Rituximab therapy after pediatric hematopoietic stem cell transplantation can cause prolonged B-cell impairment and increases the risk for infections - a retrospective matched cohort study

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SUPPLEMENT

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I. Supplement figure legends

Figure S1 - B cell recovery and function is impeded by rituximab treatment after pediatric HSCT.

(A) CD4+ & CD8+ T cell recovery and IgG & IgM blood level development over time after HSCT for RTX and Ctrl groups. Mean & standard error of mean per group and day after HSCT. Patients were allocated to either rituximab or control group for each time point depending on rituximab therapy initiation and a time- and group-matched mixed model analysis was computed in R version 1.4.1717 (R foundation) for group comparison. (B, C) Comparison of the primary endpoints "Time point of B cell recovery" and "Last day of IgG substitution" between subgroups of rituximab and control group who did or did not have an EBV infection. Independently from rituximab treatment, EBV infection did not delay B cell reconstitution or independence of immunoglobulin substation significantly in either RTX or Ctrl group. (D) Fine and Gray competitive risk analysis was performed to calculate appropriate hazard ratios for time-to-event end points in R version 1.4.1717 (R foundation). For consideration of potential bias caused by early group allocation and achievement of endpoints before rituximab treatment, this analysis was performed with reference to the first day of rituximab treatment. Curves depict the rate of patients at a certain time point after rituximab treatment initiation that achieved either "B cell reconstitution", "IgG levels >5g/l without IgG substitution" or "Receiving no more IgG substitutions for rituximab or control groups". "n=" refers to the number of patients that did not achieve the endpoint or did not have a dropout or end of observation period yet at the respective time point. *HSCT: Hematopoietic stem cell transplantation, RTX: Rituximab (group), Ctrl: Control group, EBV: Epstein Barr virus, dHSCT: HSCT treatment timeline day (d0 = day of HSCT),* HR: hazard ratio, CI: confidence interval. *Significance levels: *** : p<0.001; ** : p<0.01; * : p<0.05; n.s. : not significant*

Figure S2 - Primary end points do not correlate with number of rituximab doses received, but a subgroup of 9 patients could be identified who developed especially prolonged B cell impairment after rituximab treatment.

(A) Correlation analysis for the "number of received rituximab doses", "time point of first rituximab treatment" and "maximal measured EBV copy number in the blood" against different primary endpoints ("time point of B cell recovery", "time to B cell recovery after last rituximab dose received", "time point of IgG levels >5g/l without IgG substitution" and "time point of last IgG"). Every dot represents one patient out of the rituximab group. Patients with a dropout event before reaching the end point of interest were excluded from the analysis. For correlation analysis, Spearman correlation was computed in *GraphPad PRISM 8 & 9 (GraphPad Software, San Diego, USA)* **(B)** Testing of different subgroup definitions for prolonged B cell damage via allocation of patients from the rituximab and control groups to different subgroups. I - Immunoglobulin-substitution after B cell recovery, II- Immunoglobulin-substitution at least 365 days after last RTX, III- IgG <2SD at least 365 days after last RTX, IV- Immunoglobulin-substitution at least 365 days after last RTX and until end of OP. **(C)** Inverse Kaplan-Meier curve depicting the rate of patients that achieved independence of IgG substitution from the group of patients that were observed at least 365 after last rituximab treatment (or an equivalent time point in the control group). **(D)** CD3+, CD4+, CD8+ T cell & CD19+ B cell, and IgG & IgM blood level development over time after HSCT for prolonged B cell damage, rituximab-control and non-rituximab control groups. Mean & standard error of mean per group and day after HSCT. *RTX: Rituximab (group), EBV: Epstein Barr virus, Max.: maximal.* PBD: prolonged B cell damage subgroup, *RTX-Ctrl: rituximab-control subgroup.* dHSCT: HSCT treatment timeline day (d0 = day of HSCT). *Significance levels: **** : p<0.0001, *** : p<0.001, ** : p<0.01; * : p<0.05; n.s. : not significant.*

II. Supplement Figures

Figure S1

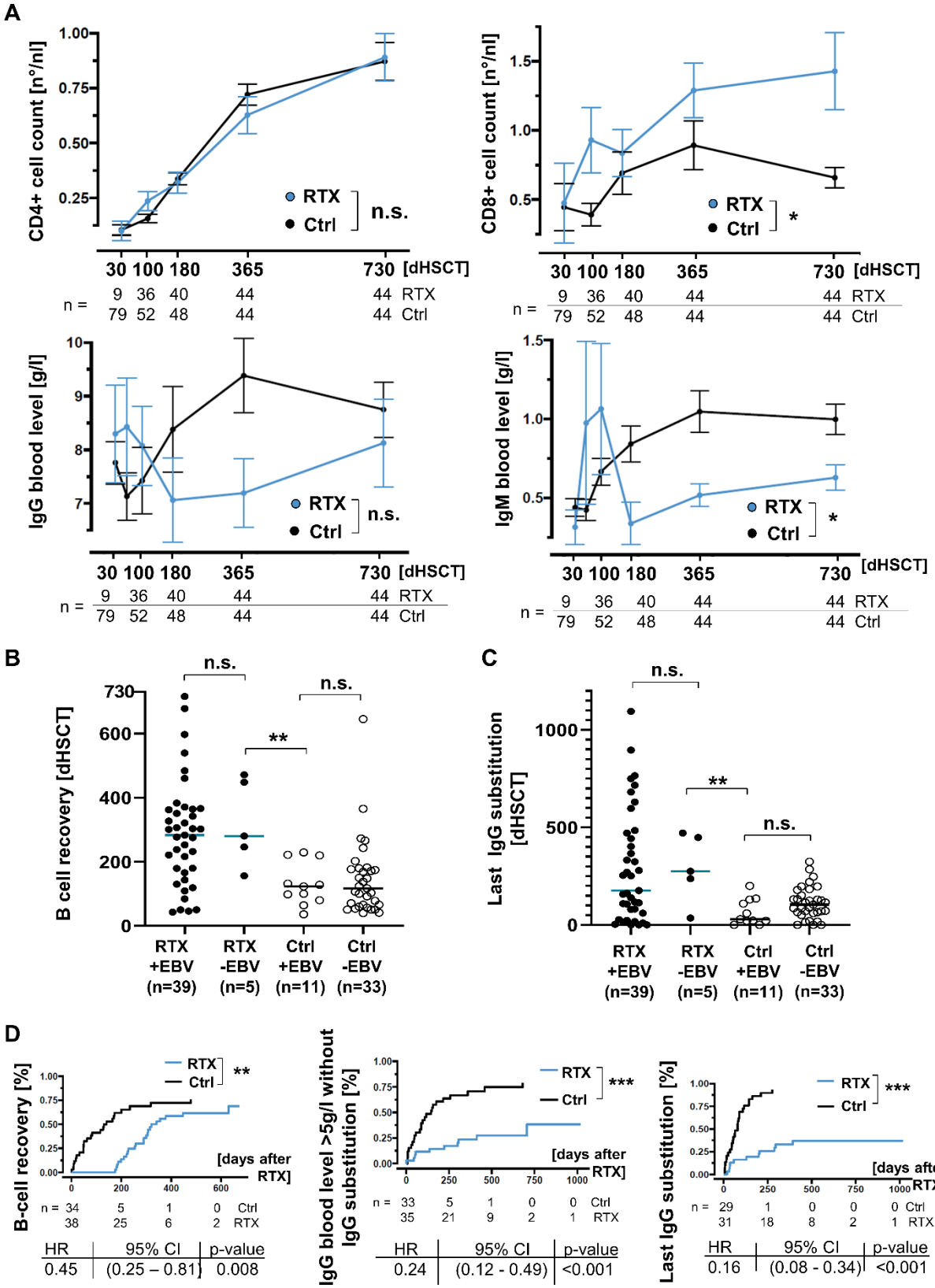
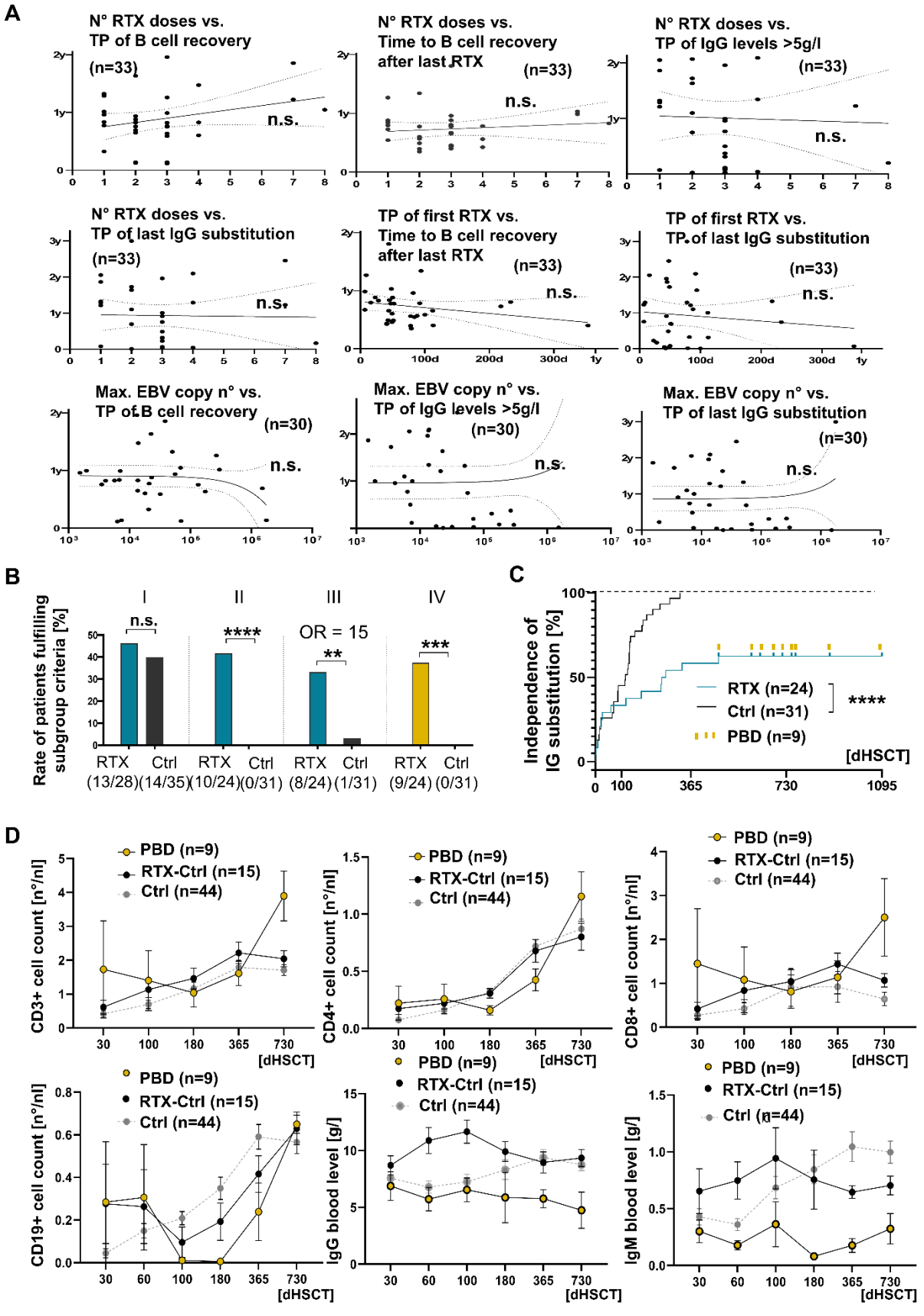


Figure S2


A
N° RTX doses vs. TP of B cell recovery
(n=33)
n.s.
N° RTX doses vs. Time to B cell recovery after last RTX
(n=33)
n.s.
N° RTX doses vs. TP of IgG levels >5g/l
(n=33)
n.s.
N° RTX doses vs. TP of last IgG substitution
(n=33)
n.s.
TP of first RTX vs. Time to B cell recovery after last RTX
(n=33)
n.s.
TP of first RTX vs. TP of last IgG substitution
(n=33)
n.s.
Max. EBV copy n° vs. TP of B cell recovery
(n=30)
n.s.
Max. EBV copy n° vs. TP of IgG levels >5g/l
(n=30)
n.s.
Max. EBV copy n° vs. TP of last IgG substitution
(n=30)
n.s.
B
Rate of patients fulfilling subgroup criteria [%]
I
II
III
IV
n.s.
****
OR = 15
**
***
RTX (13/28)
Ctrl (14/35)
RTX (10/24)
Ctrl (0/31)
RTX (8/24)
Ctrl (1/31)
RTX (9/24)
Ctrl (0/31)
C
Independence of IG substitution [%]
RTX (n=24)
Ctrl (n=31)
PBD (n=9)
****
[dHSCT]
D
CD3+ cell count [n°/nl]
CD4+ cell count [n°/nl]
CD8+ cell count [n°/nl]
CD19+ cell count [n°/nl]
IgG blood level [g/l]
IgM blood level [g/l]
PBD (n=9)
RTX-Ctrl (n=15)
Ctrl (n=44)
[dHSCT]

III. Supplement tables

Table S1 - Comparison of study cohorts

A - General Characteristics	Rituximab group	Control group	p-value [a]
Observation period [days] [b]	481 (84 - 1095)	785 (51 - 1095)	0.07
Cumulative days observed [days]	25434	31489	
Number of transplantations in 2018-2020	31	21	
Sex, n (%)			
Male	25 (56.8)	23 (52.3)	0.75
Female	19 (43.2)	21 (47.7)	
Age at transplantation [years] [b]	8 (0 - 24)	5 (0 - 26)	0.27
Patients per age group, n (%)			
<1	2 (4.5)	3 (6.8)	
1-5	21 (47.7)	20 (45.5)	
6-11	6 (13.6)	7 (15.9)	
12-17	11 (25)	11(25)	
18+	4 (9)	3 (6.8)	
Age mismatch for matched-control pairs, n (%)	24 (55,5)		
Age mismatch between adjacent age-groups, n (% of mismatches)	15 (62,5)		
Bodyweight at transplantation [kg] [b]	27 (3-90)	21.4 (5.1-90.6)	0.87
HSCT count, n (%)			
1	42 (95.5)	41 (93.2)	
2	1 (2.3)	2 (4.5)	
3	1 (2.3)	1 (2.3)	
Type of HSCT, n (%)			
MUD	31 (70.5)	31 (70.5)	>0.99
MSD	8 (18.2)	8 (18.2)	
MMRD/haploidentical	3 (6.8)	3 (6.8)	
MRD	2 (4.5)	2 (4.5)	
Source of HSC, n (%)			
BM	20 (45.5)	23 (52.3)	0.25
PBSC	24 (54.5)	21 (47.7)	
Malignancy, n (%)			
Benign	24 (54.5)	20 (45.5)	0.125
Malign	20 (45.5)	24 (54.5)	
HSCT indication, n (%)			
Acute myeloid leukemia	1 (2.3)	5 (11.3)	
Beta thalassemia major	2 (4.5)	2 (4.5)	
Burkitt Lymphoma	2 (4.5)	0 (0)	
Chronic granulomatous disease (CGD)	2 (4.5)	5 (11.4)	
B-cell acute lymphoblastic leukemia	10 (22.7)	10 (22.7)	
Diamond-Blackfan anemia	1 (2.3)	1 (2.3)	
Fanconi anemia	2 (5)	1 (2.3)	
Hemophagocytic lymphohistiocytosis (HLH)	1 (2.3)	0 (0)	
Hyper IgM Syndrome Type I	1 (2.3)	0 (0)	
ICF Syndrome Type 3	1 (2.3)	0 (0)	
Immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) -like Syndrome	1 (2.3)	0 (0)	
Myelodysplastic syndrome (MDS)	3 (6.8)	6 (13.6)	
Glanzmanns thrombasthenia	1 (2.3)	0 (0)	

Hunter syndrome	1 (2.3)	1 (2.2)	
Neuroblastoma	1 (2.3)	1 (2.2)	
Osteopetrosis	1 (2.3)	0 (0)	
Severe aplastic anemia	1 (2.3)	0 (0)	
Sickle cell disease	6 (13.6)	7 (15.9)	
Severe combined immunodeficiency (SCID)	1 (2.3)	1 (2.3)	
STK4 deficiency	1 (2.3)	0 (0)	
T-cell acute lymphoblastic leukemia	1 (2.3)	2 (4.5)	
T-lymphoblastic lymphoma	1 (2.3)	0 (0)	
Yokohama hemoglobinopathy	1 (2.3)	0 (0)	
Castleman disease	0 (0)	1 (2.3)	
X-linked adrenoleukodystrophy	0 (0)	1 (2.3)	
Graft manipulation, n (%)			
None[c]	15 (34.1)	24 (54.5)	
CD34 selection	6 (13.6)	4 (9.1)	
CD34 selection + CD3 addback	2 (4.5)	3 (6.8)	
CD3/CD19 depletion	1 (2.5)	1 (2.5)	
TCRab/CD19 depletion	5 (11.4)	4 (9.1)	
TCRab/CD19 depletion+ CD3 addback	15 (34.1)	6 (13.6)	
TCRab depletion, + CD3 addback	0 (0)	2 (4.5)	
TCRab depletion (any)	20 (45.5)	12 (27.3)	0.008
Blood group (recipient/donor), n (%)			
0-/0-	2 (4.5)	0 (0)	
0+/0+	8 (18.2)	10 (22.7)	
0+/AB-	1 (2.3)	0 (0)	
0+/B+	2 (4.5)	0 (0)	
A-/B+	1 (2.3)	0 (0)	
A+/0-	2 (4.5)	1 (2.3)	
A+/0+	5 (11.4)	5 (11.4)	
A+/A-	1 (2.3)	0 (0)	
A+/A+	10 (22.7)	9 (20.5)	
A+/AB+	1 (2.3)	0 (0)	
AB-/0+	1 (2.3)	0 (0)	
AB+/A+	1 (2.3)	1 (2.3)	
B-/AB+	1 (2.3)	0 (0)	
B+/0+	1 (2.3)	2 (4.5)	
B+/A+	1 (2.3)	2 (4.5)	
B+/AB-	1 (2.3)	0 (0)	
B+/B+	2 (4.5)	1 (2.3)	
A-/0+	0 (0)	1 (2.3)	
A+/B+	0 (0)	2 (4.5)	
AB+/A-	0 (0)	1 (2.3)	
AB+/AB+	0 (0)	1 (2.3)	
B-/A+	0 (0)	1 (2.3)	
B+/0-	0 (0)	1 (2.3)	
HLA x/12, n (%)			
5/6	3 (6.8)	3 (6.8)	
9	8 (18.2)	8 (18.2)	
10	31 (70.5)	31 (70.5)	
12	2 (4.5)	2 (4.5)	
Most common conditioning therapy, n (%)			
Fludarabine, treosulfan, thiotepa,	17 (38.6)	13 (29.5)	
total body irradiation, etoposide,	6 (13.6)	5 (11.3)	

Conditioning regimen containing serotherapy (i.e. anti-thymocyte globulin or alemtuzumab), n (%)	40 (90.9)	36 (81.8)	
Immune suppression regimen containing ciclosporin, n (%)	41 (93.1)	40 (90.9)	
Transplanted CD34+ cells [x10^6/kg BW] [b]	10.21 (2.2 - 55)	7.9 (0.9 - 79)	0.91
Drop out events:			
Relapses, n (%)	3 (6.8)	6 (13.6)	
Non-relapse related deaths, n (%)	6 (13.6)	4 (9.1)	
Non-relapse graft rejections, n (%)	2 (4.5)	0 (0)	
Non-relapse re-transplantations, n (%)	2 (4.5)	0 (0)	
Cause of death, n (%)			
Septic shock	2 (25)	1 (16.6)	
Multi organ failure	3 (37.5)	3 (50)	
Brain death	1 (12.5)	1 (16.6)	
CNS aspergillosis	1 (12.5)	0 (0)	
Not specified	1 (12.5)	1 (16.6)	

B - Rituximab related data and therapy response	Rituximab group	Control group	p-value [a]
First RTX [days after HSCT] [b]	55 (-29 to +353)	-	
Patients with an observation period >365 days after the last RTX, n (%)	24 (54.5)	31 (70.5)	
RTX cycles, n (%) [c]			
1	39 (88.6)	0 (0)	
2	5 (11.4)	0 (0)	
Total n° of RTX doses, n (%) [d]			
1	10 (22.7)	0 (0)	
2	9 (20.5)	0 (0)	
3	14 (31.8)	0 (0)	
4	7 (15.9)	0 (0)	
6	1 (2.3)	0 (0)	
7	2 (4.5)	0 (0)	
8	1 (2.3)	0 (0)	
RTX therapy indication, n (%)			
EBV	41 (83.6)	0 (0)	
Autoimmune hematologic disease	4 (9.1)	0 (0)	
Conditioning/treatment	2 (4.5)	0 (0)	
Conditioning/AIHD	1 (2.3)	0 (0)	
Immune encephalitis	1 (2.3)	0 (0)	
EBV serostatus (recipient/donor), n (%)			
Pos/pos	29 (65.9)	21 (47.7)	
Pos/neg	3 (6.8)	4 (9.1)	
Neg/pos	6 (13.6)	7 (15.9)	
Neg/neg	0 (0)	5 (11.4)	
Pos/missing	2 (4.5)	1 (2.3)	
Neg/missing	1 (2.3)	0 (0)	
Missing/pos	2 (4.5)	4 (9.1)	
Missing/Missing	1 (2.3)	2 (4.5)	
At least one positive	42 (95.5)	37 (84)	0.18
Patients with EBV infection/reactivation	38 (86.4)	11(25)	0.00002
Episodes of EBV infections, n (%) [e]			
0	6 (13.6)	33 (75)	
1	34 (77.3)	10 (22.7)	
2	2 (4.5)	0 (0)	

3	1 (2.3)	0 (0)	
4	1 (2.3)	0 (0)	
5	0 (0)	1 (2.3)	
Maximal EBV viral load [copies/ml] [b]	22600 (1530 - 3100000)	4055 (1860 - 19100)	0.005
Time point of 1st RTX (Ctrl: 1st peak) in patients with EBV infection/reactivation [days after HSCT] [b]	70 (23 - 314)	102 (47 - 418)	0.029
Patients with an EBV load <2000 copies/ml 28 days after 1st RTX (Ctrl: 1st Peak), n (%)	36/38 (94.7)	11/11 (100)	
Time until EBV viral load drops below <50% of value at time point of 1st RTX dose (Ctrl: 1st peak) [days] [b]	2 (0 - 42)	4 (2 - 7)	0.027
Start of RTX therapy (Ctrl: 1st peak) despite EBV viral load <10000copies/ml, n (%)	14/38 (36.8)	7/11 (63.6)	
Start of RTX therapy (Ctrl: 1st peak), despite EBV load <10000copies/ml in patients with systemic steroid treatment, n (%)	10/17 (58.8)	3/4 (75)	
Start of RTX therapy (Ctrl: 1st peak) despite EBV viral load <3000copies/ml, n (%)	5/38 (13.2)	2/11 (18.2)	

C - Immune reconstitution	Rituximab group	Control group	p-value [a]
B cell count <0,01/nl at d365, n (%)	4 (9.1)	0 (0)	
Patients with IgG substitution, n (%)	41 (93.2)	39 (88.6)	
Cumulative IgG dose [g/kg BW] [b]	2.57 (0 - 28.05)	1.04 (0 - 11.33)	0.002
Before first RTX	0.57 (0 - 4.61)	0.46 (0 - 3.59)	0.054
After first RTX	1.66 (0 - 26.85)	0.42 (0 - 11.33)	0.002
Patients that received more than 0,5g/kg BW IgG after first RTX, n (%)	27 (61.4)	19 (43.2)	0.152
IgG levels >5g/l without substitution [days after HSCT] [b]	278 (4 - 1095)	118 (4 - 722)	0.002
Tetanus toxoid IgG 2y after HSCT [IU/ml] [b]	1.1 (0.08 - 5.87) (n=32) [f]	1.4 (0.3 - 9.5) (n=33) [f]	0.22
Pneumococcal capsular polysaccharide IgG1 2y after HSCT [IU/ml] [b]	34 (5 - 102) (n=26) [f]	42 (7 - 172) (n=22) [f]	0.09
Pneumococcal capsular polysaccharide IgG2 2y after HSCT [IU/ml] [b]	7.9 (2.5 – 36.2) (n=26) [f]	11 (2.3 - 83.1) (n=23) [f]	0.21
Neutrophil count >500/µl [days after HSCT] [b]	17 (9 - 38)	19 (8 - 40)	0.22
Neutrophil count >1000/µl [d] [b]	19 (9 - 79)	22 (9 - 292)	0.25
Platelet count >50000/µl without transfusion [days after HSCT] [b]	49 (11-600)	26 (5-451)	0.03
Lymphocyte count at d365 [/nl] [b]	2.17 (0.48 - 5.79)	2.21 (0.9 - 6.37)	0.75
Total chimerism at d365 [%] [b]	100 (32 - 100)	100 (15 - 100)	0.25
CD34 chimerism d365 [%] [b]	100 (30 - 100)	100 (25 - 100)	0.27

D - Secondary complications	Rituximab group	Control group	p-value [a]
Duration of hospitalization for HSCT [days] [b]	61.5 (27 - 411)	59.5 (20 - 191)	0.27
Re-hospitalizations per patient [b, g]	2 (0 - 34)	1 (0 - 9)	0.64
Patients that developed a severe adverse event that was survived, n (%) [h]	6 (13.6)	2 (4.5)	0.29

Patients with a stem cell boost, n (%)	3 (6.8)	6 (13.6)	0.37
Patients with donor lymphocyte infusions, n (%)	6 (13.6)	9 (20.5)	0.55
Number of viral infections (without EBV) with >2000 copies/ml per patient, n (%)			
0	16 (36.4)	28 (63.6)	
1	15 (34.1)	7 (15.9)	
2	11 (25)	5 (11.4)	
3	1 (2.3)	3 (6.8)	
4	0 (0)	1 (2.3)	
5	1 (2.3)	0 (0)	
Patients with ADV infection (>2000copies/ml blood), n (%)	11 (25)	10 (22.7)	0.99
Patients with CMV infection (>2000copies/ml blood), n (%)	14 (31.8)	7 (15.9)	0.14
Patients with HHV6 infection (>2000copies/ml blood), n (%)	0 (0)	0 (0)	0.99
Patients with BKV infection (>2000copies/ml blood/urin), n (%)	14 (31.8)	9 (20.5)	0.27
Patients with antivirals other than rituximab, n (%) [i]	25 (56.8)	14 (31.8)	0.024
Ganciclovir	14 (31.8)	7 (15.9)	
Valganciclovir	7 (15.9)	5 (11.4)	
Foscarnet	12 (27.2)	5 (11.4)	
Cidofovir	11 (25)	11 (25)	
Brincidofovir	4 (9.1)	0 (0)	
Patients with initiation of antivirals other than rituximab, n (%) [i]			
Before first RTX	18 (40.9)	7 (15.9)	0.003
After first RTX	14 (31.8)	10 (22.7)	0.39
Patients receiving virus-specific T cells, n (%)	9 (20.5)	4 (9)	0.23
Neutropenia relapses <1000/µl blood [b, j]	2 (0 - 19)	1 (0 - 8)	0.02
Before first RTX	0 (0 - 5)	0 (0 - 4)	0.15
After first RTX	1 (0 - 17)	0 (0 - 8)	0.09
Mean duration of elevated CRP levels [days] [b]	20 (7 - 160)	23 (3 - 46)	0.9
Patients who developed relevant acute GvHD, n (%) [k]	11 (25)	9 (20.5)	0.98
Patients that received systemic antimycotic treatment in therapeutic dose, n (%)	15 (34.1)	19 (43.2)	0.52
Cumulative number of systemic steroid treatments [b]	1 (0 - 7)	1 (0 - 4)	0.04
Before first RTX	0 (0 - 3)	0 (0 - 3)	0.28
After first RTX	0 (0 - 5)	0 (0 - 3)	0.03
Systemic steroid treatments after HSCT per patient, n (%)			
0	16 (36.4)	22 (50)	0.04
1	11 (25)	15 (34.1)	
2	7 (15.9)	3 (6.8)	
3	6 (13.6)	1 (2.3)	
4	2 (4.5)	3 (6.8)	
5	1 (2.3)	0 (0)	
7	1 (2.3)	0 (0)	
Patients with extra-corporal photopheresis (ECP) procedures, n (%)	10 (22.7)	6 (13.6)	0.45

Patients with applications of vedolizumab, basiliximab or infliximab, n (%)	6 (13.6)	4 (9.1)	0.72
Development of VOD after HSCT	9 (20.5)	7 (15.9)	0.8
Red blood cell concentrates per patient [b]	9 (1 - 143)	6 (1 - 50)	0.17
Total amount of red blood cell concentrates transfused, n	720	445	
Platelet concentrates per patient [b]	16 (1 - 177)	9 (0 - 109)	0.06
Total platelet concentrates transfused, n	1212	730	
E - prolonged B cell damage subgroup analysis	**PBD group**	**RTX-Ctrl group**	**p-value [a]**
General characteristics			
Observation period [days] [b]	716 (471 - 1095)	972 (444 - 1095)	0.052
Age at transplantation [year] [b]	8 (2 - 19)	12 (4 - 21)	0.2
Gender, n (%)			
Male	5 (55.5)	8 (53.3)	
Female	4 (44.4)	7 (46.7)	
Bodyweight at transplantation [kg] [b]	26.5 (11.5 - 90)	30.3 (16.3 - 85.7)	
Type of HSCT, n (%)			
MUD	5 (55.5)	9 (60)	
MSD	4 (44.4)	3 (20)	
MMRD/haploidentical	0 (0)	3 (20)	
Graft manipulation: TCRab depletion (any), n (%)	4 (44.4)	5 (33.3)	0.68
Source of HSC, n (%)			
BM	3 (33.3)	9 (60)	0.4
PBSC	6 (66.7)	6 (40)	
Drop out, n (%)	0 (0)	0 (0)	
HSCT count per patient, n (%)			
1	8 (88.9)	14 (93.3)	
2	1 (11.1)	0 (0)	
3	0 (0)	1 (6.7)	
Malignancy, n (%)			
Benign	4 (44.4)	9 (60)	
Malign	5 (55.6)	6 (40)	
HSCT indication, n (%)			
Acute myeloid leukemia	0 (0)	1 (6.7)	
Beta thalassemia major	0 (0)	1 (6.7)	
B-cell acute lymphoblastic leukemia	3 (33.3)	4 (26.7)	
Fanconi anemia	1 (11.1)	0 (0)	
Hyper IgM Syndrome	1 (11.1)	0 (0)	
ICF Syndrome Type 3	0 (0)	1 (6.7)	
Myelodysplastic syndrome	1 (11.1)	1 (6.7)	
Glanzmanns thrombasthenia	0 (0)	1 (6.7)	
Osteopetrosis	1 (11.1)	0 (0)	
Sickle cell disease	1 (11.1)	5 (33.3)	
T-cell acute lymphoblastic leukemia	1 (11.1)	0 (0)	
Yokohama hemoglobinopathy	0 (0)	1 (6.7)	
PBD - RTX related data			
RTX doses per patient, n (%) [d]	Mean: 2.6	Mean: 3.1	0.2
1-2	6 (66.7)	5 (33.3)	
3-4	2 (22.2)	9 (60)	

7-8	1 (11.1)	1 (6.7)	
RTX cycles, n (%) [c]			
1	7 (77.8)	15 (100)	0.13
2	2 (22.2)	0 (0)	
First RTX [days after HSCT] [b]	48 (9 - 94)	51 (41 - 353)	0.35
Last RTX [days after HSCT] [b]	58 (9 - 302)	79 (57 - 372)	0.4
Indication of rituximab therapy, n (%)			
EBV	8 (72.7)	15 (100)	
AIHD	1 (9.1)	0 (0)	
Conditioning/treatment	0 (0)	0 (0)	
Conditioning/AIHD	1 (9.1)	0 (0)	
Immune encephalitis	1 (9.1)	0 (0)	
Max. EBV viral load after HSCT [copies/ ml] [b]	13550 (1530 - 1770000)	23100 (4050 - 299000)	0.16
Patients with an EBV load <2000 copies/ml 28 days after 1st RTX, n (%)	9 (100)	14 (93.3)	>0.99
Start of RTX therapy despite EBV load <10000copies/ml, n (%)	3 (33.3)	5 (33.3)	
Start of RTX therapy despite EBV load <3000copies/ml, n (%)	2 (22.2)	2 (13.3)	0.61
PBD - Reconstitution			
B cell count < 0,01/nl at d365, n (%)	3 (33.3)	0 (0)	0.04
Lymphocyte count/nl at d365 [b]	2.02 (0.5 - 4.4)	2.54 (0.9 - 4.6)	0.14
CD3+ T cell count/nl at d365 [b]	1.17 (0.6 - 3.5)	1.85 (0.5 - 3.8)	0.27
CD4+ T cell count/nl at d365 [b]	0.34 (0.17 - 1.1)	0.52 (0.4 - 1.4)	0.049
CD8+ T cell count/nl at d365 [b]	0.62 (0.19 - 3.5)	1.2 (0.14 - 3.1)	0.53
B cell count/nl at d365 [b]	0.04 (0 - 1.2)	0.4 (0.05 - 1.3)	0.057
B/T ratio at d365 [b]	0.05 (0 - 0.5)	0.2 (0.01 - 1.1)	0.22
IgG levels >5g/l without IgG substitution [days after HSCT] [b]	-	73 (4 - 757)	
Tetanus toxoid IgG 2y after HSCT [IU/ml] [b]	0.86 (0.34 - 1.6) (n=8) [f]	1.3 (0.1 - 6.3) (n=15) [f]	0.47
Pneumococcal Capsular Polysaccharide IgG1 2y after HSCT [IU/ml] [b]	33.4 (6.4 - 62.8) (n=7) [f]	36.8 (5.2 - 74) (n=12) [f]	0.55
Pneumococcal Capsular Polysaccharide IgG2 2y after HSCT [IU/ml] [b]	11.5 (2.5 - 25.4) (n=7) [f]	7.1 (1.3 - 36.2) (n=12) [f]	0.9
First time platelet count >50000/µl without transfusion, [days after HSCT] [b]	85 (17 - 402)	29 (13 - 600)	0.2
First time neutrophil count >500/µl [days after HSCT], median (range)	17 (9 - 25)	17 (10 -. 33)	>0.99
First time neutrophil count >1000/µl [days after HSCT], median (range)	18 (9 - 79)	19 (10 - 67)	0.99
Patients with decrease of donor chimerism <50%, n (%)	1 (11.1)	2 (13.3)	
PBD - Secondary complications			
Duration of hospitalization for HSCT [days], median (range)	87 (38 - 411)	49 (33 - 201)	0.27
Cumulative duration of re-hospitalizations after HSCT [days], [b, g]	15 (1 - 141)	18 (2 - 45)	0.74
Before first RTX	0 (0 - 2)	0 (0 - 28)	0.48
After first RTX	14 (1 - 141)	16 (2 - 38)	0.55

Patients that developed a severe adverse event that was survived, n (%) [h]	2 (22.2)	2 (13.3)	
Number of viral infections (w/o EBV) with >2000copies/ml blood after first rituximab (or equivalent TP) per patient, n (%)			
0	5 (55.5)	12 (90.9)	0.034
1	2 (22.2)	1 (6.7)	
2	2 (22.2)	2 (13.3)	
3	0 (0)	0 (0)	
Patients with PTLD, n (%)	0 (0)	2 (13.3)	0.51
Patients with any non-EBV viral infection (>2000 copies/ml in blood), n (%)	9 (100)	9 (60)	0.052
Before first RTX	8 (88.9)	9 (60)	0.19
After first RTX	3 (33.3)	0 (0)	0.042
Patients with ADV infection (>2000copies/ml blood), n (%)	4 (44.4)	3 (20)	0.36
Patients with CMV infection (>2000copies/ml blood), n (%)	4 (44.4)	4 (26.7)	0.38
Patients with BKV infection (>2000copies/ml blood/urin), n (%)	5 (55.5)	6 (40)	>0.99
Patients with antivirals other than rituximab, n (%) [i]	9 (100)	7 (46.7)	0.01
Ganciclovir	5 (55.6)	4 (26.7)	
Valganciclovir	5 (55.6)	1 (6.7)	
Foscarnet	3 (33)	6 (40)	
Cidofovir	2 (22.2)	2 (13.3)	
Brincidofovir	1 (11.1)	3 (20)	
Patients with initiation of antivirals other than rituximab, n (%) [i]			
Before first RTX	8 (88.9)	7 (46.7)	0.08
After first RTX	7 (77.8)	2 (13.3)	0.003
Patients who received virus specific T cells, n (%)	0 (0)	3 (20)	0.27
Neutropenia relapses (<1000/µl blood) [b, j]	4 (0 - 7)	1 (0 - 6)	0.03
Patients with positive blood culture findings, n (%)	5 (55.6)	8 (53.3)	>0.99
Before first RTX	2 (22.2)	5 (33.3)	0.67
After first RTX	5 (55.6)	5 (33.3)	0.4
Patients receiving systemic antimycotic treatment in therapeutic dose, n (%)	3 (33.3)	1 (6.7)	0.13
Patients who developed relevant acute GvHD, n (%) [k]	4 (44.4)	3 (20)	0.36
Patients that developed relevant chronic GvHD, n (%)	2 (22.2)	2 (13.3)	0.61
Number of systemic steroid treatments after HSCT per patient, n (%)			
0	2 (22.2)	7 (46.7)	
1	2 (22.2)	3 (20)	
2	2 (22.2)	3 (20)	
3	2 (22.2)	1 (6.7)	
4	0 (0)	1 (6.7)	
5	0 (0)	0 (0)	
7	1 (11.1)	0 (0)	
Patients with ECP procedures, n (%)	4 (44.4)	3 (20)	

Patients with application of vedulizumab, basiliximab or infliximab after HSCT, n (%)	2 (22.2)	0 (0)	
Development of VOD after HSCT, n (%)	1 (11.1)	3 (20)	
Red blood cell concentrates per patient [b]	12 (2 - 143)	6 (1 - 15)	0.09
Platelet concentrates per patient [b]	19 (3 - 94)	12 (3 - 41)	0.31

[a] To compare cohorts the Wilcoxon signed rank test was used for continuous data and the McNemar test for binary data. When matching was impossible (high abundance of missing values or subgroup analysis), the Mann-Whitney U and Fishers exact tests were used. Test statistics were created using SPSS version 28.0(IBM SPSS Statistics, Armonk, USA), GraphPad PRISM 8 & 9 (GraphPad Software, San Diego, USA).

[b] Median (range)

[c] A cycle of repetitive rituximab dosing was defined to have ended when no rituximab application occurred for more than 28 days.

[d] Rituximab dose: 375 mg/m^2 body surface area

Including Erythrocyte depletion & Plasma depletion only

[e] One episode was defined as continuous EBV viral load >2000 copies/ml blood in consecutive measurements

[f] n excludes all patients with dropout events but includes all available data until the 20th of January 2022 even if outside of the observation period.

[g] re-hospitalizations for rituximab application only were not included

[h] Oriented on common terminology criteria for adverse events (CTCAE) version 5.0 Grade 4: Complications that necessitated transfer to ICU, Catecholamines to prevent cardiac failure, dialysis, invasive ventilation.

[l] aciclovir was excluded from this analysis as all patients regardless of study cohorts received prophylactic aciclovir for herpes simplex infection prevention

[j] New drop of neutrophil blood level below 1000/µl after previous regeneration of neutrophils (>1000/µl) for at least 7 days (independent of granulocyte colony stimulating factor treatment)

[k] This includes every episode of acute GvHD for which patients received systemic treatment like steroids or extracorporal photopheresis.

RTX: rituximab, Ctrl: control (group), HSCT: Hematopoietic stem cell transplantation, MUD: Matched unrelated donor, MSD: Matched sibling donor, MMRD: Mismatch related donor, MRD: Matched related donor, HSC: Hematopoietic stem cell, *BM: Bone marrow, PBSC: Peripheral blood stem cells,* AIHD: Autoimmune hematologic disease, *BW: body weight. ADV: Adenovirus, CMV: Cytomegalovirus, HHV6: Human herpes virus 6, BKV: BK virus, VOD: Veno-occlusive disease.* PBD: prolonged B cell damage (subgroup), RTX-Ctrl: non-PBD control subgroup from rituximab group, *PTLD: post-transplant lymphoproliferative disease*