ARTICLE

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Clinical outcomes of third-line therapy for aGvHD with gastrointestinal involvement after steroids and ruxolitinib failure

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CHRONOS is a multicenter, retrospective, cohort study involving acute graft-versus-host disease (aGvHD) adult patients with gastrointestinal (GI) symptoms, steroid- and ruxolitinib refractory, who initiated third-line therapy between 30 May 2019 and 30 September 2024. Primary endpoints were all-organ overall response rate (ORR) and GI-specific ORR (GI-ORR) around 28 days after treatment initiation. Secondary endpoints included duration of response, real-world progression-free survival (rwPFS) of underlying malignancy, and overall survival (OS). Fifty-nine patients from 16 sites in Europe were included. On Day 28, ORR was 36% (95% CI: 24–49%), GI-ORR was 37% (95% CI: 25–51%); 29% (95% CI: 11–49%) of responders lost response within 30 days, and 52% (95% CI: 29–72%) within 90 days. Median rwPFS and median OS were both 86 days (95% CI: 54–128 days). Median OS was higher in responders than in non-responders (186 versus 45 days). Over the 12-month follow-up period, 41 patients died, mainly due to aGvHD progression ($n = 25$), and infectious complications ($n = 9$). Within 3 months, Grade ≥ 2 infectious events occurred in 51% of patients; Grade 3–4 thrombocytopenia and neutropenia in 64% and 32%, respectively. These findings demonstrate limited effectiveness of third-line therapy in this cohort of steroid- and ruxolitinib-refractory aGvHD patients with GI symptoms.

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INTRODUCTION

Acute graft-versus-host disease (aGvHD) is a leading cause of non-relapse mortality after allogeneic hematopoietic cell transplantation (allo-HCT) [1–3], occurring in 30% to 60% of patients [4–8]. aGvHD typically affects the skin, the gastrointestinal (GI) tract, and the liver [9, 10]. GI involvement increases the risk of non-relapse mortality after allo-HCT [11–13].

Systemic corticosteroids are the standard first-line treatment for patients with Grade II–IV aGvHD (Mount Sinai Acute GvHD International Consortium [MAGIC] classification) [14]. The proportion of steroid-refractory (SR) patients (those who do not respond to high-dose corticosteroids or are unable to taper steroids after an initial response) can reach 70% among those with Grade III–IV aGvHD [15, 16]. Moreover, responses are not durable for approximately half of the initial responders to steroids [17]. An SR status is associated with a high mortality [11, 18–20] whereas steroid dependency exposes patients to the risk of serious adverse effects including infectious complications [21].

Ruxolitinib, a Janus kinase inhibitor, has become the standard second-line therapy for patients with SR-aGvHD. In the REACH2 randomized controlled phase III study (NCT02913261), the overall response rate (ORR) at Day 28 was higher in the ruxolitinib group than in the control group (62% versus 39%) but decreased to 40% at Day 56, underscoring a substantial unmet medical need for those patients who failed to respond at Day 28, or worsened afterwards [22]. In addition, a retrospective study involving 57 patients with SR-GI-aGvHD who received ruxolitinib as second-line therapy showed an ORR of 45% at Day 28 and 48% at Day 56 [23].

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No approved third-line therapy for SR-aGvHD patients is available [24]. Several therapeutic options have been explored, many of which were used off-label, but existing evidence largely limited to single–center or multicenter studies in heterogenous patient populations [25]. In the seminal retrospective study by Abedin et al., including 43 patients, response rates to third-line therapy at any timepoint were 36% among 33 ruxolitinib- and steroid-resistant-aGvHD patients and slightly higher (40%) when ruxolitinib-resistant and 10 ruxolitinib-intolerant patients were mixed [24]. This study had some limitations including that responses were not evaluated at predefined timepoint. Consequently, the real-world outcomes of these therapies remain poorly characterized.

SUBJECTS AND METHODS

CHRONOS is a multicenter, international, non-interventional retrospective cohort study initiated by MaaT Pharma (Lyon, France) and conducted in Austria, Belgium, France, and Spain. Data were protected in conformity with the European General Data Protection Regulation. Patients received written information and were given the option to opt out of the use of their personal data in Austria and France whereas in Belgium and Spain, the independent ethics committee granted an informed consent waiver. The study was conducted in compliance with the principles of Good Pharmacoepidemiology Practice [26].

A systematic, multistep approach was employed to identify eligible patients and select participating transplant sites. Data for all consecutive patients who received third-line therapy between 30 May 2019 and 30 September 2024, with evaluable outcomes, were extracted from medical charts in reverse chronological consecutive order and staff at participating sites enter them into an electronic case report. Eligible patients had to be at least 18 years old, have undergone allo-HCT, irrespective of donor type, stem cell source, conditioning regimen, or GvHD prophylaxis and present with aGvHD with GI involvement (Grade II–IV as per MAGIC criteria [27, 28]) at the time of initiation of third-line therapy. Inclusion criteria also included resistance to or dependence on first-line steroids and resistance to or dependence on second-line ruxolitinib [29]. Patients must have received any systemic aGvHD treatment in the third-line setting (excluding fecal microbiotherapy due to lack of commercial availability in the third line setting). Their records had to contain information on treatment response to third-line therapy for those alive at predefined timepoints, and information on vital status (see Supplementary Appendices for the complete list of inclusion and non-inclusion criteria).

This study was designed to be purely descriptive, without any formal hypothesis testing or inter-group comparisons. The primary endpoints were the all-organ ORR and GI-ORR at Day 28 after third-line therapy initiation, including complete response, very good partial response, and partial response [30]. aGvHD staging and grading were based on the MAGIC criteria [27] and assessed by the site investigators as were responses (see Supplementary Appendices for definitions of response). Unlike prospective trials with protocol-mandated visit schedules, real-world retrospective analyses must utilize existing clinical assessments, which may not align precisely with predetermined timepoints. For the primary analysis, we defined the Day 28 response as the assessment occurring closest to Day 28, regardless of the time interval. When multiple assessments were equidistant from Day 28, we selected the earlier assessment. This approach maximizes the evaluable patient population and reflects real-world practice patterns. To evaluate the impact of assessments performed distant from Day 28, we conducted a sensitivity analysis restricting responses to those within ±7 days (Day 21–35). This more restrictive approach tested whether our findings remained robust when limiting the analysis to responses temporally proximate to the standard Day 28 endpoint used in prospective aGvHD trials (see Supplementary Appendices for illustration).

Secondary endpoints included the all-organ and GI response rates at Day 56 after initiation of third-line therapy using the same visit window methodology as the primary endpoint, cumulative incidence of loss of response (LOR), real-world progression-free survival (rwPFS), overall survival (OS), and incidence of chronic GvHD (cGvHD). LOR was defined as the cumulative incidence of loss of response accounting for the competing risks of cGvHD and mortality without evidence of progression. All response assessments, as well as competing risk events were defined by the investigators at each site and captured directly within the case report form. rwPFS, OS, and incidence of cGvHD were estimated up to 12 months

Table 1. Eligible study population.

	$N = 59$
Age, years [IQR]	59 ± 11 [49; 64]
Male gender	32/59 (54%)
Primary hematological disease	
Myeloid malignancies	
Chronic myeloid leukemia	1
Chronic myelomonocytic leukemia	3
Myelofibrosis	3
Myeloproliferative neoplasm	1
Myelodysplasia	20*
Acute myeloid leukemia	18
Lymphoid malignancies	11/59 (19%)
Non-Hodgkin lymphoma	1
Hodgkin disease nodular sclerosis	1
T-cell lymphoma	2
Acute lymphoid leukemia	4
Chronic lymphocytic leukemia	2
Myeloma	1
Others	2/59 (3%)
Vexas syndrome	1
Systemic mastocytosis	1
HCT-CI score	
0	19/51 (37%)
1	7/51 (14%)
2	3/51 (6%)
3	12/51 (24%)
4	8/51 (16%)
5	2/51 (4%)
HCT conditioning regimen	
Myeloablative	22/59 (37%)
Reduced intensity	26/59 (44%)
Non myeloablative	11/59 (19%)
Anti-thymocyte globulin	29/58 (50%)
Post-transplant cyclophosphamide	23/59 (39%)
Stem cell source	
Peripheral blood	52/59 (88%)
Bone marrow	7/59 (12%)
HLA-matching donor type	
Matched unrelated	24/59 (41%)
Matched related	14/59 (24%)
Haploidentical	15/59 (25%)
Mismatched unrelated	6/59 (10%)
aGvHD Type***	
Classical	47/59 (80%)
Late onset / Post-donor lymphocyte	6/59 (10%)
Late onset	6/59 (10%)

Results are expressed as median (interquartile range) or n/N observed (%)
* one patient with an overlapping form myelodysplasia/myeloproliferative neoplasm;
**$N = 57$: one patient with benign disease and one with missing data;
*** classic aGvHD if first episode within 100 days of transplantation, late onset aGvHD if first episode more than 100 days after allo-HCT;
aGvHD acute Graft-versus-Host Disease, *HLA* Human Leukocyte Antigen, *HCT* Hematopoietic Cell Transplantation.

after initiation of third-line therapy. Incidence of key events, such as Grade 3–4 neutropenia, thrombocytopenia and Grade ≥2 infectious events was estimated up to 3 months after initiation of third-line therapy.

Data were analyzed by a third-party statistician (Inovia Bio, London, United Kingdom) using the R programming language. The sponsor was blinded to the results until the analysis was completed. Missing data were not imputed, and all patients were included in the statistical analysis. The index date was the date of third-line therapy initiation. Response rates were calculated along with a two-sided 95% confidence interval (95% CI; Clopper–Pearson method). The observational period was defined as the time between the index date and 12 months after the index date, date of the event of interest, date of death or date of loss to follow-up, whichever occurred first. OS and rwPFS were estimated using the Kaplan–Meier method. rwPFS events were underlying malignancy relapse, progression of underlying malignancy, or death due to any cause, whichever occurred first. Cumulative incidence of LOR (assessed as the cumulative incidence of loss of response with non-relapse death or cGvHD as competing risks) was assessed for responders only and was defined as the time from first documented response (at least a partial response) until the date of a documented aGvHD progression, the starting date of additional systemic therapies for aGvHD, or lost to follow-up, whichever occurred first. For cumulative incidence of LOR, death or cGvHD without prior observation of aGvHD progression were considered as competing risks (Fine and Gray proportional-hazards model). In this retrospective study, response status, loss of response and competing risk events were ascertained directly from data captured directly within the case report form. Incidence rates of cGvHD, Grade ≥ 2 infectious events, and Grade 3–4 neutropenia and thrombocytopenia were calculated as the number of patients diagnosed with the event of interest divided by the total number of patients at risk at each evaluation.

RESULTS

Twenty-two European sites were activated. Based on their medical charts, 59 patients from 16 sites were eligible for the study in France ($n = 30$), Spain ($n = 15$), Austria ($n = 8$), and Belgium ($n = 6$). Six sites did not identify eligible patients. Characteristics of patients are summarized in Tables 1 and 2.

All patients received corticosteroids as first-line therapy for a median duration of 9 days (Interquartile range [IQR]: 6–16 days) before initiation of ruxolitinib. Fifty-seven patients (97%) were SR and 2 patients (3%) experienced corticosteroid dependence. All patients underwent a second-line therapy with ruxolitinib at a median dose of 20 mg/day (range 5–40 mg/day) before initiation of third-line therapy. Three patients (5%) were intolerant of ruxolitinib; the others reported progression ($n = 36$, 61%), lack of improvement ($n = 14$, 24%), loss of response ($n = 5$, 9%), or absence of complete response or very good partial response at Day 28 after ruxolitinib initiation ($n = 1$, 2%).

Median time from initial aGvHD diagnosis to the initiation of third-line therapy was 32 days (IQR: 20–53 days). Patients underwent various therapies in the third-line setting: extracorporeal photopheresis ($n = 17$), etanercept ($n = 14$), vedolizumab ($n = 10$), infliximab ($n = 6$). Other third-line agents were used less frequently and included anti-thymocyte globulin ($n = 4$) (see Supplementary Appendices for details), alpha-1-proteinase inhibitor ($n = 3$), alemtuzumab ($n = 2$), inolimomab ($n = 2$), methotrexate ($n = 2$), and single patients each receiving either allogeneic mesenchymal stem cells, mycophenolic acid, sirolimus, or teduglutide. 47 patients (80%) were still under ruxolitinib after the third-line treatment initiation.

At the Day 28 evaluation, 21 patients achieved an all-organ response (36%, 95% CI: 24–49%). Twenty-two patients achieved a GI response, with a GI-ORR of 37% (95% CI: 25–51%) at Day 28 (Table 3). The median time from third-line therapy initiation to the assessment used for Day 28 response was 29 days (IQR: 27–32), demonstrating that most assessments occurred close to the Day 28 target. The prespecified sensitivity analysis of the primary endpoint showed an all-organ response of 39% (95% CI: 25–54%) and a GI response of 41% (95% CI: 27–56%).

At the Day 56 evaluation, an all-organ response was observed in 12 patients (20%, 95% CI: 11–33%) and 13 patients had a GI-ORR of 22% (95% CI: 12–35%). The cumulative incidence of LOR — defined as a documented loss of response due to disease progression or initiation of a new line of therapy—was calculated treating death or development of cGvHD without documented loss of response as competing risks and revealed that 29% (95% CI: 11–49%) of responders at Day 28 lost their response within the next 30 days, and 52% (95% CI: 29–72%) within 90 days. The same results were observed for the GI-aGvHD response: 28% (95% CI: 11–48%) of responders lost their response within 30 days, and 52% (95% CI: 29–71%) within 90 days (Fig. 1a, b).

A prespecified subgroup analysis showed that a hematopoietic cell transplantation-specific comorbidity index score of 0 ($n = 19$) was associated with an all-organ ORR and GI-ORR of 63% (95% CI: 38%–84%) and 68% (95% CI 43–87%), respectively, while for patients ($n = 12$) with a score of 3, all-organ ORR and GI-ORR was 0% (95% CI: 0–27%) for both. Patients with Grade III aGvHD had a GI-ORR of 44% (95% CI: 26–65%) and all-organ ORR of 41% (95% CI: 22–61%). Patients with Grade IV had a GI-ORR and all-organ ORR of 25% for both (95% CI: 11–45%). These results should be viewed in the context of the study's limited sample size.

At the end of the 12-month follow-up period, OS was 29% (Fig. 2) and 41 patients experienced a rwPFS event. Patients died mostly from aGvHD ($n = 25$), infectious complications ($n = 9$), hematological relapse or progression of the underlying malignancy ($n = 1$), or unspecified cause ($n = 6$). The median rwPFS was 86 days (95% CI: 54–128) as was the median OS. Indeed, only one patient experienced a relapse of underlying malignancy during the observation period and died.

In patients who had achieved a response by Day 28, median OS was 186 days (95% CI: 115 days-not reached) *versus* 45 days in

Table 2. GI-aGVHD staging and grading at start of third-line therapy.

Stage	Skin	Liver	Lower GI	Upper GI
0	37/59 (67%)	52/59 (88%)	1/59 (2%)	48/59 (81%)
1	4/59 (7%)	5/59 (9%)	3/59 (5%)	11/59 (19%)***
2	7/59 (12%)	1/59 (2%)	18/59 (31%)	
3	7/59 (12%)	-	11/59 (19%)	
4	4/59 (7%)*	1/59 (2%)	26/59 (44%)**	
aGVHD overall grade	I	II	III	IV
	-	4 (7%)	27 (46%)	28 (48%)

*4 patients with bullous formation;

**11 patients with ileus, 21 patients with severe abdominal pain, 15 patients with hematochezia.

*** 11 patients with persistent nausea/vomiting/anorexia; *GI-aGvHD* acute Graft-versus-Host Disease with gastrointestinal involvement, *GI* gastrointestinal.

Table 3. aGvHD therapies and clinical outcomes.

		***N* = 59**
First-line treatment with corticosteroids		
Corticosteroid resistance		57/59 (97%)
Corticosteroid dependance		2/59 (3%)
Second-line treatment with ruxolitinib		
Ruxolitinib resistance		56/59 (95%)
Ruxolitinib intolerance		3/59 (5%)
Third-line therapy*****		
Median time since a GvHD diagnosis [IQR],days		32 [20–53]
Extracorporeal photopheresis		17
TNFα inhibitors		20
Vedolizumab		10
Other systemic therapies		17
Clinical outcome at Day 28 evaluation		
All-organ Response		
All-organ ORR	21 (36%; 24%–49%)	
Complete Response	12 (20%; 11%–33%)	
Very Good Partial Response	4 (7%; 3%–16%)	
Partial Response	5 (9%; 3%–19%)	
Lack/Loss of Response	26 (44%; 31%–58%)	
Death	12 (20%; 11%–33%)	
GI-aGvHD Response		
GI-ORR	22 (37%; 25%–51%)	
Complete Response	13 (22%; 12%–35%)	
Very Good Partial Response	6 (10%; 3.8%–21%)	
Partial Response	3 (5%; 1%–14%)	
Lack/Loss of Response	25 (42%; 29%–56%)	
Death	12 (20%; 11%–33%)	
Clinical outcome at Day 56 evaluation		
All-organ Response		
All-organ ORR	12 (20%; 11%–33%)	
Complete Response	8 (14%; 6%–25%)	
Very Good Partial Response	1 (2%; 0%–9%)	
Partial Response	3 (5%; 1%–14%)	
Lack/Loss of Response	24 (41%; 28%–54%)	
Death	23 (39%; 27%–53%)	
GI-aGVHD Response		
GI-ORR	13 (22%; 12%–35%)	
Complete Response	8 (14%; 6%–25%)	
Very Good Partial Response	4 (7%; 2%–16%)	
Partial Response	1 (2%; 0%–9%)	
Lack/Loss of Response	23 (39%; 27%–53%)	
Death	23 (39%; 27%–53%)	

Results are expressed as median [IQR] and n/N observed (%); response rate is expressed as n/N observed (%; 95% CI); * total > 59 due to combinations of treatments.

aGvHD acute Graft-versus-Host Disease, *CS* Corticosteroids, *CR* Complete Response, *GI* Gastrointestinal, *VGPR* Very Good Partial Response, *ORR* Overall Response Rate. See supplementary data for definitions.

non-responders (95% CI: 28–86 days) suggesting a better survival in responders. Same results were found between GI-responders and GI-non-responders (Table 4). However, as this is a descriptive study, without matching or multivariate adjustment, no formal comparison between these groups was made. To further explore post-baseline survival conditional on response status while mitigating immortal time bias, a Day 28 landmark analysis was performed among patients alive and under observation at the landmark. In this analysis, median post-landmark OS was longer among responders ($n = 21$; 12 events) with a median of 158 days (95% CI: 78 days–not reached), compared with 51 days (95% CI: 23 days–not reached) among non-responders ($n = 26$; 17 events), (see Supplementary Appendices for the landmark overall survival curves).

No confirmed case of cGvHD was recorded. Grade ≥ 2 infectious events were reported in 30 patients (51%, 95% CI: 37–64%) within 3 months after initiation of third-line therapy. Infections were viral (25%, 95% CI: 15–37%), bacterial (bacteremia and sepsis, 22%, 95% CI: 12–32% each) and fungal (9%, 95% CI: 4–19%). Based on the maximum grade in case of repeated events, infectious events were Grade 3 in 44% of cases (95% CI: 27–62%), Grade 4 in 12% (95% CI: 3–27%) while Grade 5 (fatal) accounted for 27% (95% CI: 13–44%).

Thirty-eight patients (64%, 95% CI: 51–76%) experienced Grade 3–4 thrombocytopenia within the same period. Grade 3-4 neutropenia was reported in 19 patients (32%, 95% CI: 21–46%).

Additional subsequent lines of therapy were initiated in a subset of patients demonstrating ongoing refractory or progressive aGvHD after third-line initiation ($n = 32$). These therapies were usually discontinued quickly.

DISCUSSION

This retrospective, multicenter cohort study represents the largest systematic evaluation to date of third-line systemic therapy for SR and ruxolitinib-refractory aGvHD with GI involvement. Data on the effectiveness of third-line therapy in this population are scarce, partly due to the relatively small number of patients with aGvHD undergoing such therapy. Most patients that failed second-line therapy did not receive a third-line treatment as evidenced by the real-world US study from Abedin et al., which reported that among 307 SR patients receiving ruxolitinib, only 43 (14%) received third-line therapy [24]. This is mainly due to frailty or premature death. Similarly, a retrospective study involving 151 patients with post-HCT aGvHD from Finland, Sweden and France showed that only 23 patients (15%) progressed to third-line therapy [31].

In our study, third-line therapy was initiated shortly after the diagnosis of aGvHD. The recruited cohort was critically ill, with 93% presenting Grade III/IV aGvHD and exhibiting skin (33%) and/or liver (12%) involvement in addition to GI symptoms at the time of third-line initiation. The heterogeneity of therapies used in this setting underscored the lack of standard care within clinical practice. Our results also underscore the limited effectiveness of available third-line options in this immunocompromised, high-risk, and treatment-refractory population. Twelve patients died prior to Day 28, primarily due to aGvHD progression ($n = 7$) emphasizing the severity of disease and early mortality risk. The all-organ ORR and GI-ORR declined dramatically between Day 28 and Day 56 demonstrating that even among patients with initial therapeutic benefit, the underlying disease biology remains aggressive. All-organ and GI early response to third-line therapy may be prognostic since both OS and rwPFS were higher in the subgroup of early responders. However, confounding factors impacting survival cannot be excluded. Infectious events were frequent and often severe, with 22% of Grade ≥ 2 bacteremia and sepsis events each, and infectious complications were the leading cause of death after aGvHD. It has been demonstrated that refractory aGvHD patients, particularly those with GI symptoms, are prone to developing infections [32]. Given the retrospective nature of this study and the complexity of the clinical picture of these patients, we cannot exclude that some patients reported to die from aGVHD actually died from infectious events. The high incidence of Grade 3–4 thrombocytopenia and neutropenia reflects the substantial comorbidity of this population

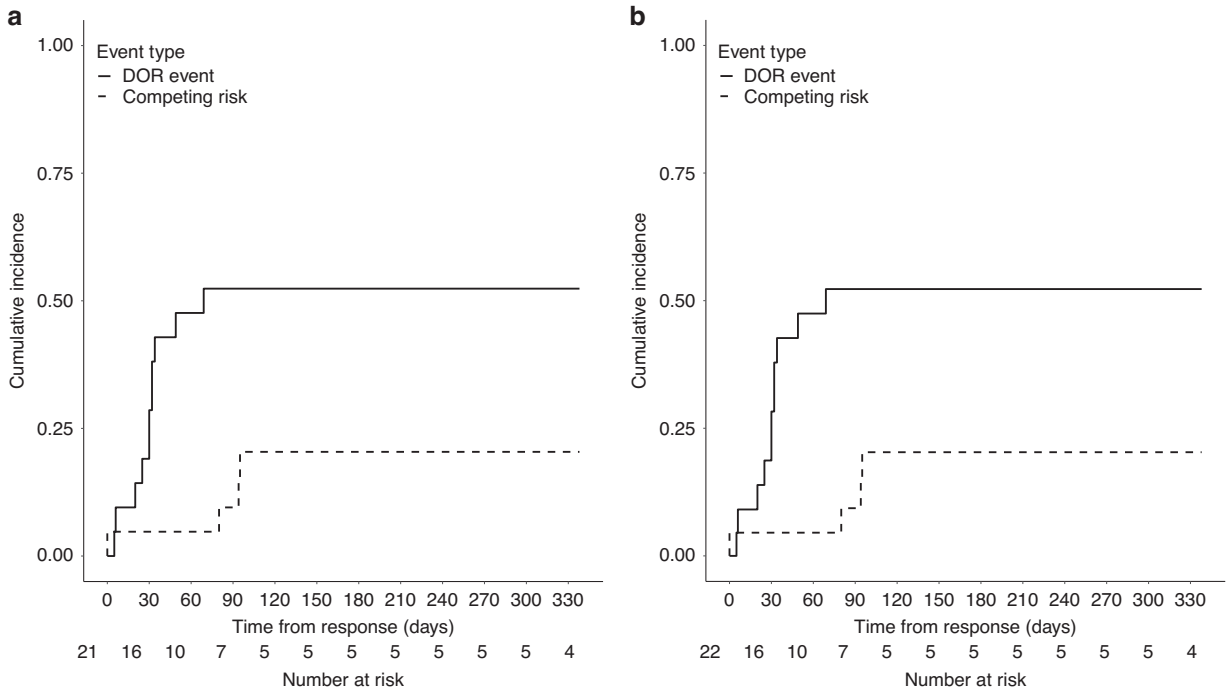

Fig. 1 Cumulative incidence of loss of response. a Cumulative incidence of loss of all organ aGVHD response accounting for competing risks; **b** Cumulative incidence of loss of GI aGVHD response accounting for competing risks.

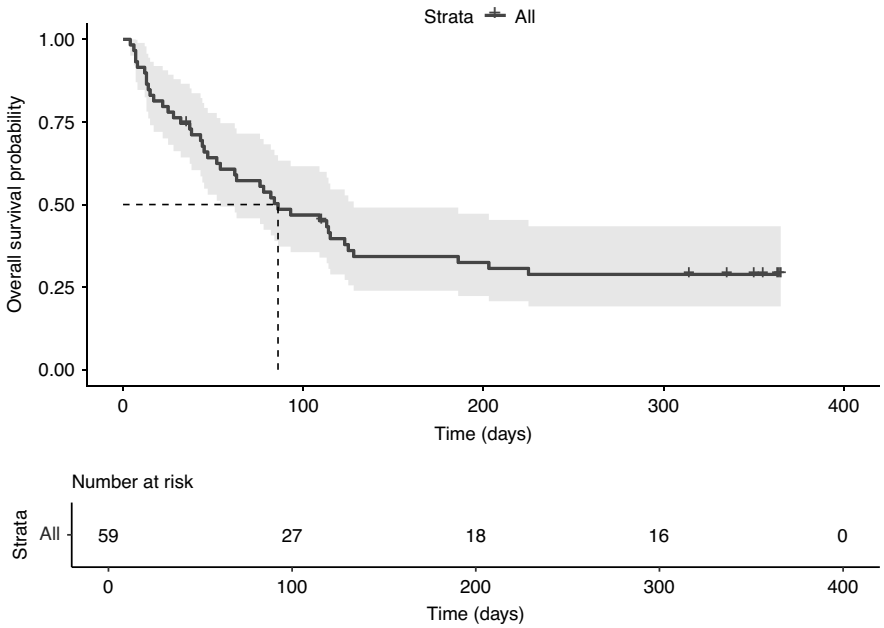

Fig. 2 Overall survival.

exacerbated by the concurrent post-transplant aGvHD treatment and may reflect that 80% of patients were still receiving ruxolitinib after the start of the third-line therapy [21], a well-documented clinical practice [29, 33–36].

The seminal multicenter retrospective study by Abedin et al. [24] sheds light on treatment outcomes for patients following treatment failure to ruxolitinib in SR-aGVHD. A total of 64 SR-aGvHD patients treated with ruxolitinib met the criteria for ruxolitinib resistance ($n = 48$) or intolerance ($n = 16$). Of the 48 patients with ruxolitinib resistance, 15 died prior to initiation of additional therapy; the remaining 33 patients received third-line therapy. Among them, the all-organ ORR was 36%. Among the 16 patients who stopped ruxolitinib due to intolerance, 10 underwent third-line therapy; of these 5 (50%) exhibited a partial response. In the combined population of both ruxolitinib-resistant and ruxolitinib-intolerant patients, all-organ ORR was 40%. However, response was not evaluated on a fixed day but at any time, potentially including patients whose response before Day 28 was not sustained at Day 28 or Day 56. In addition, only 78% of Abedin's patients presented with GI-aGvHD at initiation of ruxolitinib against 100% of our patients. Though our study population may represent a more severely ill cohort, together

Table 4. Secondary outcomes.

	Patients	Events	Median (days)	95% CI (days)
Progression-free survival	59	41	86	[54–128]
Overall survival	59	41	86	[54–128]
All-organ responders to 3rd-line therapy	21	12	186	[115–NA]
All-organ non-responders to 3rd-line therapy	38	29	45	[28–86]
GI-aGvHD responders to 3rd-line therapy	22	12	186	[115–NA]
GI-aGvHD non-responders to 3rd-line therapy	37	29	45	[28–86]

GI-aGVHD acute Graft-versus-Host Disease with gastrointestinal involvement, *CI* Confidence Interval, 3rd third.

with a more stringent definition of response, response rates were comparable between the two studies reinforcing the conclusion that outcomes to third-line therapies are principally limited.

The prognosis of SR-GVHD patients resistant to or intolerant of ruxolitinib is poor as demonstrated by a median OS of 86 days (95% CI: 54–128 days). OS was worse in the Abedin study, which reported a median OS of 28 days (range 15–253 days; 21 days for ruxolitinib refractoriness, 50 days for ruxolitinib intolerance). OS was 20% after 6 months, 16% after 12 months, and 10% after 24 months [24]. The poor prognosis and abbreviated survival may partly explain why none of our patients developed cGvHD.

Our results suggest that response to third-line therapy on Day 28 may be associated with higher survival. This is consistent with a retrospective study evaluating the effectiveness of bone marrow mesenchymal stromal cells harvested from multiple donors in SR-aGvHD patients who were not controlled with ruxolitinib. In this study, the Day 28 response rate was highly predictive of survival at 6, 12, and 24 months [37]. Of the 79 patients classified as non-responders to the stromal cell preparation at Day 28, 64 (81%) died within 24 months following infusion compared with 49% of the 77 patients who were responders. It should be noted that the population included 123 adults and 33 children.

While our study has several strengths, including a pre-specified protocol, monitoring by an independent contract research associate, uniform outcome assessment, and blinding, the results should also be interpreted in the context of its limitations. These include potential selection bias as a function of the inclusion criteria, as well as the fact that half of the study participants were recruited in France, potentially limiting the generalizability of the population. Equally, the risk of misclassification cannot be fully excluded, although this risk was mitigated by the medical staff training, use of a standardized electronic case report form, careful medical review of the data, implementation of the widely validated MAGIC criteria, and clear definitions of clinical outcomes. Furthermore, the findings of this study should be viewed in light of the sample size, particularly when considering inferences about subgroups, and the variability of third-line treatments. It is worth noting that fecal microbiotherapy, not available commercially, was not included in this study and the use of mesenchymal stem cell therapy was extremely rare. The latter may be related to the strict inclusion criteria of our study that focus on third line treatment, after steroid and ruxolitinib failure (no association with other therapies was allowed, including ECP). MSC was, however, the most frequent subsequent additional therapies after initiation of a third-line treatment.

These findings illustrate the absence of standardization and the limited benefit of available third-line interventions, emphasizing the need for novel treatments. New hopes have been raised by recent encouraging results with fecal microbiotherapy or infusion of mesenchymal stem cells [37–39]. Despite being the gold standard for the development of new drugs, randomized controlled clinical trials are hardly feasible in this setting. By establishing clear benchmarks, this study will facilitate the evaluation of novel therapeutic approaches.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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AUTHOR CONTRIBUTIONS

EP, FM, MM contributed to the conception of the study. EP, HJP, BKM, IF contributed to the design of the study, analysis and interpretation of the data. HJP supervised the project. HJP, FM, MM wrote and critically revised the manuscript. JC, JAPS, MC, CCL, DM, AS, PAC, SF, LG, LLC, A Benzaquen, HL-W, JC, RD, AH, PT, A Bauhofer, HPJ, MB contributed to the collection of data. All authors reviewed and approved the manuscript.

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ADDITIONAL INFORMATION

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