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ARTICLE

Long-term outcomes of ruxolitinib therapy in steroid-refractory graft-versus-host disease in children and adults

I. S. Moiseev[1] · E. V. Morozova[1] · T. A. Bykova[1] · O. V. Paina[1] · A. G. Smirnova[1] · A. A. Dotsenko[1] · E. S. Borzenkova[1] · A. N. Galimov[1] · Ya. V. Gudognikova[1] · K. A. Ekushov[1] · P. V. Kozhokar[1] · A. A. Osipova[1] · O. V. Pirogova[1] · T. A. Rudakova[1] · O. U. Klimova[1] · N. Yu Tcvetkov[1] · E. A. Kulagin[1] · E. A. Surkova[2] · S. V. Lapin[2] · G. G. Rodionov[3] · S. I. Moiseev[3] · Yu. A. Serov[1] · L. S. Zubarovskaya[1] · B. V. Afanasyev[1]

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Abstract

Acute and chronic steroid-refractory graft-versus-host disease (srGVHD) is a life-threatening complication of allogeneic stem cell transplantation. There are a number of reports on case series describing efficacy of ruxolitinib in both acute and chronic srGVHD. We conducted a prospective study (NCT02997280) in 75 patients with srGVHD (32 acute, 43 chronic, 41 adults, and 34 children). Patients with chronic GVHD had severe disease in 83% of cases, and acute GVHD patients had grade III–IV disease in 66% of cases. The overall response rate (ORR) was 75% (95% CI 57–89%) in acute GVHD and 81% (95% CI 67–92%) in chronic. Overall survival was 59% (95% CI 49–74%) in acute group and 85% (95% CI 70–93%). The major risk factors for lower survival were grade III–IV gastrointestinal involvement (29% vs 93%, $p = 0.0001$) in acute form and high disease risk score in chronic (65% vs 90%, $p = 0.038$). Toxicity was predominantly hematologic with 79% and 44% of grade III–IV neutropenia in acute and chronic groups, respectively. There was no difference between adults and children in terms of ORR ($p = 0.31$, $p = 0.35$), survival ($p = 0.44$, $p = 0.12$) and toxicity ($p > 0.93$). The study demonstrated that ruxolitinib is an effective option in acute and chronic srGVHD and can be used both in adults and children.

Introduction

Steroid-refractory graft-versus-host disease (srGVHD) is still one of the major causes of mortality after allogeneic stem cell transplantation. A recent study indicated that there was little progress in long-term survival of patients with GVHD requiring systemic treatment and mortality still exceeds 50% [1]. Patients with GVHD failing first-line steroids have even worse prognosis. Despite multiple agents and methods were tested in clinical trials currently there is no standard of care for both acute [2] and chronic srGVHD [3]. The efficacy of immunosuppressive regimens is usually counterbalanced with excessive mortality due to secondary infections. One of the promising approaches is the use of kinase inhibitors, which target GVHD pathogenesis pathways. Recently ibrutinib, a Bruton-kinase inhibitor, was approved for chronic srGVHD [4]. Also, the muticenter confirmatory studies of ruxolitinib, a Janus-kinase (JAK) inhibitor, are ongoing both in acute and chronic srGVHD [5].

Ruxolitinib was developed for the treatment of myelofibrosis as the JAK pathway activation is one of the events in the pathogenesis of this disease [6]. However cases of abnormal infectious complications in myelofibrosis patients treated with ruxolitinib have initiated a series of studies that demonstrated the multiple immunosuppressive mechanisms of ruxolitinib, which are involved in development and resistance in GVHD [7, 8]. Subsequently the retrospective multicenter clinical study of ruxolitinib both in acute and

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✉ I. S. Moiseev
moisiv@mail.ru

[1] R.M. Gorbacheva Memorial Institute of Hematology, Oncology and Transplantation, Pavlov University, Saint-Petersburg, Russian Federation

[2] Laboratory of Autoimmune Diagnostics, Pavlov University, Saint-Petersburg, Russian Federation

[3] Nikiforov's All-Russian Center for Emergency and Radiation Medicine, Saint-Petersburg, Russian Federation

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chronic srGVHD demonstrated very promising response rate and survival [9]. This created a basis for further clinical research in this area. So far there are no published large prospective studies of ruxolitinib therapy in srGVHD patients. Also the preregistration research program involves only children older than 12 years of age [5] and data on small children will be scarce even after the completion of these studies. So we conducted a prospective single-center trial in patients from 1 year of age with acute or chronic srGVHD.

Patients and methods

The prospective single-center open-label study (NCT02997280, clinicaltrials.gov) was conducted from 2016 to 2018 in First Pavlov Medical University. The study was approved by the ethical committee of the Ministry of Health of Russian Federation and the local ethical committee and performed as clinical approbation program No.2016–29–1. All patients or their legal guardians gave informed consent for the participation in the study. The inclusion criteria were age from 1 to 70 years, the presence of steroid-refractory GVHD based on EBMT/ELN definition [10], Karnofsky index >30% and ability for oral drug intake. The exclusion criteria were severe organ dysfunction (liver function tests not related to liver GVHD > 5× upper limits of normal (ULN) and creatinine >2 × ULN) or requirement of vasopressor support at the time of enrollment. Seventy-five patients were enrolled. Thirty-two patients had acute GVHD and 43 had chronic GVHD (Table 1). Half of the patients were children. At the time of enrollment among the patients with acute srGVHD 11 had grade II disease, 10 had grade III disease, and 11 had grade IV disease. Skin was involved in 91%, gastrointestinal (GI) tract in 56%, and liver in 37% (Supplementary Fig. 1SA). Among patients with chronic GVHD 14% had moderate disease and 86% had severe disease. Most commonly skin (91%), mouth mucosa (81%), GI (56%), liver (51%), and eyes (74%) were involved. Lung involvement was observed in 40% of patients with 13% of moderate and severe cases (Supplementary Fig. 1SB). Among patients with clinically significant chronic skin GVHD 39% had scleroderma. Median number of previous lines in acute GVHD patients was 1 (range 1–2), in chronic GVHD patients was 2 (range 1–5).

Ruxolitinib was administered at a starting dose of 10 mg bid for adults and children with weight >40 kg and 0.15 mg/kg bid for children with a weight less than 40 kg. Patients were allowed to continue any previous therapy which attending physician considered necessary. Nonsystemic adjuvant therapy was not considered treatment failure. Dose modifications were allowed in case of grade 4 leukopenia, neutropenia, or thrombocytopenia. Under study protocol ruxolitinib was continued as long as there was no evidence of GVHD progression. Ruxolitinib was interrupted or discontinued in case of any other nonhematological grade 4 adverse event (AE). In the analysis if a patient discontinued ruxolitinib for a period due to response but then had a flair or chronic GVHD with restarted ruxolitinib treatment it was not considered a "discontinuation event".

Under study protocol the attending physician was allowed to continue any concurrent treatment administered before ruxolitinib if it was considered beneficial. Administration of any new method or escalation of steroids was considered treatment failure. In acute GVHD the following agents were co-administered with ruxolitinib: calcineurin

Table 1 Characteristics of patients.

Parameter	Acute srGVHD ($N = 32$)	Chronic srGVHD ($N = 43$)
Age, median, range, years Adults/children, %	17 (1–67) 47%/53%	21 (2–62) 61%/39%
Gender m/f, %	63%/37%	56%/44%
Diagnosis	Hereditary diseases 28% ALL 16% CML 16% AML 12.5% MDS 12.5% AA 6% HD 6% JMML 3%	AML 40% ALL 30% HD 10% AA 5% Hereditary diseases 5% NHL 5% CML 5%
DRI, median	2	2
Matched related donor Matched unrelated donor Haploidentical donor	6% 59% 35%	16% 70% 14%
HLA-matching <10/10	50%	35%
Graft source	BM 74% PBSC 26%	BM 33% PBSC 67%
Myeloablative conditioning	50%	60%
PTCy in GVHD prophylaxis ATG in GVHD prophylaxis	75% 31%	60% 33%
First allogeneic SCT Second allogeneic SCT	87.5% 12.5%	90.7% 9.3%
GVHD severity at baseline	Grade II 34.5% Grade III 31% Grade IV 34.5%	Moderate 14% Severe 86%
Time from GVHD onset to ruxolitinib, days, median (range)	16 (5–113)	376 (28–3219)
History of TA-TMA	12.5%	8%

Hereditary diseases = Hurler syndrome, Fanconi anemia, thalassemia, Wiskott–Aldrich syndrome

PTCy posttransplantation cyclophosphamide, *ATG* antithymocyte globulin, *TA-TMA* transplant-associated thrombotic microangiopathy, *AML* acute myeloid leukemia, *ALL* acute lymphoblastic leukemia, *CML* chronic myeloid leukemia, *MDS* myelodysplastic syndrome, *AA* aplastic anemia, *HD* Hodgkin's disease, *JMML* juvenile myelomonocytic leukemia.

inhibitors in 59%, sirolimus in 64%, mycophenolate mofetil in 15%, extracorporeal photopheresis in 12%, and anti-TNF antibodies in 9%. Median steroid dose was 1 mg/kg at enrollment. In chronic GVHD ruxolitinib was co-administered with calcineurin inhibitors in 37%, sirolimus in 42%, ECP in 17%, and rituximab in 12%.

Clinical definitions

Time to disease relapse, nonrelapse mortality (NRM), overall survival (OS), and failure-free survival (FFS) were defined as the time from ruxolitinib initiation to the event. Events for FFS were relapse, death, or escalation of immunosuppression. Disease relapse was defined as morphologic or cytogenetic evidence of disease with pretransplantation characteristics, or morphologic evidence without pretransplantation characteristics. The acute GVHD [11] was graded based on 1995 Consensus Conference criteria and chronic GVHD based on 2005 National Institute of Health (NIH) criteria [12]. The response in acute GVHD was assessed based on 2009 joint statement criteria [13]. The response in chronic GVHD was assessed by NIH 2014 criteria [14]. Disease risk was measured using Armand et al. score for malignant diseases and it was set to zero for nonmalignant [15]. The primary endpoint of the study was overall response rate (ORR) based on these criteria. Cytomegalovirus (CMV) reactivation was documented in case of >500 copies/ml detected by RQ-PCR. The secondary endpoints were OS, toxicity based on NCI CTCAE 4.03, infections complications, incidence of relapse of underlying disease. Among AEs routinely hematological toxicity, liver and kidney toxicity along with infectious complications were collected. Also severe AE considered by the attending physician related to ruxolitinib and not GVHD were documented.

Laboratory studies

In all patients at least one sample of EDTA plasma was collected after 5–7 days of treatment, centrifuged within 2 h after collection at 1000 *g* for 15 min at 4 °C temperature, aliquoted and stored at −70 until the day of the assay. All samples were collected before the next dose of ruxolitinib. A high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) method was used to determine the minimal (Cthrough) concentration of ruxolitinib in human plasma. Methodology was previously described in detail [16]. Analyzes were performed using HPLC Agilent 1200 with triple quadrupole mass spectrometer Agilent 6460 with system of ionization—electro spray (Agilent technology, USA). Levels of interleukin-8 (IL-8), interferon gamma (IFN-γ), interleukin 17 (IL-17), and interleukin 1β (IL-1β) were measured using commercially available enzyme-linked immunosorbent assay kits (Cytokine LLC, RF) according to the manufacturer's instructions.

Statistical analysis

In the study group description OS and FFS was performed using Kaplan–Meier methodology. The comparisons were made using the log-rank test. Cumulative incidence analysis was used for the incidence of relapse, NRM, discontinuation of ruxolitinib, and discontinuation of all immunosuppression. The comparisons were made using Gray test. The competing risks for discontinuation of ruxolitinib were administration of new systemic therapy or relapse. The competing risk for discontinuation of immunosuppression was relapse. Nonparametric data were analyzed with Chi-square, Fisher exact and Mann–Whitney tests according the type of data in each group. Exact confidence limits were calculated. Association between quantitative parameters was performed with Spearman correlation.

Results

Response to ruxolitinib

Patients with acute srGVHD ORR was 75% (95% CI 57–89%), including 63% of patients with complete response (CR) (95% CI 44–79%) and 12% with partial response (PR) (95% CI 4–29%) (Fig. 1a). The absence of response or progressive disease was observed in 25% of patients. Median time to PR in patients with acute GVHD was 20 days (range 1–112). Median time to CR was 53 days (range 9–255). There was a trend with longer time to PR in patients with GI involvement (median 26 days vs 19 days, $p = 0.08$), while other organ involvement had no impact on time to CR ($p = 0.36$). Overall severity of GVHD was not associated with time to PR ($p = 0.91$) and CR ($p = 0.99$). None of the transplantation and donor characteristics were predictive for response in acute GVHD patients. Also no difference in response was observed according to the concomitant immunosuppressive agents ($p > 0.2$). No differences in ORR was observed in adults and children ($p = 0.31$) (Supplementary Table S1, Supplementary Fig. S2). Nonetheless, patients with grade III–IV GVHD ($p = 0.0292$) had significantly reduced ORR. The severity of skin ($p = 0.0868$) was not predictive for ORR, but liver GVHD severity ($p = 0.032$) and grade IV GI GVHD ($p = 0.003$) were associated with worse response rate. Also overall severity of acute GVHD ($p = 0.0190$) and the presence of grade IV GI GVHD ($p = 0.0081$) were predictive for lower CR rate.

Patients with chronic srGHVD had ORR of 81% (95% CI 67–92%), including 21% of CR (95% CI 10–36%) and

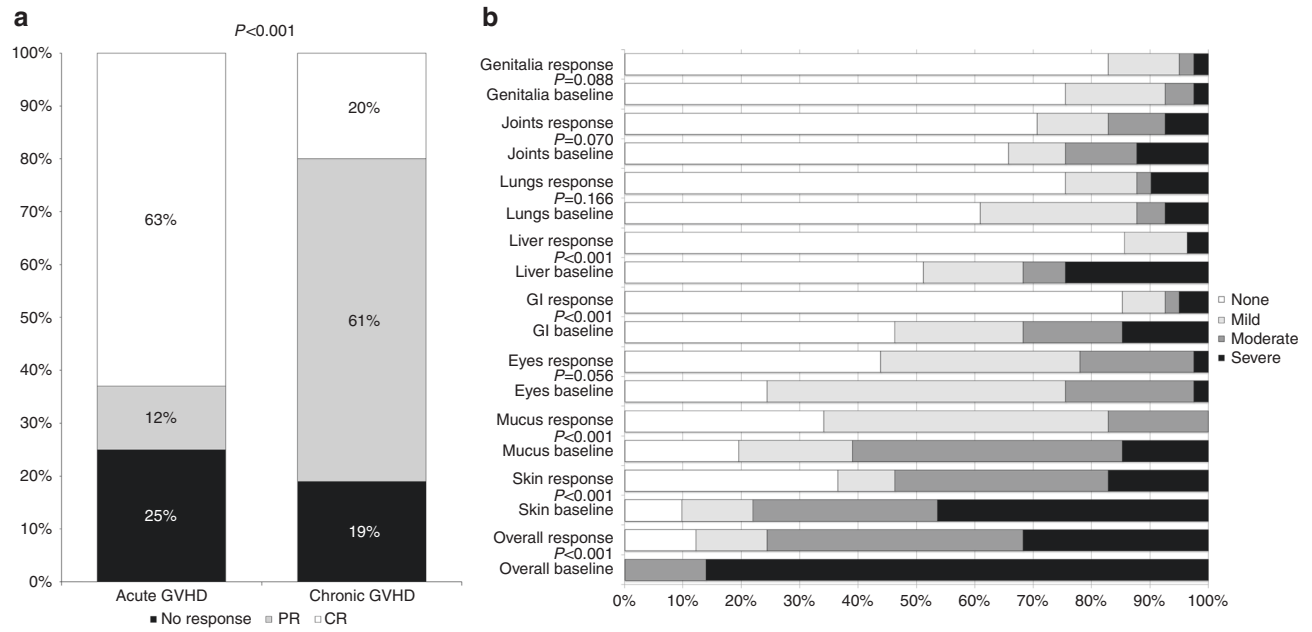

Fig. 1 **a** Best response to ruxolitinib in acute and chronic srGVHD. **b** Comparison of organ severity at baseline and at last follow-up or before initiation of another therapy line.

60% of PR (95% CI 44–75%). Median time to PR was 71 days (range 18–783) and median time to CR was 425 (27–635 days). None of the transplantation and donor characteristics were predictive for response also in the chronic GVHD patients. History of acute GVHD also had no impact on ORR ($p = 0.44$), however there was a trend to shorter time to PR in patients without history of acute GVHD (50 vs 89 days, $p = 0.060$). No difference in response was observed if patients continued previously administered CNIs ($p = 0.7$), sirolimus ($p = 0.4$), imatinib ($p = 0.35$), rituximab ($p = 0.85$), or ECP ($p = 0.082$). No differences in response was observed in adults and children ($p = 0.35$) (Supplementary Table S1). Initial severity of organ involvement was not predictive for response except for lung GVHD severity ($p = 0.0023$). The analysis of final severity revealed that there was a significant reduction in the scores of skin severity ($p < 0.001$), mouth mucosa ($p < 0.001$), GI tract ($p < 0.001$), and liver ($p < 0.001$). On the other hand, the changes in the severity scores of eyes ($p = 0.056$), lungs ($p = 0.166$), joints ($p = 0.070$), and genitalia ($p = 0.088$) were not significant (Fig. 1b). The best organ responses were observed for the mouth mucosa with only 17% having moderate disease and none severe, for GI tract with only 2% having moderate disease and 5% severe, and for the liver with only 4% having severe disease and none moderate at last follow-up. Due to significant proportion of patients with scleroderma in the study group, there were only 47% of patients in whom there was either complete skin response or conversion to mild clinical symptoms. Surprisingly, there were 15% of patients with mild bronchiolitis obliterans who had a complete resolution of lung GVHD. On the other hand, patients with moderate lung disease progressed to severe form, which overall resulted in nonsignificant changes in severity. There was a slight increment of mean FEV1 values during first-year evaluations ($63 \pm 4\%$, $63 \pm 2\%$, $69 \pm 9\%$, $71 \pm 4\%$), but the difference was not significant ($p > 0.27$, Supplementary Fig. S3) due to opposite dynamics in these patients. The evaluation of joint response revealed that despite there was a reduction in the scleroderma severity limiting joint motility there were cases of aseptic necrosis and rheumatoid-arthritis like disease. This resulted in nonsignificant changes in the joint severity scores.

Long-term outcomes

Median follow-up was 28 months, range 23–47 months. OS in the acute GVHD group was 59% (95% CI 49–74%), including 34% NRM (95% CI 19–51%) and 6% of relapse (95% CI 1–18%). In the chronic GVHD group survival was significantly higher, 85% (95% CI 70–93%), $p = 0.0028$ (Fig. 2a). NRM was observed in 7% (95% CI 2–18%) of patients and relapse of malignancy was documented in 10% (95% CI 3–22%). The difference between acute and chronic groups remained significant (HR 0.23, 95% CI 0.08–0.63, $p = 0.004$) when corrected for underlying disease risk (HR 2.9, 95% CI 1.0–8.0, $p = 0.044$) and response to ruxolitinib (HR 0.2, 95% CI 0.07–0.55). The major factor predicting survival in acute GVHD group was grade III–IV GI involvement (29% vs 93%, $p = 0.0001$, Supplementary

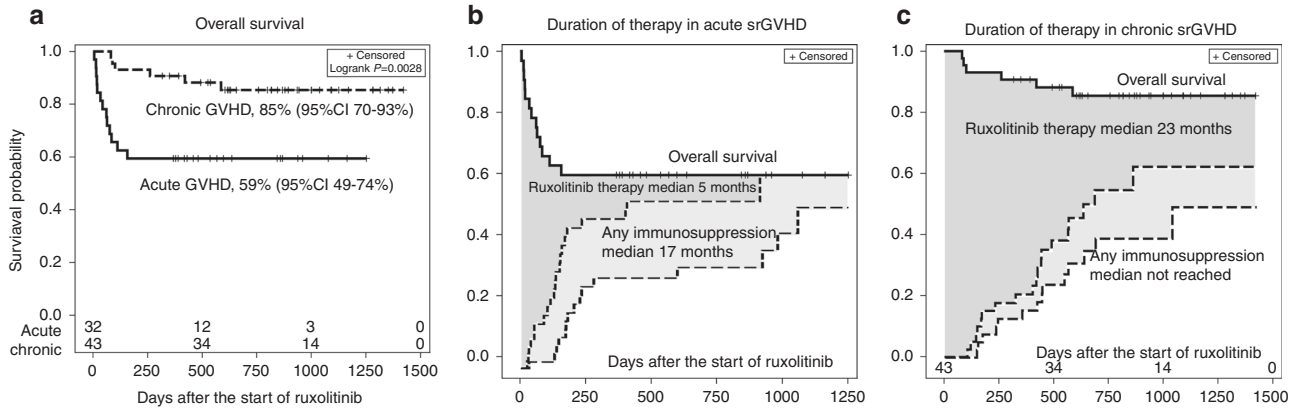

Fig. 2 **a** Comparison of overall survival in acute and chronic srGVHD patients. **b** Duration of immunosuppression in acute GVHD patients. **c** Duration of immunosuppression in chronic GVHD patients.

Fig. S4). No differences in survival was observed between adults and children (65% vs 53%, $p = 0.44$). Neither overall severity of chronic GVHD, nor organ involvement, nor the age of patients were predictive for OS. Highest predictive value had underlying disease risk ($p = 0.038$, Supplementary Fig. S4).

In acute srGVHD patients FFS was 56% (95% CI 38–71%, Supplementary Fig. S4). Median duration of ruxolitinib therapy was 5 months. Median time to discontinuation of all immunosuppressive treatment was 17 months. Nine patients (28%) had an overlap syndrome. Overall chronic GVHD developed in 12 patients (38%) treated for acute srGVHD and was severe in 13%, moderate in 16%, and mild in 9% of patient. The overlap syndrome and chronic GVHD determined the necessity of long-term immunosuppression. At the end of follow-up 88% of patients discontinued ruxolitinib and 80% discontinued all immunosuppressive treatments (Fig. 2b). There was no difference in the duration of ruxolitinib therapy between adults and children ($p = 0.12$). The only predictive parameter determining the duration of therapy was the type of donor, it was significantly longer after haploidentical transplantation than after MRD and MUD ($p = 0.006$).

In chronic srGVHD patients FFS was 74% (95% CI 57–86, Supplementary Fig. S5). Median duration of ruxolitinib therapy was 23 months and median duration of immunosuppression was not reached. At last follow-up 53% of patients discontinued ruxolitinib and 45% were immunosuppression-free (Fig. 2c). The duration of therapy was also dependant on the type of donor with shorter duration after MRD transplantation and longer duration after MUD and haploidentical ($p = 0.0189$). There was a trend to more frequent successful completion of therapy in patients without scleroderma (75% vs 33%, $p = 0.0552$). The other clinical parameters, including age ($p = 0.7399$), did not predict the duration of therapy.

Toxicity and complications

Although there were preexisting cytopenias before ruxolitinib administration, clearly hematological toxicity was the most common complication. In patients with acute GVHD 86% had hemoglobin below 80 g/l or transfusion dependence. Also 53% exhibited grade 4 leukopenia, 41% grade 4 neutropenia, and 77% grade 4 thrombocytopenia, which was a significant change from the baseline incidences ($p < 0.001$). Liver and kidney toxicities were uncommon and predominantly related to concomitant medications (Table 2, Supplementary Fig. S6). Sixty-five percent of patients received systemic antibiotics and 35% ganciclovir and the start of ruxolitinib. After ruxolitinib initiation 59% had either persistence or de novo CMV reactivation. Seventy-four percent received additional antibiotic treatment (a median of two courses), 62% additional systemic antiviral treatment with ganciclovir or foscavir, and 32% additional antifungal treatment besides prophylaxis with pozaconasol. Uncommon infections included *Moraxella* spp. meningitis, BK-viral encephalitis, sepsis *Elizabethkingia meningoseptica*, EBV-associated proliferative colitis. The detailed description of severe AEs and severe infections is presented in Supplementary Table S2.

In chronic GVHD patients ruxolitinib was tolerated significantly better than in acute with grade 4 hematological toxicity observed only in less than 15% of patients. Nonetheless, the significant decrease from baseline was observed for white blood cell count, neutrophils, and platelets ($p < 0.001$). Viral reactivations were observed less frequent, but one case of CMV-associated and one of rhinosyncitial virus-associated pneumonias were documented. Rare complications included lung tuberculosis 3 years after the SCT, esophageal adenocarcinoma in the same patient 4 years after SCT, myasthenia gravis with respiratory failure, generalized *Mycobacterium avium*

Table 2 Toxicity of ruxolitinib in srGVHD.

Adverse event	Grades	Acute GVHD		Chronic GVHD	
		Before ruxolitinib administration. % of patients	On ruxolitinib therapy. % of patients	Before ruxolitinib administration. % of patients	On ruxolitinib therapy. % of patients
Anemia	Normal	11.8%	2.9%	61.0	14.6
	Grade 1	17.7%	2.9%	17.1	34.2
	Grade 2	38.2%	8.8%	17.1	24.4
	Grade 3	28.1%	79.4%	4.9	26.8
	Grade 4	6.3%	6.3%	0.0	0.0
Leukopenia	Normal	52.9%	2.9%	82.9	26.8
	Grade 1	11.8%	5.9%	12.2	34.2
	Grade 2	26.5%	2.9%	2.4	22.0
	Grade 3	8.8%	35.3	2.4	12.2
	Grade 4	0.0%	52.9%	0.0	4.9
Neutropenia	Normal	35.3%	5.9	61.0	14.6
	Grade 1	26.5%	0.0	26.8	34.2
	Grade 2	20.6%	14.7	4.9	7.3
	Grade 3	5.9%	38.2	4.9	36.6
	Grade 4	11.8%	41.2	2.4	7.3
Lymphopenia	Normal	8.8%	0.0%	41.5	19.5
	Grade 1	32.4%	5.9%	43.9	36.6
	Grade 2	20.6%	8.8%	12.2	17.1
	Grade 3	20.6%	29.4%	0.0	22.0
	Grade 4	17.7%	55.9%	2.4	4.9
Thrombocytopenia	Normal	17.7%	0.0%	80.5	68.3
	Grade 1	17.7%	5.9%	12.2	7.3
	Grade 2	8.8%	2.9%	2.4	2.4
	Grade 3	35.3%	14.7%	2.4	7.3
	Grade 4	20.6%	76.5%	2.4	14.6
Liver function tests abnormal[a]	Normal	76.2%	33.3%	90.5%	66.7%
	Grade 1	14.3%	28.6%	4.8%	33.3%
	Grade 2	9.5%	19.1%	0.0%	0.0%
	Grade 3	0.0%	9.5%	4.8%	0.0%
	Grade 4	0.0%	9.5%	0.0%	0.0%
Creatinine abnormal	Normal	85.7%	61.9%	85.7%	71.4%
	Grade 1	9.5%	23.8%	9.5%	23.8%
	Grade 2	0.0%	14.3%	4.8%	0.0%
	Grade 3	4.8%	0.0%	0.0%	0.0%
	Grade 4	0.0%	0.0%	0.0%	4.8%
Hemorrhagic cystitis		17.7%	17.7%	12.2%	2.4%
CMV reactivation		38.2%	58.8%	65.9%	17.0%

[a]Assessed only in patients without established diagnosis of liver GVHD.

infection and persistent clonal large granular lymphocytosis (Table 2, Supplementary Table S2).

The severity of neutropenia was affected by CMV reactivation ($p = 0.07$), treatment with ganciclovir ($p = 0.0006$), and higher initial steroid dose ($p = 0.0017$). The same factors predicted the severity of thrombocytopenia ($p < 0.01$). Concentration of ruxolitinib was not predictive for severe hematological toxicity ($p = 0.55$). There was no difference in the severity of hematological toxicity between adults and children ($p = 0.93$).

The mean starting dose of ruxolitinib was 0.24 mg/kg. During treatment the dose of ruxolitinib was significantly more often tapered due to toxicity in acute GVHD patients compared with chronic (38% vs 18%, $p = 0.0453$). The

mean dose after tapering was 0.19 mg/kg in acute and 0.21 mg/kg in chronic GVHD. There was no difference in the starting dose ($p = 0.13$) and final dose ($p = 0.83$) between adults and children. In patients in whom the dose was tapered the grade of cytopenia decreased in 50% of patients.

Laboratory assays

Patients with acute GVHD had significantly lower ruxolitinib Cthrough on day +7 than patients with chronic (median of 1 vs 75 ng/ml, $p = 0.02$). The difference in concentrations was related to grade III–IV GI GVHD. These patients had lower Cthrough levels (median 1 ng/ml) compared with acute GVHD patients without severe GI involvement (median 55 ng/ml), $p = 0.06$. There was no difference between adults and children ($p = 0.93$). Also no statistically significant association between Cthrough levels and response was observed ($p = 0.43$).

There was a significant variability in the IL-1, IL-8, IL-17, and interferon gamma concentrations in the first 2 weeks after initiation of ruxolitinib. Only interferon gamma demonstrated a rapid decline (Supplementary Fig. S7). Nonetheless, the direction of changes was similar, and there was a positive correlation between changes in interferon gamma concentrations and IL-8 ($p = 0.0240$), IL-17 ($p = 0.0124$). Due to the significant variability none of the cytokines tested were predictive for response ($p > 0.2$).

Discussion

In this prospective study of ruxolitinib in steroid-refractory acute and chronic GVHD we have demonstrated high response rate and favorable survival. These outcomes are very similar to previously published retrospective studies by Zeiser et al. [9] and Escamilla Gómez et al. [17]. However the survival rates of acute GVHD patients differ between these two studies. The most likely reason is higher percentage of patients with GI GVHD in the Escamilla Gómez et al. study where there was 95% of these patients. In the present study the same observation was made that patients without grade III–IV GI GVHD have an excellent long-term survival reaching 93% while only third of patients with severe acute GI GVHD survive. These results are somewhat better than reported outcomes of best available therapy for severe acute GI GVHD in some leading centers [18], however there is a significant need for improvement. The results highlight the importance of combination approaches in this group of patients. Given the significance of gut microbiota and high incidence of association between GI GVHD and colonization with multidrug resistant bacteria [19], fecal microbiota transplantation is one of the promising approaches for combination therapy [20].

Ruxolitinib is currently approved for acute but not chronic GVHD. However in this study we observed fair response rate for both acute and chronic disease. Acute and chronic GVHD have significant differences in pathogenesis. The key events in the acute form is disruption of natural barriers, release of danger-associated molecular patterns along with autoantigens, increased toll-like receptor signaling and presentation by dendritic cells, cytokine dysregulation and "storm" leading to proliferation of alloreactive clones of T-cells and tissue damage [21, 22]. Chronic GVHD pathogenesis comprises several events including T-regulatory cell depletion, disruption of central tolerance, abnormalities in the B-cell maturation processes, and several others [23–25]. One of the reasons why ruxolitinib works in both acute and chronic GVHD is that it targets the JAK signaling, which mediates many cytokine pathways including IL-2, IL-6, IL-10 as well as T-cell proliferation and antigen presentation by dendritic cells [7, 8]. Thus the beneficial effects of ruxolitinib might be mediated via different pathways. Further studies are required to elucidate the exact mechanisms. Understanding of these mechanisms will allow to predict response beyond the disease severity.

Current consensus statements recommend using day 28 response as a surrogate marker of efficacy in acute GVHD [13, 26], however in the present study we observed that with ruxolitinib the median time to PR was very close to this time point and in severe GI GVHD time to PR occurred in several cases beyond several months. Thus for target agents, like ruxolitinib, the timing for response assessment might be longer. Although the idea of implementing ruxolitinib as immunosuppressive agent came from cases of opportunistic infections in myelofibrosis patients [27] and there is a concern of infection-related mortality from prolonged ruxolitinib administration, in this study we have not seen significant NRM beyond 2 months of therapy. Thus the conclusion of the study is that ruxolitinib in acute form should be continued until CR if there is no severe hematological toxicity or no clear evidence of GVHD progression. For chronic GVHD the recommended time to assess objective response is 2–5 months [14], but in our study, especially in patients with scleroderma, we have seen the delayed objective PR even beyond 1 year. However the clinical benefit for the majority of patients was observed during first 3–6 months even without PR criteria. The study indicated that the absence of objective response without evidence of GVHD progression should not be an indication to switching therapy, the responses could be observed with longer follow-up.

The chronic GVHD part of the study gives the first information about organ response after ruxolitinib treatment. Currently only data on extracorporeal photopheresis efficacy exists for srGVHD patients. Several studies demonstrated that skin and oral mucosa improvement was

most prominent [28, 29], however in this study we have observed significant number of CRs in GI, liver, and oral GVHD. There were not many complete skin responses because half of the patients in the study had severe scleroderma and this form with conventional treatment is associated with slow if any responses [30]. These results are comparable with the previously described patient series [31]. Interesting results were observed for bronchiolitis obliternas. Since the first report by George Santos group [32] this form was considered an eminently progressive disease and the treatment was to slow the deterioration of lung function. However in this study the majority of patients with mild lung disease had a CR, while patients with moderate and severe disease had either stabilization or deterioration of lung function. Overall there was a mean improvement in FEV1 parameter (Supplementary Fig. S6). These results strengthen the necessity of early ruxolitinib therapy in case of steroid-refractory lung involvement.

Currently there is limited data on discontinuation of immunosuppression in srGVHD patients. In the general cohort of chronic GVHD patients the median time to discontinuation of immunosuppressive therapy was 1.6 years [33]. In the present study this time was very similar despite the fact that this was a refractory cohort of patients, which indicates the efficacy of ruxolitinib in this setting. Discontinuation of systemic immunosuppression is the key goal of therapy, because the majority of deaths occur due to secondary infectious complications [34]. Our study indicates that immunosuppression can be discontinued in this population of patients. Surprisingly, the time to discontinuation was comparable in acute and chronic GVHD patients due to significant number of overlap syndromes and subsequent chronic GVHD in the acute group.

One of the major points of this study is the identical results in adults and children starting from 1 year of age. Previous pediatric patients' series reported comparable response rates and survival to the adult population [35, 36], but in this study we prospectively demonstrated the absence of differences in mean dosage, response rate, toxicity, complications, and long-term outcomes between adults and children. Ruxolitinib can be used in young children population, including patients with hereditary diseases.

Toxicity was predominantly hematologic which is consistent with the previously published data [9]. It was significantly more prominent in acute GVHD patients than in chronic, which is, partly related rather to viral reactivations and antiviral treatments than to direct hematological toxicity [37]. CMV reactivation and ganciclovir treatment are the long known risk factors of cytopenias after SCT [28]. The absence of hematological recovery in half of the patients after ruxolitinib taper might indicate complex etiology of these cytopenias.

In conclusion, the study demonstrated high efficacy and favorable outcomes of ruxolitinib in adults and children with srGVHD.

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Compliance with ethical standards

Conflict of interest ISM received consultancy and lecturer honoraria from Novartis. EVM received lecturer honoraria from Novartis. The other authors have no conflicts of interest.

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