

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

by Michael Launspach, Dennis Temel, Emily Ohlendorf, Felix Zirngibl, Bianca Materne, Lena Oevermann, Hedwig E. Deubzer, Anton G. Henssen, Annette Künkele, Patrick Hundsdörfer, Horst von Bernuth, Axel Prüß, Angelika Eggert, Arend von Stackelberg, Peter Lang, and Johannes H. Schulte

Received: April 10, 2022.

Accepted: September 13, 2022.

Citation: Michael Launspach, Dennis Temel, Emily Ohlendorf, Felix Zirngibl, Bianca Materne, Lena Oevermann, Hedwig E. Deubzer, Anton G. Henssen, Annette Künkele, Patrick Hundsdörfer, Horst von Bernuth, Axel Prüß, Angelika Eggert, Arend von Stackelberg, Peter Lang, and Johannes H. Schulte. 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.

Haematologica. 2022 Sept 22. doi: 10.3324/haematol.2022.281134 [Epub ahead of print]

Publisher's Disclaimer.

E-publishing ahead of print is increasingly important for the rapid dissemination of science. Haematologica is, therefore, E-publishing PDF files of an early version of manuscripts that have completed a regular peer review and have been accepted for publication. E-publishing of this PDF file has been approved by the authors. After having E-published Ahead of Print, manuscripts will then undergo technical and English editing, typesetting, proof correction and be presented for the authors' final approval; the final version of the manuscript will then appear in a regular issue of the journal. All legal disclaimers that apply to the journal also pertain to this production process.

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

Michael Launspach^{1,2,3,4}, Dennis Temel¹, Emily Ohlendorf^{1,2}, Felix Zirngibl^{1,2}, Bianca Materne⁵, Lena Oevermann^{1,2}, Hedwig E. Deubzer^{1,2,3,4}, Anton G. Henssen^{1,2,3,4,6}, Annette Künkele^{1,2,3,4}, Patrick Hundsdörfer^{1,7}, Horst von Bernuth^{2,8,9,10}, Axel Prüß¹¹, Angelika Eggert^{1,2,3,4}, Arend von Stackelberg^{1,2}, Peter Lang¹², Johannes H. Schulte^{1,2,3,4}

¹ Department of Pediatric Oncology and Hematology, Charité - Universitätsmedizin Berlin, Berlin, Germany.

² Berlin Institute of Health at Charité - Universitätsmedizin Berlin, Germany

³ The German Cancer Consortium (DKTK), Partner Site Berlin, Berlin, Germany.

⁴ The German Cancer Research Center (DKFZ), Heidelberg, Germany.

⁵ Institute of Biometry and Clinical Epidemiology, Charité - Universitätsmedizin Berlin, Berlin, Germany.

⁶ Experimental and Clinical Research Center, Max Delbrück Center for Molecular Medicine and Charité - Universitätsmedizin Berlin, Berlin, Germany

⁷ Department of Pediatrics, Helios Klinikum Berlin-Buch, Berlin, Germany

⁸ Department of Pediatric Respiratory Medicine, Immunology and Critical Care Medicine, Charité - Universitätsmedizin Berlin, Berlin, Germany

⁹ Labor Berlin - Charité Vivantes GmbH, Department of Immunology, Berlin, Germany

¹⁰ Berlin-Brandenburg Center for Regenerative Therapies (BCRT), Charité - Universitätsmedizin Berlin, Berlin, Germany

¹¹ Institute of Transfusion Medicine, Charité - Universitätsmedizin Berlin, Berlin, Germany.

¹² Department of Hematology/Oncology and General Pediatrics, Children's University Hospital, University of Tuebingen, Tuebingen, Germany

Running head: Rituximab therapy after pediatric HSCT

Corresponding author: Michael Launspach, email:
michael.launspach@charite.de

Authors' contributions:

ML conceptualized the study, collected and analyzed data and prepared the manuscript. DT collected and analyzed data and prepared the manuscript. EO collected data and reviewed the manuscript. BM performed the mixed-model and fine and grey analysis, gave input on statistical aspects and possible sources of bias and reviewed the manuscript. FZ, LO, HED, AGH, AK, PH, HvB, AE, AvS & PL participated

in designing the study, discussing the data and reviewed the manuscript. AP contributed data and reviewed manuscript. JHS conceptualized the study and reviewed the data, results and manuscript. All authors have read and agreed to the published version of the manuscript.

Data sharing statement:

All data collected and analyzed in this study as well as detailed test statistics can be received upon request. This excludes data that falls under data privacy restrictions (e.g. exact date of transplantation).

Word count & objects:

Main text: 1500 words, Tables: 1, Figures: 2, Supplementary file: 1 table, 2 figures

Acknowledgments

The authors would like to thank Karin Pretzel for collecting and providing blood transfusion related data.

The authors would like to thank Kathy Astrahantseff for proofreading the manuscript.

ML is participant in the BIH Charité Clinician Scientist Program funded by the Charité - Universitätsmedizin Berlin & the Berlin Institute of Health (BIH).

HED and AK are participants in the BIH Charité Advanced Clinician Scientist Pilot program funded by the Charité - Universitätsmedizin Berlin and the Berlin Institute of Health (BIH).

Rituximab, the monoclonal antibody directed against CD20, is established in treatment regimens against CD20+ non-Hodgkin lymphomas and is used increasingly in refractory systemic autoimmune disorders.(1-4) Besides, it is applied as treatment in patients with Epstein-Barr virus (EBV) infection/reactivation, post-transplant lymphoproliferative disease (PTLD) and autoimmune complications following hematopoietic stem cell transplantation (HSCT).(5-8) Documentation of immunological consequences and impact on immune reconstitution in pediatric HSCT patients is, however, sparse. Previously published studies on small cohorts suggest delayed B-cell recovery, need for prolonged immunoglobulin substitution and an increased risk for secondary infections.(9, 10) To further elucidate rituximab implications in this setting, we performed a retrospective analysis of 44 pediatric patients who received allogeneic HSCT in our center between 2015 and 2020, and who were treated with rituximab within 365 days after HSCT. We compared this cohort against matched HSCT patients who didn't receive rituximab within four weeks before, or after HSCT. Despite similar overall survival, we observed that rituximab therapy significantly delayed B cell recovery, extended immunoglobulin deficiency and led to longer re-hospitalization durations and more bacterial infections despite immunoglobulin replacement therapy. In a subgroup (9 patients, 38%), we observed prolonged immunoglobulin deficiency >365 days after rituximab treatment, suggesting that rituximab harbors a significant risk for prolonged B cell impairment.

Rituximab patients were matched in a best-match approach with control patients. For matching, eight transplant-relevant parameters from the JACIE essential data list were chosen. The pool of possible matches was then filtered for one parameter after another which led to high correct-match-rates for top priority parameters (HSCT matching 100%; Graft source 95%) but lower success in low-priority parameters (**Figure 1A**). After matching, the control group showed a longer cumulative observation duration. This can be explained by a more frequent use of rituximab in later years. No difference in the overall outcome was observed (**Figure 1B, Table S1A**). Most patients received the first rituximab dose before day +100 (82%) and indication for initiating rituximab treatment was mainly EBV infection/reactivation (41 (84%)) (**Table S1B**). Analysis of EBV blood level development shows that rituximab was highly effective against EBV infection with 95% treatment success (**Figure 1C, Table S1B**). Both patients that did not respond completely, developed PTLD. In total, PTLD occurred in 6.1% (3/49) of patients with EBV levels >2000 copies/ml measured via PCR and all 3 patients had received preemptive rituximab treatment (Rituximab 7.9% (3/38) vs Control 0% (0/11), p>0.99) (**Table 1A**). At the same time, we observed that rituximab patients generally were at a higher PTLD risk due to significantly earlier EBV infection/reactivation after HSCT and higher maximal viral loads (**Figure 1C and 1D**).⁽¹¹⁾ The EBV level at

start of rituximab treatment, however, was quite variable (0 - 1,770,000 copies/ml). For 14 (37%) patients, rituximab treatment was initiated at an EBV load <10,000copies/ml. The 11 control patients (25%) with EBV infection/reactivation (all <20,000copies/ml) were treated with ganciclovir and/or foscarnet only, and all resolved their EBV reactivations. Regarding immune reconstitution, our data shows that rituximab treatment delayed B cell recovery by a median of 162 days (day +282 (43 - 716) vs +120 (d36 - d645); Hazard ratio 2.2 (p=0.008)) and led to significantly lower B cell numbers and B cell to T cell ratios at day +365. However, by day +720, the majority of patients had recovered to similar B cell levels (Figure 2A-D and S1; Table 1A and S1C). To evaluate B cell damage beyond normal B cell count recovery, we analyzed immunoglobulin levels and IgG substitution dependence. Rituximab treatment led to reaching IgG blood levels >5g/l without IgG substitution significantly later (median day +278 (4 - 1095) vs. +118 (4 - 722); Hazard ratio 4.2 (p<0.001)) and the last documented IgG substitution happened significantly later (median day +254 (0 - 1095) vs. +109 (0 - 324); Hazard ratio 6.25 (p<0.001)). While IgG recovery and B cell recovery coincided in both groups (Rituximab: last IgG recovery median day +278 and B cell recovery +282; Control: day +118 and +120), significant differences in IgM levels were still measureable even two years after HSCT, pointing towards a prolonged impairment not only of B cell numbers but also function (Figure S1A; Table 1A). Additionally, we evaluated the question of a correlation between rituximab doses received and rituximab initiation time point with primary endpoints, but no correlation could be found (Figure S2A). Investigating secondary complications, we found a significantly higher cumulative duration of re-hospitalizations in the rituximab group (median 20 vs. 9 days). In line, we noted significantly more non-EBV viral infections in the rituximab group, but as this was true independently from rituximab initiation, we suggest a general increased risk for viral infections in the rituximab group (Table 1A and S1D). However, despite similar use of myelotoxic antiviral agents (e.g. foscarnet) after rituximab treatment initiation, we found significantly more neutropenia relapses, initiations of intravenous antibiotic treatment and a higher rate of patients who had positive bacterial blood culture findings (17 (39%) vs. 6 (14%)) after rituximab therapy. While we saw no difference regarding acute graft versus host disease (GvHD) incidence, that necessitated systemic treatment, moderate-severe chronic GvHD only occurred in the rituximab group (5 (11%)). 4 of these patients had received rituximab before GvHD onset or steroid treatment. A possible explanation could be that these patients were multimorbid patients with a high co-incidence of complications (Table 1A and S1D). We then followed previous reports of prolonged B cell impairment after rituximab treatment in non-HSCT-related situations.(4, 10, 12) Out of 24 patients who were observed longer than 365 days after rituximab treatment ended, we identified 9 (38%) who had unresolved

immunoglobulin deficiency. We compared these patients (= prolonged B cell damage group (PBD)) to the other 15 and found that B cell recovery and function were severely impeded in the PBD group (**Figure S2B-D; Table 1B and S1E**). 3 (33%) PBD patients had unmeasurable B cells counts at day +365. Regarding prolonged functional impairment, we observed significantly lower IgG and IgM levels despite receiving more IgG substitutions and significantly more PBD patients received IgG substitutions after B cell recovery. They also developed more complications after initiation of rituximab treatment as suggested by significantly more re-hospitalizations, a higher rate of non-EBV viral infections and more initiations of intravenous antibiotic treatments. Apart from significantly faster rituximab therapy response and a tendency towards younger age (8 (2 - 19) vs. 12 (4 - 21) years), no significant differences were found when looking for possible risk factors (**Figure S2; Table 1B and S1E**). Follow up on IgG substitution beyond the observation period on 06/30/2022 showed that 3 out of the 9 PBD patients had become independent of IgG substitutions. This leaves 6 (25%) patients with a prolonged B cell damage with continuous dependence on IgG substitutions beyond two years after HSCT. A complication that has so far been described only in case reports.(9, 10)

Although age-, graft manipulation- and cGvHD mismatches create potential bias as immune reconstitution influencing confounders, our study confirms for the first time in a large pediatric cohort that rituximab therapy <365 days after HSCT leads to a delay in B cell recovery of both B cell numbers and function.(5, 9, 12, 13) In line with *Ottaviano et al.*, who observed prolonged hypogammaglobinemia after rituximab treatment in a non-HSCT-related setting, the faster rituximab therapy response in the PBD subgroup supports the hypothesis of an increased rituximab sensitivity at the time point of first rituximab application.(4) This also fits with our finding that B cell impairment did not correlate with the number of rituximab doses received. Regarding secondary infections, our results clearly point towards an increased risk for secondary bacterial infections after rituximab initiation which is in line with *Petropoulou et al.*, although no increase in mortality could be observed in our pediatric cohort.(14) In contrast to the findings of *Arai et al.*, we could not confirm a decreased alloimmunity after rituximab treatment.(6) We conclude that rituximab harbors a significant risk for prolonged B cell impairment and bacterial infections when administered shortly after HSCT. It remains unclear whether regular IgG substitution can completely mitigate the adverse side effects, but similar overall survival suggests that IgG substitution and appropriate treatment of complications can compensate the damage. We postulate that rituximab treatment within 365 days after HSCT poses a 20-40% risk to develop especially prolonged B cell impairment. This risk should be discussed in a shared decision-making process with caretakers when considering

initiation of rituximab treatment. To propose a solution for prolonged B cell impairment, donor stem cell boosts could be evaluated further in cases without GvHD (9). Our findings furthermore support the need to research factors predisposing for rituximab sensitivity. Exact determination of each patients risk to develop prolonged B cell damage after rituximab therapy could help to identify those patients that could qualify for an alternative treatment, e.g. EBV-specific T cell transfer.(15) Finally, we urge physicians to carefully consider the initial indication for rituximab treatment and recommend to generally not start rituximab therapy too early at low EBV levels, but instead to monitor EBV levels daily in these situations.

Abbreviations

EBV	Epstein Barr virus
GvHD	Graft versus host disease
HSCT	Hematopoietic stem cell transplantation
JACIE	Joint Accreditation Committee of the International Society for Cellular Therapy (ISCT) and the European Group for Blood and Marrow Transplantation (EBMT)
PBD	prolonged B-cell damage
PTLD	Post-transplant lymphoproliferative disorder

References

1. Glass B, Hasenkamp J, Wulf G, et al. Rituximab after lymphoma-directed conditioning and allogeneic stem-cell transplantation for relapsed and refractory aggressive non-Hodgkin lymphoma (DSHNHL R3): an open-label, randomised, phase 2 trial. Lancet Oncol. 2014;5(7):757-766.
2. Salles G, Barrett M, Foà R, et al. Rituximab in B-Cell Hematologic Malignancies: A Review of 20 Years of Clinical Experience. Adv Ther. 2017;34(10):2232-2273.
3. Berghen N, Vulsteke JB, Westhovens R, Lenaerts J, De Langhe E. Rituximab in systemic autoimmune rheumatic diseases: indications and practical use. Acta Clin Belg. 2019;4(4):272-279.
4. Ottaviano G, Marinoni M, Graziani S, et al. Rituximab Unveils Hypogammaglobulinemia and Immunodeficiency in Children with Autoimmune Cytopenia. J Allergy Clin Immunol Pract. 2020;8(1):273-282.
5. Choquet S, Leblond V, Herbrecht R, et al. Efficacy and safety of rituximab in B-cell post-transplantation lymphoproliferative disorders: results of a prospective multicenter phase 2 study. Blood. 2006;107(8):3053-3057.
6. Arai S, Sahaf B, Narasimhan B, et al. Prophylactic rituximab after allogeneic transplantation decreases B-cell alloimmunity with low chronic GVHD incidence. Blood. 2012;119(25):6145-6154.

Rituximab therapy after pediatric HSCT

7. Kim BK, Kang HJ, Hong KT, et al. Successful preemptive therapy with single-dose rituximab for Epstein-Barr virus infection to prevent post-transplant lymphoproliferative disease after pediatric hematopoietic stem cell transplantation. *Transpl Infect Dis.* 2019;21(6):e13182.
8. Solomon SR, Sizemore CA, Ridgeway M, et al. Safety and efficacy of rituximab-based first line treatment of chronic GVHD. *Bone Marrow Transplant.* 2019;54(8):1218-1226.
9. Masjosthusmann K, Ehlert K, Eing BR, et al. Delay in B-lymphocyte recovery and function following rituximab for EBV-associated lymphoproliferative disease early post-allogeneic hematopoietic SCT. *Bone Marrow Transplant.* 2009;43(9):679-684.
10. Luterbacher F, Bernard F, Baleydier F, Ranza E, Jandus P, Blanchard-Rohner G. Case Report: Persistent Hypogammaglobulinemia More Than 10 Years After Rituximab Given Post-HSCT. *Front Immunol.* 2021;12:773853.
11. Gulley ML, Tang W. Using Epstein-Barr viral load assays to diagnose, monitor, and prevent posttransplant lymphoproliferative disorder. *Clin Microbiol Rev.* 2010;23(2):350-366.
12. Labrosse R, Barmettler S, Derfalvi B, et al. Rituximab-induced hypogammaglobulinemia and infection risk in pediatric patients. *J Allergy Clin Immunol.* 2021;148(2):523-532.
13. Faye A, Quartier P, Reguerre Y, et al. Chimaeric anti-CD20 monoclonal antibody (rituximab) in post-transplant B-lymphoproliferative disorder following stem cell transplantation in children. *Br J Haematol.* 2001;115(1):112-118.
14. Petropoulou AD, Porcher R, Peffault de Latour R, et al. Increased infection rate after preemptive rituximab treatment for Epstein-Barr virus reactivation after allogeneic hematopoietic stem-cell transplantation. *Transplantation.* 2012;94(8):879-883.
15. Qian C, Wang Y, Reppel L, et al. Viral-specific T-cell transfer from HSCT donor for the treatment of viral infections or diseases after HSCT [ReviewPaper]. *Bone Marrow Transplantation.* 2017;53(2):114-122.

Table 1 - Comparison of study cohorts

A - Rituximab cohort characteristics	Rituximab (n=44)	Control (n=44)	p-value^a
Patients with EBV who developed PTLD, n (%)	3/38 (7.9)	0/11 (0)	>0.99
B cell count at d365 [/nl] ^b	0.2 (0 - 1.3)	0.5 (0.1 - 2.0)	0.0003
B cell to T cell ratio at d365 ^b	0.2 (0 - 1.1)	0.4 (0 - 1.0)	0.0005
B cell recovery [days after HSCT] ^b	282 (43 - 716)	120 (36 - 645)	0.0001
Time to B cell recovery after last RTX (Ctrl: equivalent time point) [days] ^b	211 (13 - 658)	37 (0 - 479)	<0.0001
IgG serum level at d365 [g/l] ^b	6.2 (1.3 - 14)	9.1 (3.2 - 22)	0.03
IgM serum level at d365 [g/l] ^b at d730 [g/l] ^b	0.5 (0.1 - 1.7) 0.6 (0.2 - 1.1)	0.8 (0.2 - 3.6) 0.9 (0.3 - 2.7)	0.0002 0.01
Last IgG substitution [days after HSCT] ^b	254 (0 - 1095)	109 (0 - 324)	<0.0001
Cumulative re-hospitalization duration [days] ^b	20 (1 - 293)	9 (1 - 107)	0.0123
Before first RTX	3 (2 - 293)	6 (1 - 22)	0.77
After first RTX	16 (1 - 141)	9 (1 - 107)	0.033
Patients with any non-EBV viral infection (>2000 copies/ml in blood), n (%)	28 (63.6)	16 (36.4)	0.008
Before first RTX	23 (52.3)	15 (34.1)	0.08
After first RTX	9 (20.5)	5 (11.4)	0.22
Initiations of intravenous antibiotic treatment ^b	3 (1 - 14)	1 (1 - 6)	0.0004
Before first RTX	1 (0 - 5)	1 (0 - 3)	0.049
After first RTX	1 (0 - 11)	0 (0 - 6)	0.006
Patients with positive blood cultures, n (%)	26 (59.1)	13 (29.55)	0.021
Before first RTX	15 (34.1)	11 (25.0)	0.5
After first RTX	17 (38.6)	6 (13.64)	0.029
Moderate - severe chronic GvHD, n (%)	5 (11.4)	0 (0)	0.025
B - Prolonged B cell damage subgroup characteristics	PBD (n=9)	RTX-Ctrl (n =15)	p-value^a
Time until EBV viral load drops below <50% of value at RTX initiation (Ctrl: 1 st peak) [days] ^b	1 (1 - 2)	2 (1 - 42)	0.049

Rituximab therapy after pediatric HSCT

B cell recovery [days after HSCT] ^b	471 (50 - 716)	301 (43 - 460)	0.026
Time to B cell recovery after last RTX [days] ^b	306 (144 - 658)	214 (127 - 350)	0.029
IgG level [g/l] d365 ^b	6.0 (2.2 - 9.7)	9.0 (3.0 - 14)	0.025
IgM level [g/l] d365 ^b	0.1 (0.1 - 0.5)	0.6 (0.4 - 1.0)	<0.0001
Cumulative IgG dose [g/kg BW] ^b	7.9 (1.0 - 28)	0.9 (0.02 - 3.8)	0.0002
IgG substitution after B cell recovery, n (%)	6 (66.7)	3 (20)	0.036
Re-hospitalizations per patient ^{b, c}	5 (0 - 34) 0 (0 - 1)	1 (0 - 9) 0 (0 - 3)	0.036 0.56
Before first RTX	4 (0 - 34)	1 (0 - 8)	0.021
After first RTX	2 (1 - 3)	1 (0 - 3)	0.053
Initiations of intravenous antibiotic treatment ^b	4 (1 - 14)	2 (1 - 4)	0.034
Before first RTX	2 (1 - 3)	1 (0 - 3)	0.053
After first RTX	2 (1 - 11)	1 (0 - 3)	0.04

^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, 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 re-hospitalizations for rituximab application only were not included. EBV: Epstein Barr virus, PTLD: post-transplant lymphoproliferative disease, RTX: rituximab, Ctrl: control (group), HSCT: Hematopoietic stem cell transplantation, GvHD: graft versus host disease. PBD: prolonged B cell damage (subgroup), RTX-Ctrl: non-PBD-rituximab-control group (Patients from rituximab group that were observed longer than 365 days after initiation of rituximab treatment),

Figure legends

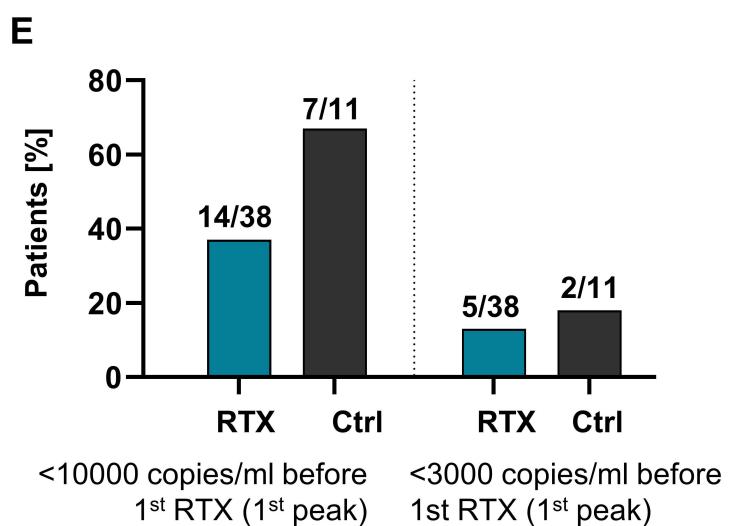
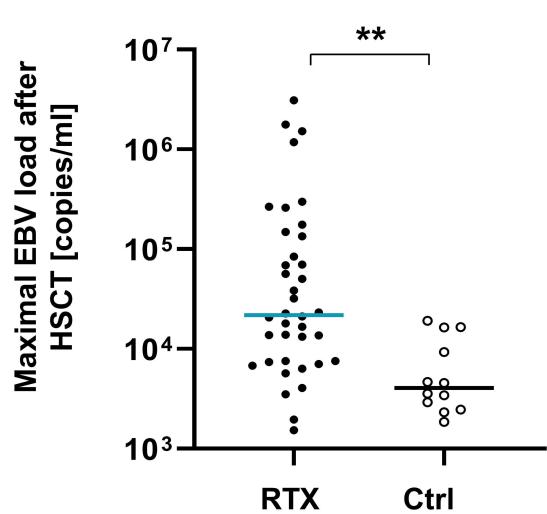
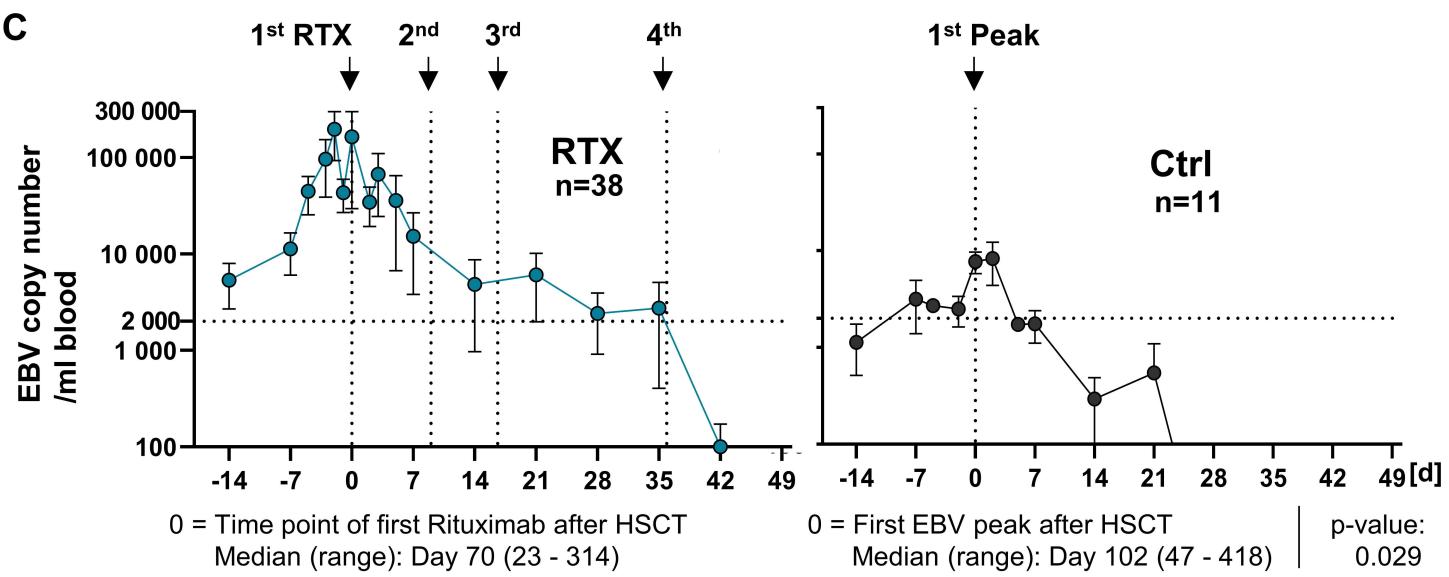
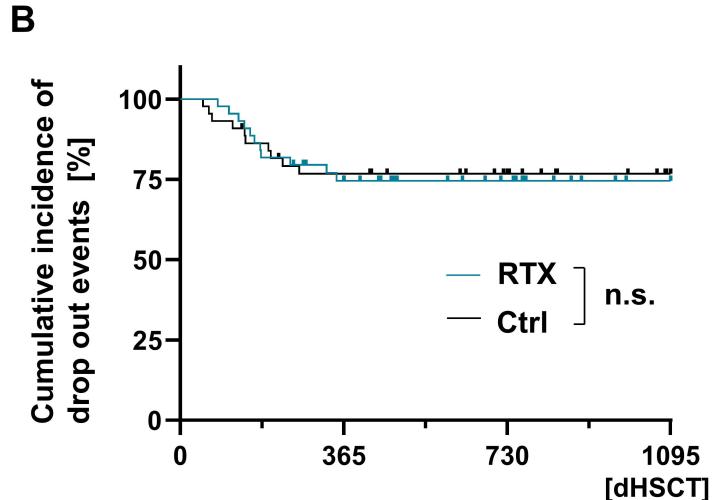
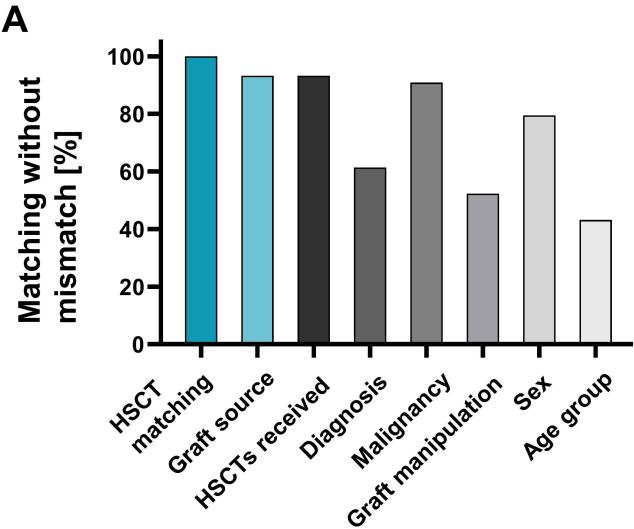
Figure 1 - Rituximab treatment is efficient for patients with high EBV viral load after HSCT but cut-off viral load for rituximab treatment initiation is unclear.

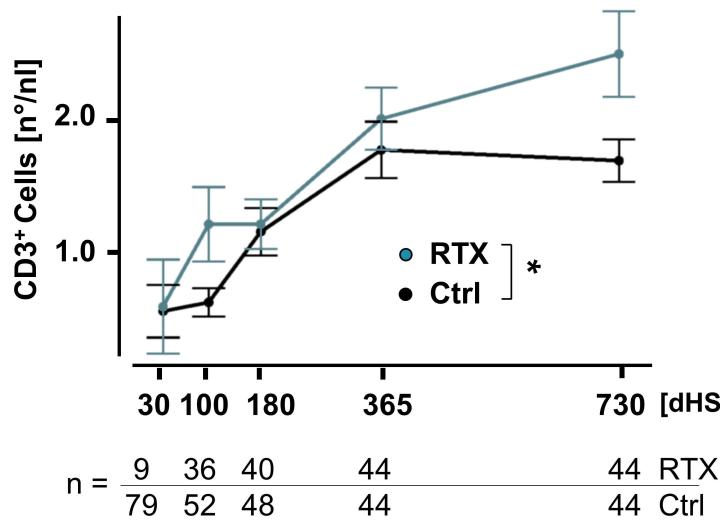
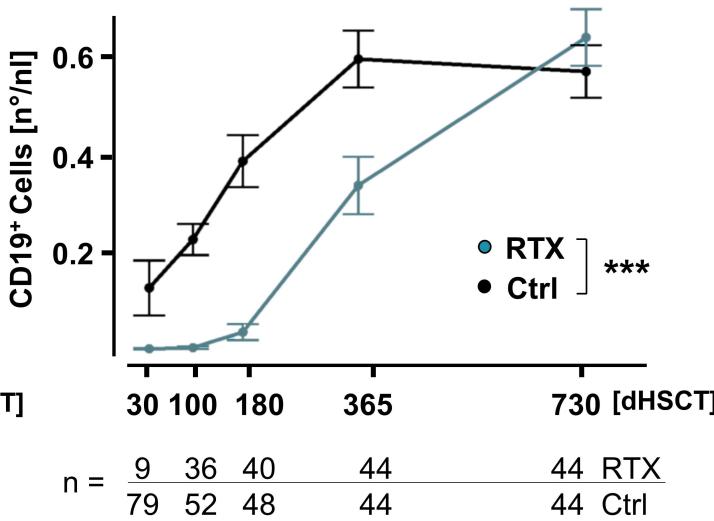
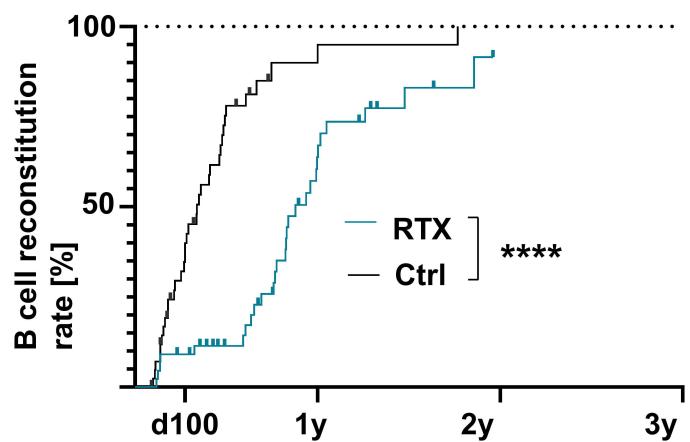
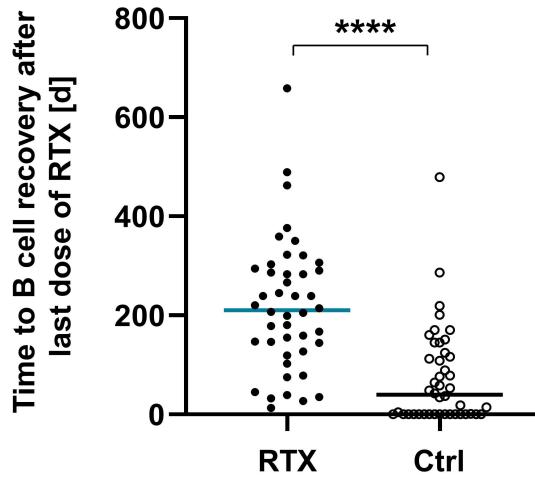
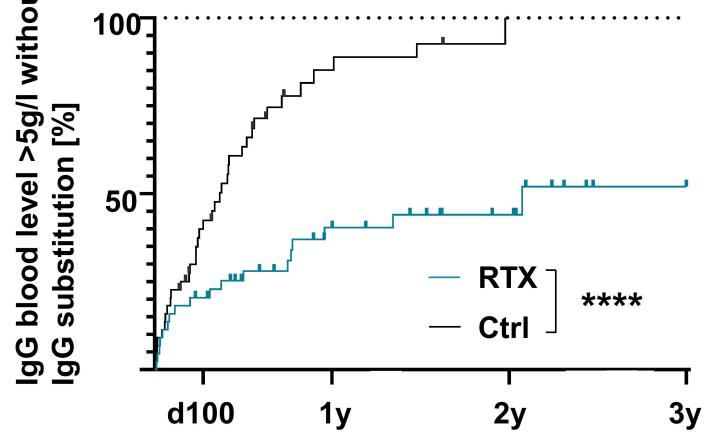
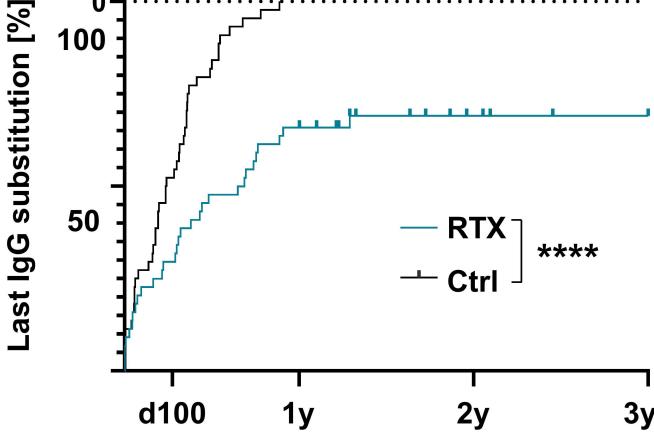
(A) Graphical overview of matching success for different matching categories. From highest matching priority to lowest: HSCT matching (matched unrelated donor, matched sibling donor or mismatch related donor), graft source (bone marrow or PBSCs), HSCTs received (*Number of HSCTs received before + 1*), exact diagnosis, malignancy (benign or malign disease as indication for HSCT), Graft manipulation, Sex and age group (<1, 1-5, 6-11, 12-17, 18+) (Table S1A). (B) Kaplan-Mayer survival curves and log-rank test for cumulative incidence of drop out events (relapse, non-relapse-related mortality, rejection and re-transplantation). (C) EBV copy number/ml EDTA blood development over time in patients with EBV infections (n=38) from the rituximab and control groups (n=11) relative to the date of the first rituximab dose in the rituximab group or the first peak in the control group. The median time points of the 2nd, 3rd and 4th rituximab doses were marked with dotted lines for the rituximab group. (D) Maximal EBV load after HSCT in copies/ml EDTA blood in rituximab and control groups. (E) Depiction of patient numbers for which rituximab treatment was initiated at <10.000 or <3.000 EBV copies/ml in the rituximab group or where the peak was <10.000 or <3.000 EBV copies/ml in the control group. *HSCT: Hematopoietic stem cell transplantation, PBSCs: Peripheral blood stem cells, EBV: Epstein Barr virus, RTX: Rituximab (group), Ctrl: Control group, dHSCT: HSCT treatment timeline day (d0 = day of HSCT). Significance levels: ***: p<0.001; **: p<0.01; *: p<0.05, n.s.: not significant*

Figure 2 - B cell recovery and function is impeded by rituximab treatment after pediatric HSCT.

(A, B) T cell (CD3+) and B cell (CD19+) recovery over time after HSCT for rituximab and control groups depicted as 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. (C, E, F) Inverse Kaplan-Meier curves depicting the rate of patients at a certain time point that achieved either (C) B cell reconstitution, (E) IgG levels >5g/l without IgG substitution or (F) receiving no more IgG substitutions for rituximab or control groups. (D) Comparison of the elapsed time to B

cell recovery after the last dose of rituximab was administered in the rituximab group and the elapsed time until B cell recovery after a time point identical to the time point of the last RTX dose for each individual matched patient for the Ctrl group. Data depicted as single patient values & median. *HSCT: Hematopoietic stem cell transplantation, RTX: Rituximab (group), Ctrl: Control group, dHSCT: HSCT treatment timeline day (d0 = day of HSCT), BW: body weight. Significance Levels: ****: p<0.0001; ***: p<0.001; **: p<0.01; *: p<0.05; n.s.: not significant*



A**B****C****D****E****F**

SUPPLEMENT

Table of contents

I. Supplement figure legends.....	1
Figure S1 - B cell recovery and function is impeded by rituximab treatment after pediatric HSCT ..	1
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.	2
II. Supplement Figures.....	3
Figure S1	3
Figure S2	4
III. Supplement tables.....	5
Table S1 - Comparison of study cohorts.....	5

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 substitution 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

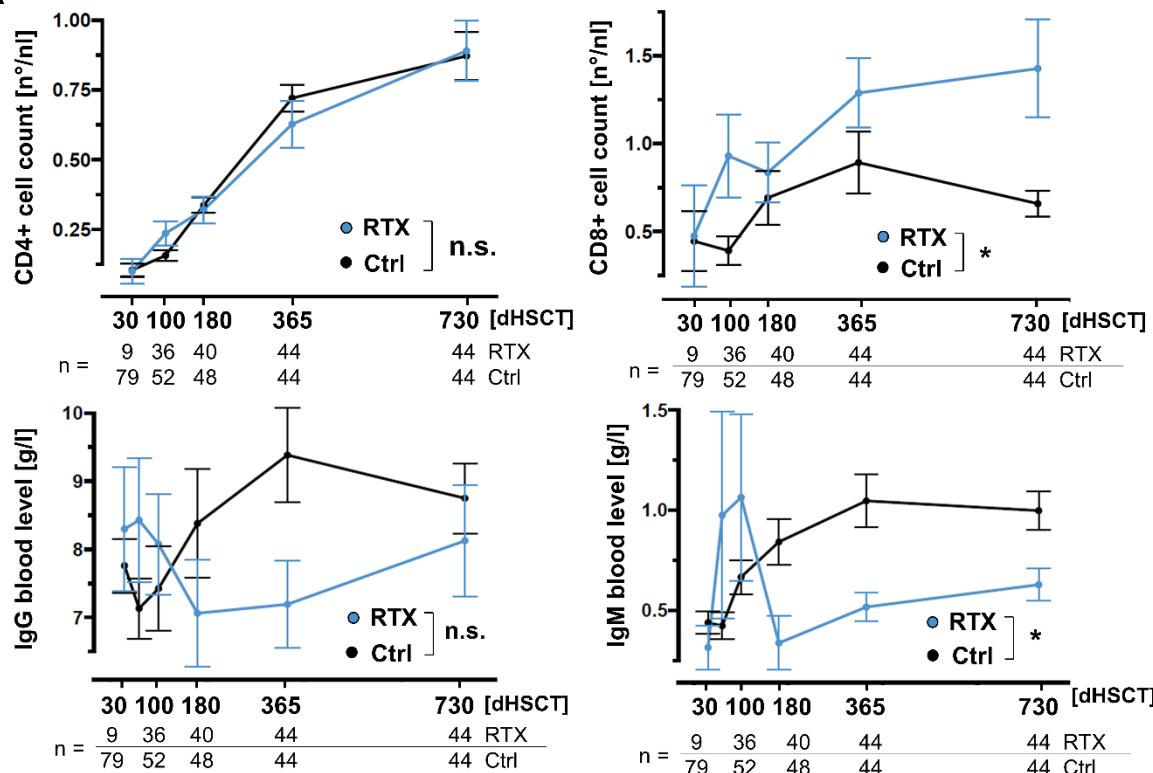
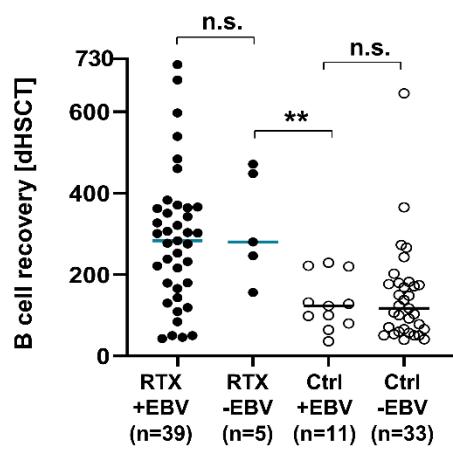
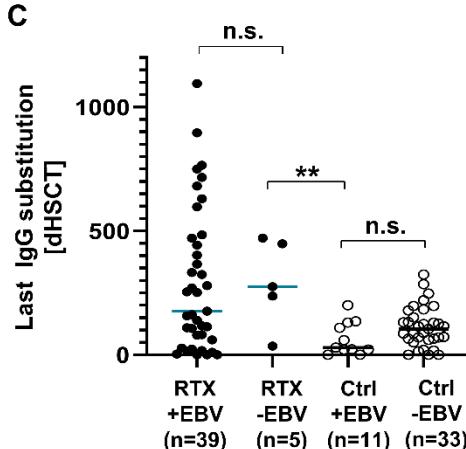
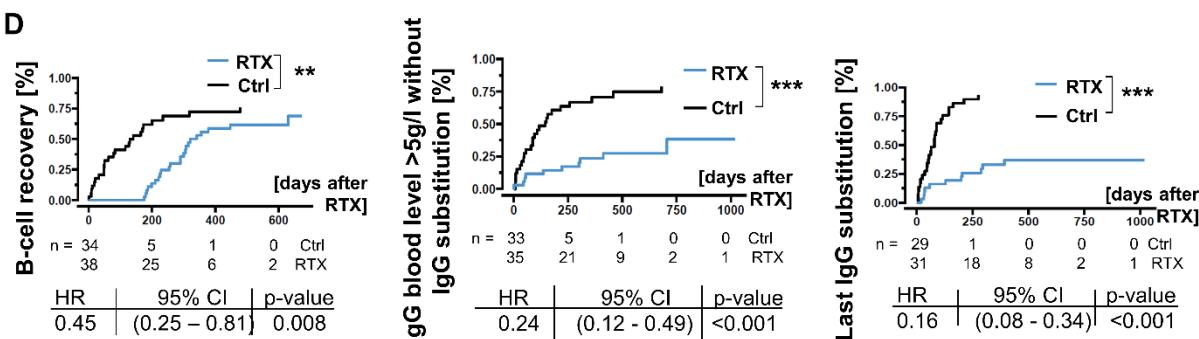
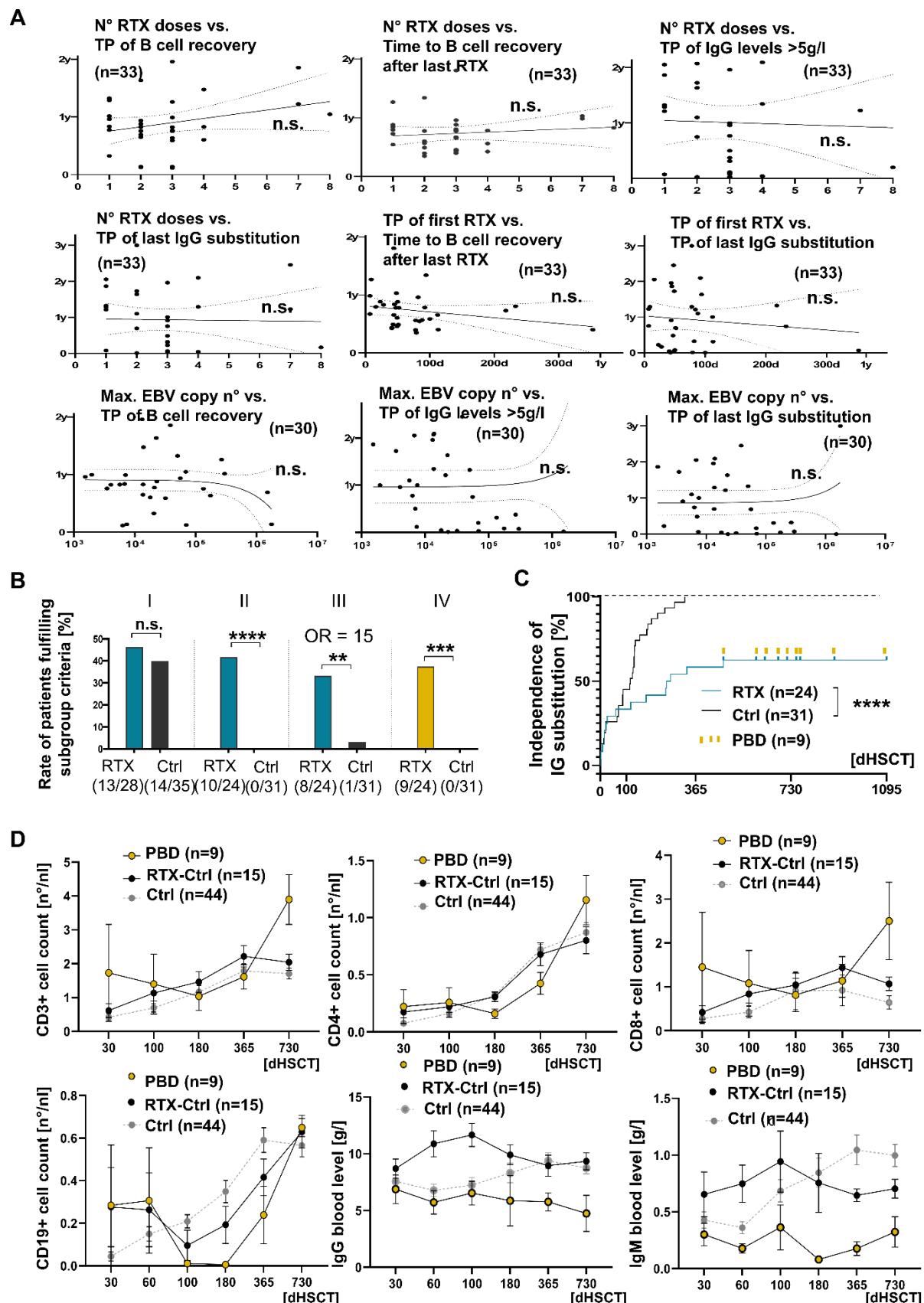
A**B****C****D**

Figure S2



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 transplants 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, thioguanine, total body irradiation, etoposide,	17 (38.6)	13 (29.5)
	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 1 st RTX (Ctrl: 1 st 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 1 st RTX (Ctrl: 1 st Peak), n (%)	36/38 (94.7)	11/11 (100)	
Time until EBV viral load drops below <50% of value at time point of 1 st RTX dose (Ctrl: 1 st peak) [days] ^b	2 (0 - 42)	4 (2 - 7)	0.027
Start of RTX therapy (Ctrl: 1 st peak) despite EBV viral load <10000copies/ml, n (%)	14/38 (36.8)	7/11 (63.6)	
Start of RTX therapy (Ctrl: 1 st 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: 1 st 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/ μ l [days after HSCT] ^b	17 (9 - 38)	19 (8 - 40)	0.22
Neutrophil count >1000/ μ l [d] ^b	19 (9 - 79)	22 (9 - 292)	0.25
Platelet count >50000/ μ l 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/ μ l 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			0.04
Before first RTX	1 (0 - 7)	0 (0 - 3)	
After first RTX	0 (0 - 5)	0 (0 - 3)	0.28
Systemic steroid treatments after HSCT per patient, n (%)			0.03
0	16 (36.4)	22 (50)	
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 1 st 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/ μ l without transfusion, [days after HSCT] ^b	85 (17 - 402)	29 (13 - 600)	0.2
First time neutrophil count >500/ μ l [days after HSCT], median (range)	17 (9 - 25)	17 (10 - 33)	>0.99
First time neutrophil count >1000/ μ l [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/ μ l 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/m² 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.

ⁱ 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 extracorporeal 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