Lower incidence of chronic graft-*versus*-host disease after ruxolitinib plus extracorporeal photopheresis *versus* ruxolitinib alone in steroid-refractory acute graft-*versus*-host disease following allogeneic stem cell transplantation

Iryna Lastovytska, Silke Heidenreich, Evgeny Klyuchnikov, Christian Niederwieser, Nico Gagelmann, Johanna Richter, Radwan Massoud, Kristin Rathje, Tetiana Perekhrestenko, Gaby Zeck, Catherina Lück, Dietlinde Janson, Christine Wolschke, Francis Ayuk and Nicolaus Kröger

Department of Stem Cell Transplantation, University Medical Center Hamburg-Eppendorf, Hamburg-Eppendorf, Germany

Correspondence: N. Kröger
nmkroeger@outlook.de

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Abstract

We compared long-term outcomes in 78 patients with steroid-refractory acute graft-*versus*-host disease (SR-aGvHD) treated at the University Medical Center Hamburg, Germany, between December 2015 and August 2022 who received either ruxolitinib alone (Ruxo, N=29) or Ruxo plus extracorporeal photopheresis (Ruxo-ECP, N=49). Patients were well balanced between both arms except for SR-aGvHD grade IV which was higher in the Ruxo-ECP group (45% *vs*. 14%, *P*<0.001). In both cohorts, steroids were tapered rapidly, and median steroid treatment was 39 days in Ruxo and 35 days in Ruxo-ECP. The overall response rate including complete remissions (CR) of aGvHD at day 28 was 90% and 31% for Ruxo *versus* 86% and 0% (*P*<0.001, respectively) for Ruxo-ECP. At six months, partial remission (PR) and CR status of evaluable patients was 11% and 50% in Ruxo-ECP *versus* 10% and 40% after Ruxo alone, respectively (*P*=0.018). At 12 months, PR and CR status was 6% and 17% in the Ruxo group, but 82% and 64% (*P*<0.001) in the Ruxo-ECP cohort, and the cumulative incidence of chronic GvHD was significantly higher after Ruxo *versus* Ruxo-ECP at 49% (95% CI: 33-69%) *versus* 24% (95% CI: 15-38%) (*P*=0.01). Reconstitution of B cells occurred significantly earlier at one and three months in the Ruxo arm. No difference in 1-year non-relapse mortality, relapse, and 2-year overall survival was observed. Despite the limitations of this retrospective single-center study, the data suggest a better long-term control of aGvHD and less chronic GvHD at one year combining ruxolitinib with ECP compared to ruxolitinib alone in SR-aGvHD.

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is a potentially curative option for patients with hematologic malignancies. The clinical benefit of HSCT is partly due to graft-*versus*-leukemia effect (GvL), in which the donor's immune response is directed against the recipient's malignant cells. Alloreactive donor T cells can also lead to graft-*versus*-host disease (GvHD), which is a leading cause of morbidity and non-relapse mortality (NRM) following transplantation.[1] Despite advances in the prevention of GvHD, approximately 50% of HSCT recipients will develop acute GvHD (aGvHD). GvHD risk depends on several factors, including donor-recipient human leukocyte antigen (HLA) disparity, patient age, source of the donor cells, conditioning regimen, and the type of GvHD prophylaxis.[2-4] History of aGvHD itself is a key risk factor for the development of chronic GvHD (cGvHD).[2] Systemic corticosteroids are the standard of care for the initial treatment of grade aGvHD and cGvHD.[5,6] However, approximately 50% of patients do not experience sustained responses to corticosteroids, and long-term survival rates among steroid-refractory (SR) patients are in general less than 50%.[7] The JAK1/2 inhibitor ruxolitinib has become the first treatment for SR-aGvHD to be approved by the US Food and Drug Administration (FDA) and the European Medicines Agency after a randomized phase III trial which compared best available therapy.[8] Ruxolitinib therapy was associated with higher overall response

rate (ORR) at day 28 (62% *vs*. 39%, *P*<0.001) and a more durable overall response (OR) at day 56 than the control group (40% *vs*. 22%, *P*<0.001). Moreover, the estimated cumulative incidence of loss of response at six months was 10% in the ruxolitinib group and 39% in the control group.[8] Furthermore, low disease recurrence rates of the underlying malignancy were observed (9.3% and 2.4% in patients with aGvHD and cGvHD, respectively), suggesting that ruxolitinib treatment did not impair the GvL effect.[8] Moreover, in the REACH2 trial, the ORR at day 56 after initiation of therapy decreased to 40%, suggesting a clear unmet medical need for patients with GvHD who did not respond at day 28 or who worsened afterwards. Extracorporeal photopheresis (ECP) is a well-established treatment for SR-GvHD.[9] Patients' mononuclear cells are exposed extracorporeally to 8-methoxypsoralen followed by UVA irradiation before being returned to the patient.[10] Though the mechanisms of ECP-induced immunoregulation are not fully understood, an important factor is the initiation of apoptosis in all lymphocytes subsets within 24-48 hours. Apoptotic cells are phagocytosed by antigen-presenting cells that lead to the suppression of T-cell reactivity, impaired cytokine release, and the induction of regulatory T cells.[10,11] We recently reported results of ruxolitinib in combination with ECP in SR-aGvHD with ORR, CR, and partial response (PR) rates of 56%, 44%, and 11%, respectively, and an increased level of regulatory T cells after combined treatment.[12] Here we report long-term outcome of ruxolitinib plus ECP (Ruxo-ECP) in comparison to ruxolitinib (Ruxo) alone in SR-aGvHD.

Methods

Study design and definition

In this retrospective study, 78 patients with SR-aGvHD treated between December 2015 and August 2022 at the Department of Stem Cell Transplantation at the University Medical Centre Hamburg-Eppendorf were included. SR-aGvHD was defined as progression after three days or no improvement in GvHD after seven days of primary treatment with prednisone (or equivalent) >2 mg/kg per day or an inability to taper the steroid dose to <0.5 mg/kg. The type of SR-aGvHD was well balanced between both cohorts, except that severe aGvHD grade IV was noted higher in the Ruxo-ECP group (45% *vs*. 16%). All patients received oral ruxolitinib at a dose of 10 mg twice daily in addition to calcineurin inhibitor and steroid therapy. In case of severe cytopenia or infections, ruxolitinib was reduced to 5 mg twice a day. Steroid treatment was rapidly tapered. ECP was initiated with a schedule of twice per week for at least the first two weeks followed by an individual reduction in ECP frequency. The majority of ECP cycles were performed with the Cellex (Therakos) System, while, in a minority, the Amicus Blue (Fresenius-Kabi) System was used.

Overall response rate was defined as proportion of patients achieving CR and PR without a requirement for additional systemic immunosuppressive therapy. CR was defined as the absence of aGvHD manifestations in all organs without increased GvHD therapy, and PR was defined as a decrease in the organs initially affected with GvHD of at least one grade without a new manifestation or worsening of at least one grade in any other organ. Non-response (NR) was defined as no change in the organs initially affected with GvHD or death, and progression was defined as the increase in GvHD grade in at least one organ with or without improvement in any other organ.[13] Failure-free survival was defined as no relapse, no death, CR/PR of aGvHD, and no moderate/severe cGvHD. cGvHD was graded according to the National Institutes of Health (NIH).[14] aGvHD was classified and graded according to MAGIC criteria.[15]

This study was conducted in accordance with German ethical legislation and a special approval of an ethics committee is not required.

Endpoints

The primary objective of our study was to compare the ORR including CR and PR of aGvHD at day 28. Secondary endpoints were ORR of aGvHD at day 56, and after six and 12 months, and the development of cGvHD after six and 12 months, NRM at one year, OS at two years, and failure-free survival at one year, respectively.

Statistical analysis

Descriptive statistics were used for patients' characteristics. The clinical characteristics of patients were expressed as median and range for continuous variables. Categorical data were compared using χ^2 tests. The survival distributions for OS and disease-free survival (DFS) were calculated using the Kaplan-Meier method from the first ruxolitinib therapy. The comparison was made using the long-rank test. OS was defined as the time from the first ruxolitinib treatment to death from any cause, censored at the last follow-up. DFS was defined as survival without any signs of relapse or progression of primary disease. cGvHD / relapse-free survival is defined as survival without cGvHD and without any signs of relapse or progression. NRM was defined as death without evidence of disease relapse. NRM and relapse, and cGvHD were analyzed as competing events using Fine-Gray methods analysis and this was performed using the IBM SPSS version 29.00 and R software.

Results

Patients' and transplant characteristics

A total of 78 patients who received ruxolitinib treatment for SR-aGvHD after allogeneic HSCT (alloHSCT) were analyzed in this study. After alloHSCT, all patients received cyclosporine or tacrolimus in combination with mycophenolate mofetil as GvHD prophylaxis. Anti-thymocyte globulin (ATG) was also given in 86% and post-transplant cyclophosphamide

in 14% (Table 1). Out of these 78 SR-aGvHD patients, 49 received additional therapy with ECP which was started after a median of nine days (range, 4-65) after ruxolitinib start. Delay of starting ECP in a few patients was due to logistical reasons, such as transfer to the intensive care unit, but no patient included in the study was refractory to ruxolitinib prior to starting with ECP. There was no randomization, and patient selection for the Ruxo cohort was carried out in the early time period (2015-2019), while in the latter time period (after 2019), the majority of patients with SR-aGvHD received ruxolitinib in combination with ECP treatment. Treatment with ruxolitinib was tapered after achievement of remission. If no aGvHD exacerbation was seen during tapering, ruxolitinib treatment was stopped. ECP was continued for 4-12 weeks individually in the Ruxo-ECP group. The main patients' characteristics are listed in Tables 1 and 2.

Median age in the Ruxo-ECP group was 62 years (range, 21-73) and 59 years (range, 19-74) in the Ruxo group. The most common diseases in this study were myeloproliferative neoplasms (MPN) and myelodysplastic syndrome (MDS). The proportion of patients with grade III-IV aGvHD was higher in the Ruxo-ECP cohort compared to the Ruxo cohort (as per study entry). The GvHD characteristics of both groups are shown in Table 2.

The median time from HCT to onset of aGvHD was 21 days (range, 8-188) in the Ruxo (ruxolitinib alone) cohort and 28 days (range, 7-198) in the Ruxo/ECP cohort. The median interval between the start of steroid first-line treatment to the beginning of ruxolitinib was 12 days (range, 4-65 days) in the Ruxo-ECP group, and ten days (range, 4-63 days) in the Ruxo group. The median days from aGvHD onset to second-line treatment with ruxolitinib was 28 days (range, 7-198 days) and 21 days (range, 8-188 days) in the Ruxo group. The duration of steroid therapy after definition of SR-aGvHD was 39 (range, 17-89) days in the Ruxo group and 35 (range, 15-68) days in the Ruxo-ECP group. The median duration of ruxolitinib therapy was longer in the Ruxo-ECP group (median 77 days; range, 13-335) than in the Ruxo group (median 46 days; range, 2-735). The median number of ECP cycles in the Ruxo-ECP arm was 15 (range, 2-76).

Response

At day 28, 31% of patients in the Ruxo arm achieved a CR and 59% showed a PR of aGvHD, resulting in an ORR of 90%. In contrast, no CR or 86% PR were observed in the Ruxo-ECP arm (P<0.001, respectively).

At day 56, the ORR and CR rate was 90% and 72% in the Ruxo cohort *versus* 86% and 19%, respectively, in the Ruxo-ECP cohort (P<0.001, respectively). However, at six months, 24% in the Ruxo group, but only 7% in the Ruxo-ECP group, lost response, while the rate of PR and CR in evaluable patients was 11% and 50% in Ruxo-ECP *versus* 10% and 40% in the Ruxo alone (P=0.003, respectively). At 12 months, the rate of PR and CR were 6% and 17% in the Ruxo alone cohort and 8% and 64% in the Ruxo-ECP cohort (P<0.006) (Tables 2 and 3). Median duration of aGvHD response was 180 days (range, 15-1,830 days) for Ruxo alone and 180 days (range, 28-2010 days) for Ruxo-ECP (P=0.9).

Table 1. Patients' characteristics: ruxolitinib plus extracorporeal photopheresis *versus* ruxolitinib.

Variable	Ruxo-ECP	Ruxo
Total patients, N (%)	49 (63)	29 (37)
Median patient age in years	62	59
Interquartile range	21-73	19-74
Patient gender, N (%)		
Male	29 (59)	13 (45)
Female	20 (41)	16 (55)
AlloSCT, N (%)		
First	44 (90)	29 (100)
Second	5 (10)	0
Donor type, N (%)		
Unrelated-MUD	39 (80)	28 (97)
Related-MRD	10 (20)	1 (3)
HLA donor type, N (%)		
Match	35 (71)	25 (8)
Mismatch	13 (26)	4 (14)
Haplo	1 (2)	0
Disease, N (%)		
AML	9 (18)	6 (21)
ALL	3 (6)	1 (3)
MDS	12 (24)	7 (24)
MPN	14 (29)	8 (28)
MM	1 (2)	2 (7)
NHL	4 (8)	5 (17)
HL	1 (2)	0
MD-CMML	2 (4)	0
MP-CMML	1 (2)	0
Remission status of primary disease at transplant, N (%)		
CR	28 (57)	18 (62)
PR	2 (4)	1 (3)
PD	7 (14)	2 (7)
nCR	12 (24)	8 (27)
Transplant conditioning regimen, N (%)		
MAC	25 (51)	16 (55)
RIC	24 (49)	13 (45)
GvHD prophylaxis therapy, N (%)		
Cyclosporine-mycophenolate-based	43 (88)	24 (83)
Tacrolimus-mycophenolate-based	6 (12)	5 (17)
Antithymocyte globulin	43 (88)	24 (83)
Cyclosporine-based	6 (12)	5 (17)

alloSCT: allogeneic stem cell transplantation; ALL: acute lymphoid leukemia; AML: acute myeloid leukemia; CMML: chronic myelomonocytic leukemia; CR: complete remission; ECP: extracorporeal photopheresis; GvHD: graft-*versus*-host disease; HL: Hodgkin lymphoma; HLA: human leukocyte antigen; MAC: myeloablative conditioning; MD-CMML: myelodysplastic CMML; MDS: myelodysplastic syndrome; MM: multiple myeloma; MP-CMML: myeloproliferative CMML; MPN: myeloproliferative neoplasms; MRD: matched related donor; MUD: matched unrelated donor; N: number; nCR: never in remission; NHL: non-Hodgkin lymphoma; PD: progressive disease; PR: partial remission; RIC: reduced intensity conditioning; Ruxo: ruxolitinib.

Table 2. Graft-*versus*-host disease characteristics.

Disease characteristic	Ruxo-ECP	Ruxo alone
Grade SR-aGvHD, N (%)	49 (63)	29 (37)
II	0	11 (38)
III	27 (55)	14 (48)
IV	22 (45)	4 (14)
Grade skin GvHD, N (%)		
I	14 (29)	1 (3)
II	7 (14)	5 (17)
III	11 (22)	12 (41)
IV	3 (6)	1 (3)
Grade liver GvHD		
I	5 (10)	1 (3)
II	3 (6)	1 (3)
III	5 (10)	0
IV	0	0
Grade GI GvHD		
I	2 (4)	5 (17)
II	6 (12)	9 (31)
III	19 (39)	5 (17)
IV	22 (45)	3 (10)
Median interval between start of first-line steroid treatment and beginning of ruxolitinib in days, N (range)	12 (4-65)	10 (4-63)
Median duration of continued Ruxo treatment in days, N (range)	77 (13-335)	46 (2-735)
Median N Ruxo and ECP cycles (range)	15 (2-76)	-
Median time from Ruxo and start of ECP treatment in days, N (range)	9 (3-69)	-
Type of SR-aGvHD, N (%)		
Progression after 3 days	7 (14)	3 (10)
No improvement after 7 days	16 (33)	9 (31)
Inability to taper steroids <0.5 g/kg	26 (53)	17 (59)

aGvHD: acute graft-*versus*-host disease; ECP: extracorporeal photopheresis; GI: gastrointestinal; GvHD: graft-*versus*-host disease; N: number; Ruxo: ruxolitinib; SR: steroid refractory.

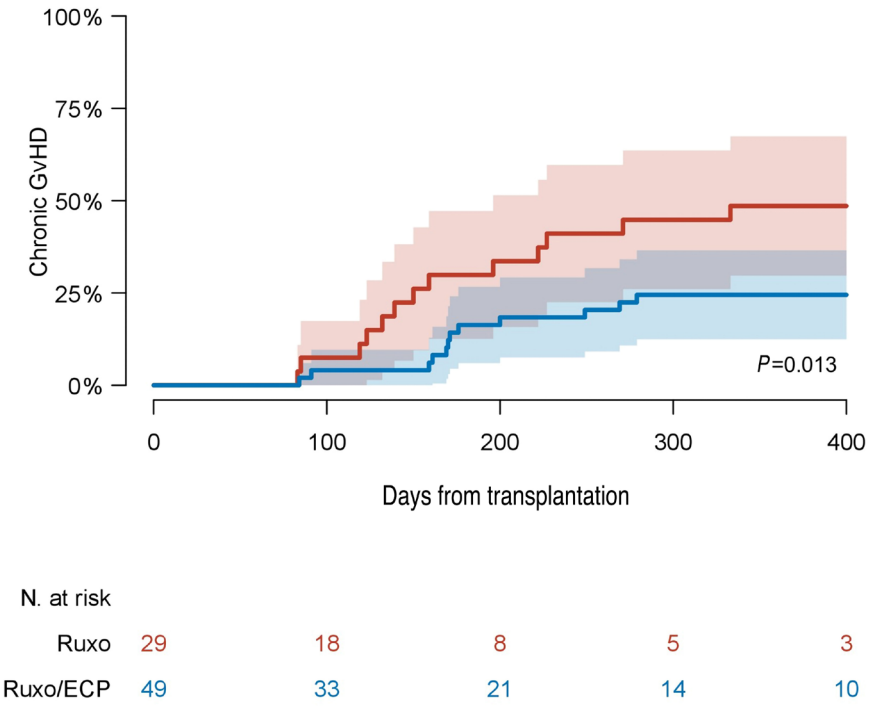


Figure 1. Cumulative incidence of chronic graft-*versus*-host disease after ruxolitinib *versus* ruxolitinib plus extracorporeal photopheresis in steroid-refractory acute graft-*versus*-host disease. ECP: extracorporeal photopheresis; GvHD: graft-*versus*-host disease; N: number; Ruxo: ruxolitinib.

Chronic graft-*versus*-host disease, and chronic graft-*versus*-host disease, relapse-free survival and failure-free survival

At six and 12 months, 25% and 20% in the Ruxo-ECP cohort and 20% and 66% in the Ruxo cohort of evaluable patients experienced cGvHD, resulting in a cumulative incidence of cGvHD at one year of 24% (95%CI: 14-27%) after Ruxo-ECP and 41% (95% CI: 31-67%) after Ruxo (*P*=0.01) (Figure 1). At one year, 12 of the 18 evaluable patients after Ruxo alone had experienced cGvHD, which was mild (N=3), moderate (N=6) or severe (N=3), while after Ruxo-ECP, only 5 of 25 evaluable patients had cGvHD, which was mild (N=2) or moderate (N=3), but no severe cGvHD was observed (Tables 3 and 4). We also defined as a combined endpoint cGvHD relapse-free survival which was in favor of the Ruxo-ECP combination at one year: 27% (95% CI: 17-40%) *versus* 8% (95% CI: 2-23%) (*P*=0.03) (Figure 2). Failure-free survival at one year was 21% (95%CI: 12-35%) in the Ruxo-ECP cohort and 4% (95%: 1-18%) after Ruxo alone treatment (*P*=0.05) (Figure 3).

After one year, 58% (14/24) of patients still received some form of immunosuppression within the Ruxo-ECP group consisting of ECP (25%), steroids (13%), calcineurin inhibitor (CNI) (47%), or ruxolitinib (33%) compared to 72% (13/18) of patients within the Ruxo group consisting of steroids (70%), CNI (50%), or ruxolitinib (12%), respectively.

Non-relapse mortality

No significant difference in NRM or relapse incidence was observed between Ruxo-ECP and Ruxo at one year: 47% (95% CI: 34-65) and 10% (95% CI: 5-21) *versus* 38% (95% CI: 22-57) and 21% (95% CI: 10-40%) (*P*=0.18 and *P*=0.1, respectively). The main cause of NRM was GvHD-related sepsis and multi-organ failure in 90% in the Ruxo and 87% in Ruxo-ECP group, followed by encephalitis in 10% for both groups, and in 3% with thrombotic microangiopathy (TMA) in the Ruxo-ECP group (Table 4). No significant difference in infectious complications was seen between the two cohorts.

Overall survival and immune reconstitution

The 2-year OS was 38% (95% CI: 22-58) after Ruxo alone and 36% (95% CI: 24-50) after Ruxo-ECP (*P*=0.75) (Table 4). In addition, we compared immune reconstitution in a subgroup of patients who received at least three months Ruxo alone (N=9) and Ruxo-ECP (N=18) and observed significantly earlier B-cell reconstitution with Ruxo alone at one and three months after treatment initiation for SR-aGvHD. An earlier, but not significant, increase in regulatory T cells was observed after Ruxo-ECP at one month, while

Table 3. Follow-up of 78 steroid-refractory acute graft-*versus*-host disease patients over 12 months.

Ruxo/ECP N=49	SR-aGvHD Ruxo alone N=29
At 28 days (±7) N=49 evaluable, N (%)	At 28 days (±7) N=29 evaluable, N (%)
Dead 0	Dead 0
ORR 42 (86)	ORR 26 (90)
CR 0	CR 9 (31)
PR 42 (86)	PR 17 (59)
Progress of aGvHD 6 (12)	Progress of aGvHD 0
Non-response 1 (2)	Non-response 3 (10)
At 56 days (±7) N=42 evaluable, N (%)	At 56 days (±7) N=22 evaluable, N (%)
Dead 7	Dead 7
ORR 36 (86)	ORR 20 (90)
CR 8 (19)	CR 16 (72)
PR 28 (67)	PR 4 (18)
Progress of aGvHD 5 (12)	Progress of aGvHD 0
Hematologic relapse 1 (2)	Hematologic relapse 2 (9)
At 6 months N=28 evaluable, N (%)	At 6 months N=20 evaluable, N (%)
Dead 14	Dead 2
CR 14 (50)	CR 8 (40)
PR 3 (11)	PR 2 (10)
Progress of aGvHD 2 (7)	Progress of aGvHD 5 (24)
Progress of cGvHD 7 (25)	Progress of cGvHD 4 (20)
Hematologic relapse 2 (7)	Hematologic relapse 2 (10)
At 12 months N=25 evaluable, N (%)	At 12 months N=18 evaluable, N (%)
Dead 3	Dead 2
CR 16 (64)	CR 3 (17)
PR 2 (8)	PR 1 (6)
cGvHD 5 (20)	cGvHD 12 (66)
Hematologic relapse 2 (8)	Hematologic relapse 2 (11)

aGvHD: acute graft-*versus*-host disease; cGvHD: chronic graft-*versus*-host disease; CR: complete remission; ECP: extracorporeal photopheresis; N: number; ORR: overall response rate; PR: partial remission; Ruxo: ruxolitinib; SR: steroid refractory.

Table 4. Outcome of steroid-refractory-acute graft-*versus*-host disease.

A. All patients.

Outcome	Ruxo-ECP N=49 % (95% CI)	Ruxo alone N=29 % (95% CI)	*P*
1-year NRM	47 (34-65)	38 (22-57)	0.18
1-year relapse	10 (5-21)	21 (10-40)	0.1
2-year OS	36 (24-50)	38 (22-58)	0.75
1-year cGvHD	25 (15-38)	51 (33-69)	0.01

B. Outcome and response of steroid-refractory acute graft-*versus*-host disease only for grade III acute graft-*versus*-host disease (N=41).

Outcome	Ruxo-ECP N=27 % (95% CI)	Ruxo alone N=14 % (95% CI)	*P*
1-year CI* NRM	42 (20-68)	34 (18-55)	0.42
1-year CI* relapse	13 (5-32)	8 (1-36)	0.59
2-year estimated OS	40 (24-59)	38 (17-65)	0.69
1-year CI* cGvHD	30 (16-49)	55 (27-80)	0.04
Response, N (%)			
Day 28	26/27 (96)	10/14 (71)	
Day 56	24/26 (92)	9/13 (69)	
Day 84	18/24 (75)	07/11 (64)	
Day 180	11/21 (5)	5/9 (56)	
Day 365	11/12 (92)	2/6 (33)	

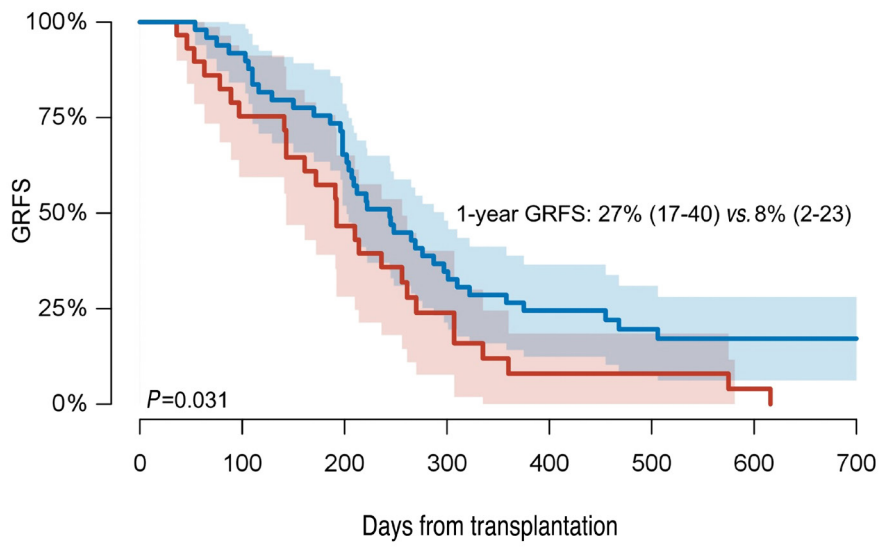
*Cumulative incidence; aGvHD: acute graft-*versus*-host disease; cGvHD: chronic graft-*versus*-host disease; ECP: extracorporeal photopheresis; N: number; NRM: non-relapse mortality; OS: overall survival; Ruxo: ruxolitinib; SR: steroid-refractory; 95% CI: 95% Confidence Interval.

the general T-cell reconstitution (CD3/μg) was significantly earlier with Ruxo alone (Figure 4A-C); no difference was seen for natural killer cells and $CD4^-$ and CD8 T cells (*data not shown*). Table 4A shows overall outcome of both cohorts and response of subgroup analysis, and Table 4B shows overall outcome and response only for SR-aGvHD grade III.

Discussion

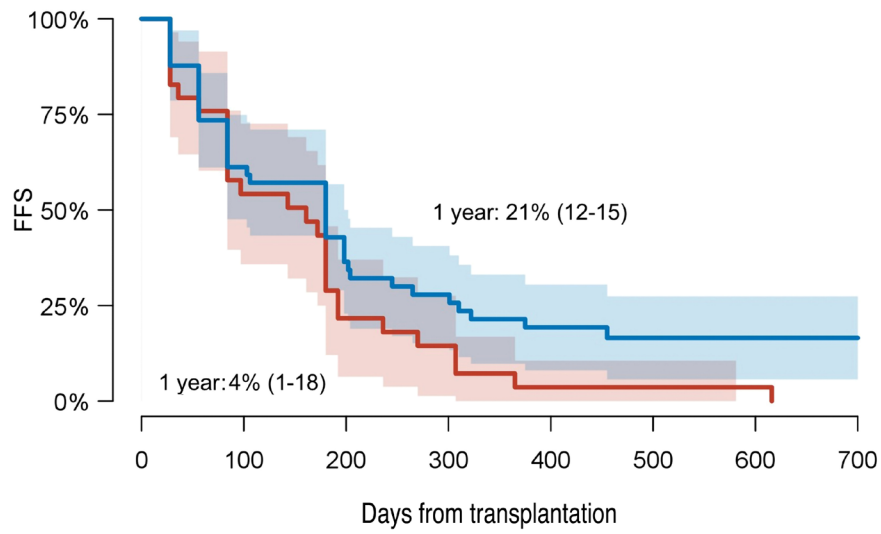
Our retrospective study was performed to analyze the efficacy of ECP in combination with ruxolitinib treatment for patients with SR-aGvHD regarding long-term outcome, evaluating response of aGvHD, development of cGvHD, and survival. In our analysis, ORR among patients with SR-aGvHD at 28 and 56 days was 86% each in the Ruxo alone group. These results are in line with other reported real-world data, expanded access programs,[16] as well as data from compassionate use programs.[17] An international collaboration has reported data on ruxolitinib treatment from 54 patients with aGvHD (grade III-IV) in a retrospective study of 19 centers in Europe and the United States in which the ORR was 81% for SR-aGvHD.[18] A Spanish group reported an outcome of ORR of 69.5% for patients with SR-aGvHD, including 22% CR and an OS of 47% at six months.[19] In two Chinese studies, Leung *et al.*[20] and Wei *et al.*[21] have reported an ORR of 86% and 86.9%, respectively. While these studies confirmed efficacy and safety of ruxolitinib in SR-aGvHD, data regarding long-term follow-up and development of cGvHD is limited. Only Leung *et al.* reported an incidence of cGvHD of 20% in patients who achieved CR of aGvHD after ruxolitinib treatment.[20]

The randomized REACH-2 study for SR-aGvHD demonstrated a decrease in response rate from 62% at day 28 to 39% at day 56, suggesting an unmet need for better long-term control of SR-aGvHD. As a result of this unmet need, identifying a combination therapy has become of clinical interest and the first studies combining ruxolitinib with ECP have already been reported.[12] In a pilot study, a CR rate of 44% has been reported by our group for the combination of ruxolitinib and ECP in SR-aGvHD which allowed rapid tapering of steroids and induced a high level of regulatory



N. at risk								
Ruxo	29	21	13	6	2	2	1	0
Ruxo/ECP	49	45	32	17	12	8	5	4

Figure 2. Chronic graft-*versus*-host disease, relapse-free survival after ruxolitinib *versus* ruxolitinib plus extracorporeal photopheresis in steroid-refractory acute graft-*versus*-host disease. ECP: extracorporeal photopheresis; GRFS: graft-versus-host diease, relapse-free survival; N: number; Ruxo: ruxolitinib.



N. at risk								
Ruxo	29	15	6	4	1	1	1	0
Ruxo/ECP	49	30	17	13	9	6	4	4

Figure 3. Failure-free survival after treatment with ruxolitinib alone and ruxolitinib plus extracorporeal photopheresis. One-year failure-free survival. Ruxolitinib monotherapy (Ruxo): 4% (range, 1-18%); ruxolitinib-extracorporeal photopheresis (ECP): 21% (12-35%); *P*=0.05. FFS: failure-free survival; N: number.

T cells, resulting in a favorable 2-year OS of 56%.[12]

Other studies have reported that ECP alone has high potential for treating patients with SR-GvHD.[9,22-24] An increase in regulatory T cells after ECP has been reported.[25] Jagasia *et al*. demonstrated an ORR of 66% *versus* 32% for ECP compared to anti-cytokine therapy as second-line treatment for SR-aGvHD.[26]

In this study, we initially observed a higher CR rate with Ruxo alone on days 28 and 56 in comparison to Ruxo-ECP, which might be explained by the increased proportion of high-grade aGvHD in the Ruxo-ECP cohort. In contrast to this, the response rate at six and 12 months after Ruxo-ECP exceeded the response rate of Ruxo alone, suggesting a latency in treatment response and a greater loss of patients between day 56 and six months in the Ruxo-ECP arm. More importantly, the incidence of cGvHD was significantly higher after Ruxo alone. The reason for a lower cGvH rate is unclear, but may be related to the faster B-cell reconstitution in the Ruxo group at months 1 and 3 of treatment in comparison to Ruxo-ECP. The important role of B lymphocytes and B-cell activating factor in the pathogenesis of cGvHD has been previously described.[27,28]

While there was no difference in NRM at one year and OS at two years between both arms, cGvHD, relapse-free survival as a novel composite endpoint in the treatment of SR-aGvHD showed a significant benefit for Ruxo-ECP. Even if quality of life was not investigated in this study, the significantly lower rate of cGvHD, and especially the lack of severe cGvHD, suggests a better quality of life in patients who received Ruxo-ECP in contrast to Ruxo alone for SR-aGvHD. The intention of the combination therapy was to start simultaneously with Ruxo and ECP after diagnosis of SR-aGvHD. The median time between start of ruxolitinib and start of ECP was nine days, representing a delay in a very few patients due to logistical reasons; however, none of the patients were refractory to ruxolitinib before starting ECP. Encouraging results in combining ruxolitinib with ECP have recently been reported also for SR-cGvHD.[29]

Our study has several limitations. First, the patients were not well balanced between both arms regarding severity of GvHD: the proportion of patients with higher grade (III-IV) aGvHD was higher in the Ruxo-ECP cohort compared to the Ruxo alone cohort. Even if it was intended that all patients receive the combination of ruxolitinib plus ECP, in some patients, start of ECP was delayed; this could have favored the Ruxo ECP arm because it would have included Ruxo-responsive patients. Additionally, this study was conducted retrospectively and a major bias cannot be excluded. Also, the reduction in immunosuppression, ruxolitinib, and ECP were at the discretion of the treating physician, and a higher CR rate on days 28 and 56 in the Ruxo cohort might have led to a faster reduction and discontinuation of ruxolitinib in this cohort, with a potential impact on the occurrence of cGvHD.

Conclusion

Despite the broad limitations of this retrospective single-center study, the data suggest a better long-term control of aGvHD and less cGvHD at one year by Ruxo-ECP than Ruxo alone in SR-aGvHD. This needs to be confirmed in a prospective randomized trial. In addition,

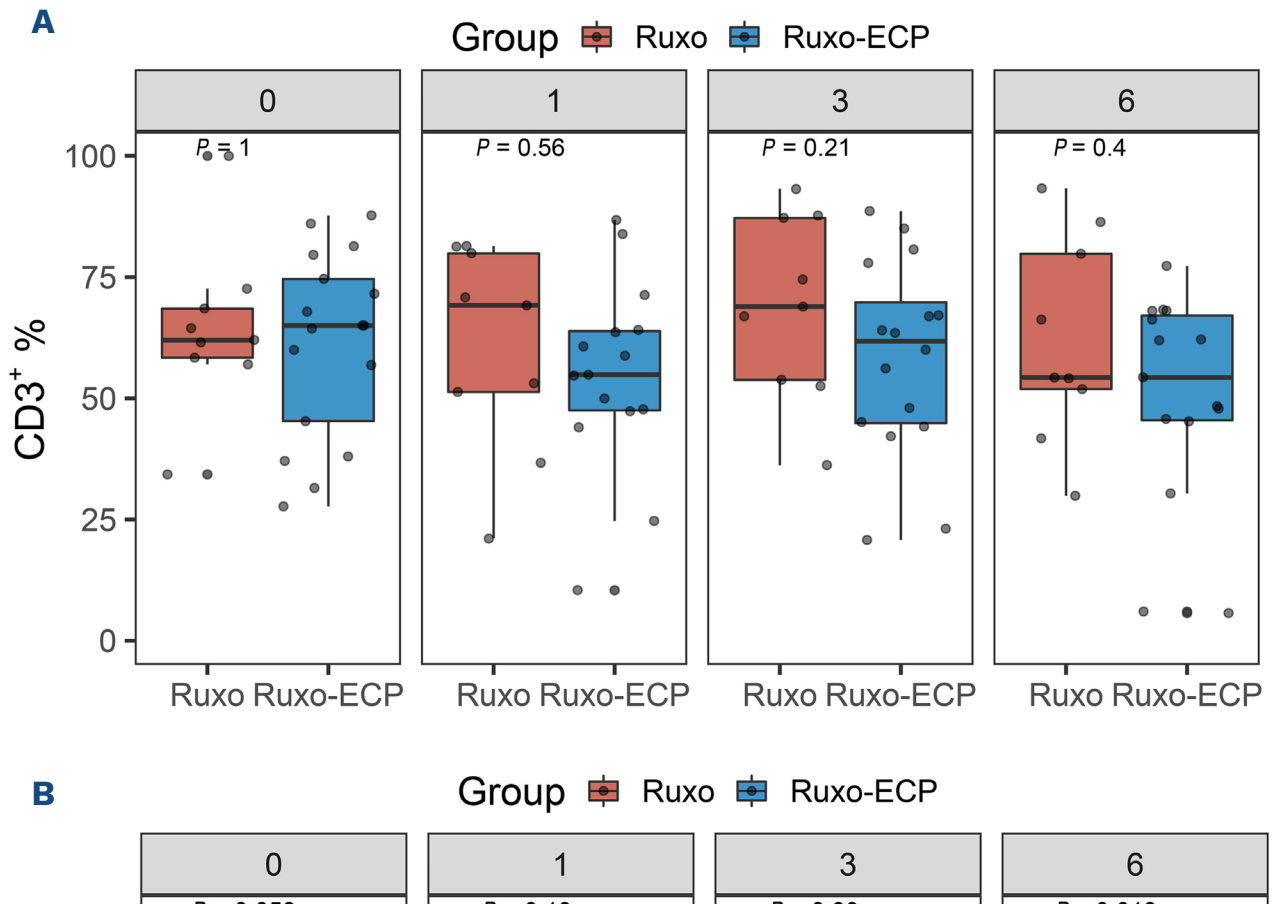


Figure 4. Immune reconstitution after treatment with ruxolitinib alone and ruxolitinib plus extracorporeal photopheresis at start, 1, 3, and 6 months. (A) For T cells, (B) for regulatory T cells, and (C) for B cells. ECP: extracorporeal photopheresis; Ruxo: ruxolitinib.

the results also provide a rationale to investigate ECP as combination partner with other agents to treat acute and chronic GvHD.

Disclosures

NK received honorarium from Mallinckrodt, Novartis, Kit/Gilead, BMS, Takeda, Medac, Neovii, and Sanofi. FA received honorarium from Abbvie, BMS, Kite/Gilead, Janssen, Mallinckrodt, Miltenyi, Novartis, Medac, and Takeda, and a research grant from Mallinckrodt. SH received a travel grant from Sanofi and honorarium from Mallinckrodt. All of the other authors have no conflicts of interest to disclose.

Contributions

IL and NK designed study, analyzed data, and wrote the manuscript. EK performed statistical analysis. EK and NG created figures. All other authors interpreted data and approved the final manuscript.

Data-sharing agreement

Please contact the corresponding author.

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