not in the control group. We hypothesize that the rather lower median anti-donor isohemagglutinin titer of the control group could prevent the finding of a correlation between the titer and the transfusion need. On the other hand, the positive correlation between the anti-donor titer and a late RBC engraftment in the rituximab group suggests that pre-transplant infusion of rituximab alone may be insufficient to prevent a delayed RBC engraftment in patients with an extremely high anti-donor isohemagglutinin titer,

where rituximab infusion could be usefully combined with other preventive strategies.

We did not find a significant difference in the incidence of immunological complications such as PRCA or post-transplant immune hemolytic anemia; this observation may be due to the limited number of patients enrolled in this study and requires further studies.

This study has several limitations that deserve to be analyzed, in particular the retrospective and monocentric nature of the study, and the heterogeneity of the patient's population. Moreover, the decision to administer rituximab in patients with major or bidirectional ABO incompatibility was started in 2016 and since then 15 out of 66 patients did not receive rituximab because of a

low anti-donor isohemagglutinins titer. We also acknowledge that the control group was not made up of consecutive patients. Despite this negative selection bias, the rituximab group seemed to have a better outcome concerning time to RBC engraftment compared with the control group, so we may speculate that a comparison between more homogeneous groups should at least confirm the results of this study. A matched analysis for anti-donor isohemagglutinins titer would resolve this issue but it was not statistically feasible due to the small number of patients enrolled.

Despite the better time to RBC engraftment, we could not demonstrate a significant reduction in the median number of RBC units transfused after transplant for patients treated with rituximab. We are tempted to speculate that this may be related to the high heterogeneity of the patient populations and the broad variability of the number of RBC units required by the two groups.

The use of rituximab in the peri-transplant setting can potentially increase the infection risk. In our study, patients who received rituximab experienced a significant impairment in the post-transplant CD19⁺ B cells reconstitution up to 180 days after transplant, which completely resolved at day +360 after transplant and the same applied to gamma globulin level. Nevertheless, this result did not translate into a greater necessity to infuse IVIG in the rituximab group, and the gamma globulin level was similar in the two groups at day +360 after transplant.

The B cell reconstitution impairment in the rituximab group was associated with an increased rate of CMV reactivations. With the use of pre-emptive therapy, none of the patients in the rituximab group experienced CMV disease. The problem of CMV reactivation could probably be mitigated by the introduction of prophylaxis with Letemovir, which was not available in the years investigated in this study. Moreover, it must be considered that rituximab and Letemovir prophylaxis significantly increase the economic burden of the transplant procedure.

Previous reports suggested that rituximab administration before transplant may have a role in reducing the risk of both acute [27, 28] and chronic GVHD [29, 30]. In our study, we did not find a significant difference in the incidence of acute or chronic GVHD in the two study groups. This could be explained by the broad use of anti-thymocyte globulin for the prophylaxis of GVHD in the two study groups.

In conclusion, the result of this study suggests that rituximab added to conditioning regimens is feasible, safe, and can improve time to RBC engraftment after a major ABO-incompatible transplant. Additional studies are needed to understand the role of rituximab on infection risk and long-term effects on engraftment and transplant outcome.

DATA AVAILABILITY

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

REFERENCES

1. Tekgündüz SA, Özbek N. ABO blood group mismatched hematopoietic stem cell transplantation. Vol. 54, *Transfusion and Apheresis Science*. Elsevier Ltd; 2016. p. 24–9.
2. Booth GS, Gehrie EA, Bolan CD, Savani BN. Clinical guide to ABO-incompatible allogeneic stem cell transplantation. Vol. 19, *Biology of Blood and Marrow Transplantation*. 2013. p. 1152–8.
3. Aung FM, Lichtiger B, Bassett R, Liu P, Alousi A, Bashier Q, et al. Incidence and natural history of pure red cell aplasia in major ABO-mismatched haematopoietic cell transplantation. *Br J Haematol*. 2013;160:798–805.
4. Ozkurt ZN, Yegin ZA, Yenicesu I, Aki SZ, Yagci M, Sucak GT. Impact of ABO-incompatible donor on early and late outcome of hematopoietic stem cell transplantation. *Transpl Proc*. 2009;41:3851–8.
5. Worel N. ABO-mismatched allogeneic hematopoietic stem cell transplantation. Vol. 43, *Transfusion Medicine and Hemotherapy*. S. Karger AG; 2016. p. 3–12.
6. Kimura F, Sato K, Kobayashi S, Ikeda T, Sao H, Okamoto S, et al. Impact of ABO-blood group incompatibility on the outcome of recipients of bone marrow transplants from unrelated donors in the Japan Marrow Donor Program. *Haematologica*. 2008;93:1686–93.
7. Canals C, Muñoz-Díaz E, Martínez C, Martino R, Moreno I, Ramos A, et al. Impact of ABO incompatibility on allogeneic peripheral blood progenitor cell transplantation after reduced intensity conditioning. *Transfus (Paris)*. 2004;44:1603–11.
8. Kanda J, Ichinohe T, Matsuo K, Benjamin RJ, Klumpp TR, Rozman P, et al. Impact of ABO mismatching on the outcomes of allogeneic related and unrelated blood and marrow stem cell transplantations for hematologic malignancies: IPD-based meta-analysis of cohort studies. *Transfus (Paris)*. 2009;49:624–35.
9. Seebach JD, Stussi G, Passweg JR, Loberiza FR, Gajewski JL, Keating A, et al. ABO blood group barrier in allogeneic bone marrow transplantation revisited. *Biol Blood Marrow Transplant*. 2005;11:1006–13.
10. Remberger M, Watz E, Ringdén O, Mattsson J, Shanwell A, Wikman A. Major ABO blood group mismatch increases the risk for graft failure after unrelated donor hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2007;13:675–82.
11. Kim JG, Sohn SK, Kim DH, Baek JH, Lee KB, Min WS, et al. Impact of ABO incompatibility on outcome after allogeneic peripheral blood stem cell transplantation. *Bone Marrow Transpl*. 2005;35:489–95.
12. Blin N, Traineau R, Houssin S, Peffault de Latour R, Petropoulou A, Robin M, et al. Impact of donor-recipient major ABO mismatch on allogeneic transplantation outcome according to stem cell source. *Biol Blood Marrow Transplant*. 2010;16:1315–23.
13. Bacigalupo A, Van Lint MT, Occhini D, Margiocco M, Ferrari G, Pittaluga PA, et al. ABO compatibility and acute graft-versus-host disease following allogeneic bone marrow transplantation. *Transplantation*. 1988;45:1091–4.
14. Keever-Taylor CA, Bredeson C, Loberiza FR, Casper JT, Lawton C, Rizzo D, et al. Analysis of risk factors for the development of GVHD After T cell-depleted allogeneic BMT: effect of HLA disparity, ABO incompatibility, and method of T-cell depletion medical college of wisconsin bone marrow transplant program, Departments of 1 Medicine, 3 Pediatrics, and 4 Radiation Oncology and the. Vol. 7, *Biology of Blood and Marrow Transplantation*. 2001.
15. Migdady Y, Pang Y, Kalsi SS, Childs R, Arai S. Post-hematopoietic stem cell transplantation immune-mediated anemia: a literature review and novel therapeutics. *Blood Adv*. 2022;6:2707–21.
16. Benjamin RJ, McGurk S, Ralston MS, Churchill WH, Antin JH. ABO incompatibility as an adverse risk factor for survival after allogeneic bone marrow transplantation. *Transfus (Paris)*. 1999;39:179–87.
17. Stussi G, Halter J, Bucheli E, Valli PV, Seebach L, Gmür J, et al. Prevention of pure red cell aplasia after major or bidirectional ABO blood group incompatible hematopoietic stem cell transplantation by pretransplant reduction of host anti-donor isoagglutinins. *Haematologica*. 2009;94:239–48.
18. Kopyńska A, Helbig G, Frankiewicz A, Grygoruk-Wiśniowska I, Kyrzc-Krzemień S. Rituximab is highly effective for pure red cell aplasia and post-transplant lymphoproliferative disorder after unrelated hematopoietic stem cell transplantation. *Wspolczesna Onkologia*. 2012;16:215–7.
19. Lee HR, Kim K, Lee SW, Song JH, Lee JH, Hwang SD. Effect of rituximab dose on induction therapy in ABO-incompatible living kidney transplantation: a network meta-analysis. *Medicine*. 2021;100:e24853.
20. Shishido S, Asanuma H, Tajima E, Hoshinaga K, Ogawa O, Hasegawa A, et al. ABO-incompatible living-donor kidney transplantation in children. *Transplantation*. 2001;72:1037–42.
21. Curley C, Pillai E, Mudie K, Western R, Hutchins C, Durrant S, et al. Outcomes after major or bidirectional ABO-mismatched allogeneic hematopoietic progenitor cell transplantation after pretransplant isoagglutinin reduction with donor-type secretor plasma with or without plasma exchange. *Transfusion*. 2012;52:291–7.
22. Quek J, Lee JJ, Lim FL, Diong C, Goh YT, Gopalakrishnan S, et al. Donor-type fresh frozen plasma is effective in preventing hemolytic reaction in major ABO incompatible allogeneic stem cell transplant. *Transfusion*. 2019;59:335–9.
23. Mehta P, Ramprakash S, Raghuram CP, Trivedi D, Dhanya R, Agarwal RK, et al. Pre-transplant donor-type red cell transfusion is a safe and effective strategy to reduce isohemagglutinin titers and prevent donor marrow infusion reactions in major ABO-mismatched transplants. *Ann Hematol*. 2021;100:2071–8.
24. Scholl S, Klink A, Mügge LO, Schilling K, Höffken K, Sayer HG. Safety and impact of donor-type red blood cell transfusion before allogeneic peripheral blood progenitor cell transplantation with major ABO mismatch. *Transfusion*. 2005;45:1676–83.
25. Chapuy CI, Kaufman RM, Alyea EP, Connors JM. Daratumumab for delayed red-cell engraftment after allogeneic transplantation. *N. Engl J Med*. 2018;379:1846–50.

26. Nam M, Hur M, Kim H, Lee TH, Lee GH, Yoon S, et al. Clinical impact of recipient-derived isoagglutinin levels in ABO-incompatible hematopoietic stem cell transplantation. *J Clin Med.* 2023;12:458.
27. Crocchiolo R, Castagna L, El-Cheikh J, Helvig A, Fürst S, Faucher C, et al. Prior rituximab administration is associated with reduced rate of acute GVHD after in vivo T-cell depleted transplantation in lymphoma patients. *Exp Hematol.* 2011;39:892–6.
28. Wei X, Xie Y, Jiang R, Li H, Wu H, Zhang Y, et al. The impact of Rituximab administered before transplantation in patients undergoing allogeneic hematopoietic stem cell transplantation: a real-world study. *Front Immunol.* 2022;13:4878.
29. Johnston HF, Xu Y, Racine JJ, Cassady K, Ni X, Wu T. et al. Administration of anti-CD20 mAb is highly effective in preventing but ineffective in treating chronic graft-versus-host disease while preserving strong graft-versus-leukemia effects. *Biol Blood Marrow Transplant.* 2014;20:1089–103.
30. van Dorp S, Pietersma F, Wöfl M, Verdonck LF, Petersen EJ, Lokhorst HM, et al. Rituximab treatment before reduced-intensity conditioning transplantation associates with a decreased incidence of extensive chronic GVHD. *Biol Blood Marrow Transplant.* 2009;15:671–8.

AUTHOR CONTRIBUTIONS

MCF and AR conceived and designed the analysis and wrote the paper. AW and MCF collected the data. CP performed the statistical analysis. AG, MCM, AA, FL, and MCF cared for patients. AR supervised the project. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary information The online version contains Supplementary Material available at <https://doi.org/10.1038/s41409-024-02247-w>.

Correspondence and requests for materials should be addressed to Maria Chiara Finazzi.

Reprints and permission information is available at <http://www.nature.com/reprints>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2024