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Real-World Experience with Ruxolitinib Therapy for Steroid Refractory Acute Graft Versus Host Disease

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Abbreviation List

aGVHD: Acute graft versus host disease
alloHCT: Allogeneic hematopoietic stem cell transplant
ATG: Anti-thymocyte globulin
CBC: Complete blood count
FDA: Food and Drug Administration
FFS: Failure-free survival
GVHD: Graft-versus-host disease
IFN-γ: Interferon gamma
IL: Interleukin
JAK: Janus Kinase
KPS: Karnofsky Performance Status
NRM: Non-relapse mortality
OS: Overall Survival
SR-aGVHD: Steroid refractory acute graft versus host disease
SR-GVHD: Steroid refractory graft versus host disease
TKI: Tyrosine kinase inhibitor
WBC: White blood cells

Abstract

Acute graft versus host disease (aGVHD) is a complication of allogeneic hematopoietic stem cell transplant (HCT) and is associated with significant morbidity and mortality. Steroid refractory aGVHD (SR-aGVHD) carries a particularly grim prognosis. Ruxolitinib has shown promise for treatment of SR-aGVHD in a phase 3 trial; however, safety and efficacy data outside of the clinical trial setting is lacking. We performed a multicenter retrospective study to examine the response to ruxolitinib and its efficacy in patients with SR-aGVHD. We included 59 patients treated with ruxolitinib for SR-aGVHD between 2015 and 2022. Of these 59 patients, 36 patients (61.0%) achieved a complete (CR) or partial response (PR) at 28 days, while 31 patients (52.5%) obtained a CR/PR at day 56. Patients that achieved a CR or PR at day 28 had a higher rate of overall survival (OS; 69.2%), compared with patients that did not (31.6%; p=0.037). OS at 12 months was 41.5%, with a median OS duration of 5.3 months. Failure free survival (FFS) at 12 months was 29.1%, with a median FFS of 2.6 months. Overall, this real-world experience data support ruxolitinib as the standard of care for SR-aGVHD in a non-controlled trial population.

Introduction

Acute graft versus host disease (aGVHD) is a frequent complication following allogeneic hematopoietic stem cell transplant (HCT) and is associated with significant morbidity and mortality.[1, 2] Although treatment outcomes have improved significantly over time, long-term survival remains poor.[3, 4] High dose corticosteroids form the backbone of treatment for aGVHD. However, up to half of patients will not respond to first-line steroids and are defined as having steroid refractory acute graft versus host disease (SR-aGVHD).[5]

Patients that do not respond to first-line corticosteroids experience worse clinical outcomes compared to patients responsive to upfront steroids therapy.[6] Historically, patients with SR-aGVHD have been treated with diverse therapies without an established standard of care. For example, patients treated with ATG for SR-aGVHD had a median survival of 3.6 months, with the majority of deaths being attributed to aGVHD or infection.[7] Despite the multiple options available for the treatment of SR-aGVHD including mycophenolate, calcineurin inhibitors, sirolimus, mesenchymal stromal cells, extracorporeal photopheresis, and monoclonal antibodies such as alemtuzumab, long- term outcomes remain disappointing.[3, 4, 6, 8, 9]

Recently, the Food and Drug Administration (FDA) approved ruxolitinib for treatment of SR-aGVHD in 2019.[10] Ruxolitinib is a tyrosine kinase inhibitor (TKI) that selectivity inhibits Janus Kinase 1 (JAK1) and Janus Kinase 2 (JAK2).[11] Multiple cytokines involved in the pathogenesis of aGVHD, including interleukin-2 (IL-2), IL-4, IL-6, IL-12, IL-15, IL-23, and interferon gamma (IFN-γ), converge on the JAK-STAT signaling pathway.[11, 12, 13] The REACH1 trial, a single-arm phase 2 trial

of ruxolitinib for the treatment of SR-aGVHD revealed a promising overall response rate of 59.4% at 28 days with an acceptable safety profile.[14] Subsequently, the REACH2 trial, a multicenter phase 3 randomized trial, found that ruxolitinib was superior to best available therapy for overall survival (OS), failure free survival (FFS), and overall response at 28 days.[11] Based on the results of these trials, ruxolitinib has received FDA approval for the treatment of SR-aGVHD.

Although the results from the REACH trials are promising, there are limited real-world data on the efficacy of ruxolitinib. In practice, some patients do not meet the strict RCT inclusion criteria (for example, due to multiple comorbidities or poor performance status) but still require treatment based on the clinical judgement of the treating team. Thus, it is unclear whether clinical trial data possess sufficient external validity for application to everyday practice.[15] Therefore, we performed a multicenter retrospective study to examine the real-world outcomes of ruxolitinib in the treatment of SR-aGVHD.

Methods

Patients and treatment

This retrospective study was designed to evaluate the efficacy and safety of ruxolitinib in SR-aGVHD patients. The study population includes patients that underwent HCT and developed SR-aGVHD between 2015 and 2022 across six Canadian transplant centers in Calgary, Toronto, Vancouver, Quebec, Ottawa, and Saskatoon. Patients were included in the study if they developed at least overall grade 2 aGVHD, met criteria for SR-aGVHD, and were started on ruxolitinib for SR-aGVHD. Steroid refractory disease was defined as 1) progressive disease after

3 days of treatment, 2) no improvement at 7 days of treatment, or 3) failure to taper methylprednisolone or prednisone less to than 0.5mg/kg/day for a minimum of 7 days.[11] Ruxolitinib was supplied through the managed access program provided by Novartis. The study was conducted in accordance with the Tri-Council Policy Statement for the ethical conduct of research involving humans and was approved by the Research Ethics Board at University Health Network, Toronto and at each institution.

Outcomes

Clinical data was gathered and recorded on standardized forms by independent reviewers across the six sites. Primary outcomes included the response rates at Day 28, Day 56, 3 months, and 6 months after initiation of ruxolitinib. Secondary outcomes included clinical benefit, overall survival (OS), failure free survival (FFS), treatment failure, and laboratory profile changes including complete blood count (CBC), biochemistry data as well as liver function profiles.

The overall response rate (ORR) and clinical benefit (CB) were assessed at day 28, day 56, 3 and 6 months after initiation of ruxolitinib, retrospectively. Responses were evaluated according to change in baseline organ staging without the use of additional systemic immunosuppression for aGVHD, with complete and partial response combined for an ORR.[11, 16] Where organ scores were not clearly delineated in the medical record, these were assigned retrospectively based on descriptions in the clinical records.

The CB was assessed considering clinical response (**Supplementary Table 1**) and defined as follows: 1) very beneficial – complete response (CR) regardless of prednisone dose reduction or

partial response (PR) with significant dose reduction (i.e. ≥ 50% reduction) of daily prednisone; 2) beneficial – PR with minor reduction of daily prednisone dose (i.e. < 50% dose reduction) or stable disease (SD) with a significant reduction of daily prednisone dose; 3) minor benefit – SD with minor reduction of daily prednisone dose; 4) no benefit – Progression or no change/increase in prednisone dose compared to the initial prednisone dose. Treatment failure was defined as 1) treatment switch due to no response/no clinical benefit or intolerance, 2) non-relapse mortality (NRM), or 3) relapse of primary disease.

To evaluate the hematologic toxicity of ruxolitinib, we collected CBCs at day 28, day 56, 3 months, and 6 months of ruxolitinib therapy. Hematologic toxicity was graded by NCI CTCAE version 5.0 criteria.[17]

Statistical analysis

Patient and disease characteristics were reported using descriptive statistics. The clinical data were locked as of August 2022.

Assessment of ruxolitinib efficacy was performed using 5 main outcome variables including ORR, CB, FFS, OS and steroid dose reduction. As described above, ORR and CB were assessed at day 28, day 56, 3 and 6 months after initiation of ruxolitinib. The daily prednisone dose (mg/day) was also captured prior to ruxolitinib initiation, and at day 28, day 56, months 3 and 6, and then converted to dose per kilogram body weight per day. The proportion of patients on daily prednisone dose ≤ 0.5 mg, ≤ 0.2, ≤ 0.1mg/kg/day and 0 (i.e. discontinued) was calculated at 5 different time points: prior to ruxolitinib initiation, at day 28, day 56, at 3 months, and at 6 months. Failure-free survival (FFS) was calculated from ruxolitinib therapy start until the event of

treatment failure or the latest follow-up, while overall survival (OS) was calculated from the day of starting ruxolitinib therapy, until death, or latest follow-up. Kaplan-Meier estimate was calculated with respect to FFS and OS and compared using log-rank test. Throughout the analysis $p<0.05$ was considered statistically significant. The survival analyses was performed using EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan).

Results

Summary of characteristics of patients and GVHD profile

We reviewed 59 patients treated with ruxolitinib for SR-aGVHD, with a median follow up of 391 days. The median age was 53 years, and 54% of patients were male. The most common indication for HCT was acute myeloid leukemia (49%). Approximately half of patients received myeloablative conditioning (51%). Matched unrelated donors were the most common donor type (44%). Most patients received ATG as part of their GVHD prophylaxis (83%), while 31% of the patients received post-transplant cyclophosphamide with or without ATG. The median onset of aGVHD was 65 days (range: 15-265) post HCT. Most patients (81%) had classic aGVHD, while the remainder (19%) had late onset aGVHD. Patients were fit prior to transplant with a median Karnofsky Performance Status (KPS) of 90% (range 70-100%) and were less fit prior to initiation of ruxolitinib with a median KPS of 70% (range 30-100%). Prior to initiation of ruxolitinib, most patients had overall grade 3 aGVHD (58%) and 17% had overall grade 4 disease (Table 1, supplementary tables 1 and 2).

Ruxolitinib therapy for steroid-refractory acute GVHD

Ruxolitinib was used as second line therapy in 18 (30%) patients, as third line therapy in 36 (61%) patients, and as fourth line therapy in 5 (9%) patients. Ruxolitinib was initiated at a median of 20 days following first line therapy for aGVHD, while 44% of the patients started ruxolitinib therapy within 14 days of starting steroid therapy for aGVHD. Forty-four (74%) patients were resistant or refractory to previous lines of therapy. Fourteen (24%) initially responded to a previous line of therapy but experienced a flare of their disease while tapering steroids. One patient (2%) initially responded but was intolerant to first- or second-line therapy. Nineteen patients (32%) started ruxolitinib at 10 mg twice daily (BID) while 40 (68%) were started on 5 mg BID, then escalated to 10mg BID within 3-7 days.

Overall response and clinical benefit to ruxolitinib treatment for steroid refractory acute GVHD

After 28 days of ruxolitinib therapy, 36 patients (61%) responded to therapy, with 8 (14%) achieving a complete response (CR) and 28 (47%) achieving a partial response (PR). At day 56, 31 patients (52%) had responded, with 12 (20%) achieving a CR and 19 (32%) achieving a PR. (**Figure 1A**). Patients that achieved a CR or PR at day 28 had a higher overall survival rate at 12 months (69%), compared with patients that did not (32%) (p=0.037).

Clinical benefit of ruxolitinib therapy, as adjudicated by clinicians with experience treating aGVHD, is presented in **Figure 1B**. At day 28, ruxolitinib was found to be either beneficial or very beneficial in 34 of 50 (68%) evaluable patients. At day 56, ruxolitinib was found to be either beneficial or very beneficial in 31 of 45 (69%) evaluable patients.

Failure-free and overall survival with ruxolitinib treatment for steroid-refractory acute GVHD

The OS rate at 12 months was 41.5% (95% CI, 27.6-54.8%) with a median OS of 5.3 months (**Figure 2A**). The 12-month FFS rate was 29.1% (16.1-43.3%) with a median FFS of 2.6 months (**Figure 2A**). Reasons for failure included NRM in 23 patients (39%), relapse of primary disease in 3 patients (5%), need for therapy switch/additional systemic therapy in 11 patients (19%), and intolerance in 2 patients (3%); **Figure 2B**.

Within 12 months after starting ruxolitinib, 16.3% (range: 7.4-28.3%) of patients were able to successfully taper off without flare of their aGVHD. By 24 months, 19.6% (9.2-32.8%) of patients were able to taper off ruxolitinib successfully (**Figure 2A**).

Adverse events with ruxolitinib therapy for steroid-refractory acute GVHD

We observed a decrease in hemoglobin, platelets, and white blood cells (WBCs) in the first two months of ruxolitinib therapy. At baseline (day 1 of ruxolitinib), mean hemoglobin concentration was 97.9$\pm$2.5 g/L, mean platelet count was 84.7$\pm$9.3x10^9 cells/L, and mean WBC count was 6.1$\pm$0.56x10^9 cells/L. By day 56, mean hemoglobin concentration decreased to 85.4$\pm$2.6 g/L, mean platelet count decreased to 59.7$\pm$11.1 x 10^9 cells/L, and mean WBC decreased to 3.4$\pm$0.56 x 10^9 cells/L. At day 56, of 41 evaluable patients 15 (37%), 14 (34%) and 10 (24%) patients experienced new onset grade 3-4 anemia, thrombocytopenia, and neutropenia, respectively.

Despite the initial drop, we observed a recovery in all three cell lineages by 6 months. Mean hemoglobin concentration increased to 104$\pm$4.3 g/L, mean platelet count increased to 144$\pm$21 x 10^9 cells/L, and mean WBC increased to 5.0$\pm$0.6 x 10^9 cells/L. (**Supplementary Figure 1**). At 6

months of therapy, out of 22 evaluable patients, 4 (18%), 3(14%), and 3 (14%) developed or remained with new onset grade 3-4 anemia, thrombocytopenia, and neutropenia, respectively.

Corticosteroid dose reduction during ruxolitinib therapy

Corticosteroid dose, measured in prednisone equivalents, was recorded starting at day 0 of ruxolitinib therapy. Corticosteroid dose decreased over time with ruxolitinib therapy. Patients started with a mean daily prednisone dose of 1.49±0.07 mg/kg at day 0 of ruxolitinib and were able to taper down to a mean dose of 0.67±0.3 mg/kg by day 56, and 0.15±0.04 mg/kg after 6 months ($p<0.001$) (**Figure 3**).

Impact of treatment outcomes according to starting dose of ruxolitinib

Forty patients (68%) received a starting dose of ruxolitinib of 5 mg bid, whereas 19 (32%) received a starting dose of 10 mg bid. There were no significant differences between the 5 mg and 10 mg starting dose groups respectively in age (51.5 vs 48.5, p=0.38), mean aGVHD grade at time of ruxolitinib initiation(3 versus 2.6, p=0.07), mean KPS at ruxolitinib initiation (76.3 versus 71.6, p=0.37), days to ruxolitinib start from aGVHD onset (48.1 versus 48.4, p=0.99) and mean platelet count prior to ruxolitinib start (79.6 vs. 88.3, p=0.67). However, those who received a starting dose of 5 mg bid were significantly more likely to have baseline grade 3-4 thrombocytopenia (23 of 40 vs. 4 of 19, p=0.01). In contrast, there were no significant differences in baseline grade 3-4 anemia and neutropenia.

At day 56 of treatment, those who received an initial dose of 5 mg bid were significantly more likely to experience new grade 3-4 anemia (14 of 29 versus 1 of 12, p=0.03). There were no significant differences in new grade 3-4 thrombocytopenia (10 of 29 versus 4 of 12, p=1.0) or neutropenia (7 of 29 versus 3 of 12, p=1.0) at day 56. There were no significant differences in any new grade 3-4 cytopenia between the two groups at 6 months on treatment.

There were no significant differences in OS or FFS observed between patients who started ruxolitinib at 10 mg bid compared to 5 mg bid. Patients who started on 10 mg BID showed a 23.1% (95% CI, 5.0-48.8%) FFS rate at 12 months with a median FFS of 3.8 months, while those started on 5 mg BID showed a 32.5% (95% CI,17.2-48.7%) FFS rate at 12 months with a median FFS of 2.6 months. **(Supplementary Figure 2).**

Discussion

Although outcomes in aGVHD have improved over time, steroid refractory disease remains a barrier to success in HCT.[18] Based on the results of the REACH trials, ruxolitinib has become the standard of care in SR-aGVHD treatment.[19] However, clinical trial participant populations may not entirely represent patient populations seen in clinical practice, limiting external validity.[15] Thus, we set out to examine the efficacy of ruxolitinib for SR-aGVHD based on a multi-centre real-world experience.

We evaluated 59 patients treated with ruxolitinib for SR-aGVHD, which is one of the larger observational cohorts of adult treated with ruxolitinib for SR-aGVHD. A 2022 systematic review on the use of ruxolitinib in both acute and chronic SR-GVHD included a single cohort study (Zeiser

et. al., 2015) with >50 patients treated with ruxolitinib for SR-aGVHD.[20, 21] While the demographics of our cohort are comparable to the ruxolitinib arm of the REACH2 trial, there are important differences between the patient populations. Firstly, in our cohort, 75% of patients were experiencing grade 3-4 aGVHD at baseline versus 64% in the REACH2 population. Secondly, ruxolitinib was used as a third- or fourth-line therapy in 70% of our patient cohort versus as a second line therapy in REACH2. Finally, our patient cohort included individuals with low baseline performance status, including patients with a KPS as low as 30, that would typically have been ineligible for participation in clinical trials. Despite these important differences in study populations, we observed similar ORR at day 28 (61% in our study, 62.3% in REACH2). However, potentially due to important differences in the study population's characteristics, OS and FFS rate at 12 months in our cohort were inferior at 5.3 and 2.6 months, compared to 11.1 and 5.0 months, respectively, in the ruxolitinib arm of REACH2.

Outcomes in our cohort compare favorably to several previously reported therapies.[3, 22] For example, amongst prior retrospective studies in SR-aGVHD with cohorts larger than 50 patients, those treated with equine ATG demonstrated a day 28 overall response rate of 54% with 1-year OS of 32% and those treated with daclizumab demonstrated a day 43 overall response of 54% and a 1-year OS of 28%.[23, 24] Additionally, we demonstrate durable responses with ruxolitinib at day 56 (52.5%), allowing for a progressive taper of prednisone over 6 months.

The present study provides novel insights into the use of ruxolitinib in a real-world setting. Importantly, we demonstrate that by 24 months of therapy, approximately 20% of patients

successfully discontinued ruxolitinib without a subsequent flare-up of aGVHD. This successful discontinuation rate may serve as a baseline for future long-term follow-up of real world or clinical trial data. It is of note that a significant proportion of our cohort (68%) received a starting dose of ruxolitinib of 5 mg bid as opposed to the 10 mg bid dosing in the REACH2 trial, possibly due to concerns regarding hematologic toxicity, particularly in the setting of baseline grade 3-4 thrombocytopenia. Yet, there was no significant difference in rates of new grade 3-4 neutropenia or thrombocytopenia between those started at 5 mg versus 10 mg dosing. In fact, those started at 5 mg dosing were more likely to develop grade 3-4 anemia than those starting at 10 mg dosing. In the entire cohort, regardless of dosing, new grade 3-4 cytopenia developed in approximately 30-40% of patients. Importantly, we did not observe a significant difference in OS or FFS between those patients started on 10mg bid versus those on 5 mg bid. These data suggest that a uniform 10 mg bid starting dose is appropriate, but careful monitoring for cytopenia, particularly in the first 2 months of therapy is important. Despite significant thrombocytopenia at baseline, platelet counts were not a barrier to the use of ruxolitinib in our cohort: only 2 of 39 failures were due to medication intolerance and hemorrhage contributed to NRM in a single patient.

Our study is limited by its retrospective nature. Although ascertainment of outcomes such as death, relapse, and trajectory of GVHD therapies are readily extracted from the medical record with accuracy, toxicity data beyond cytopenia cannot be ascertained with confidence. In addition, in the absence of a direct comparison group, it is challenging to quantify the magnitude of improvement in outcomes seen with ruxolitinib as opposed to alternative therapies for SR-

aGVHD. A strength of our analysis is the use of data from multiple centres, enhancing generalizability and limiting bias that may be introduced by single centre practice patterns.

In summary, patients treated with ruxolitinib for SR-aGVHD in second line and beyond in a multicentre real-world setting demonstrated favourable response rates; similar to rates seen in the randomized REACH2 study and better than rates described with alternative therapies in large retrospective studies. In our cohort, OS and FFS were inferior to those demonstrated in REACH2, likely owing to differences in important patient characteristics. Nevertheless, the 1-year OS in our cohort appeared to be superior to previous cohorts treated with non-ruxolitinib alternatives for SR-aGVHD. Further, we demonstrated that after 24 months of treatment, approximately 20% of patients could successfully discontinue ruxolitinib. Overall, these data support ruxolitinib as the standard of care for SR-aGVHD in a diverse, real-world, non-controlled trial population.

Author contributions:

AM and SML performed data collection, analysis, and manuscript writing. KJ and DK designed the study, provided all administrative support, supervised the project and manuscript revision. All the other authors contributed to data collection, reviewed the manuscript and approved it.

Conflicts of Interest:

DK received research grant and honoraria from Novartis, Pfizer and Paladin.

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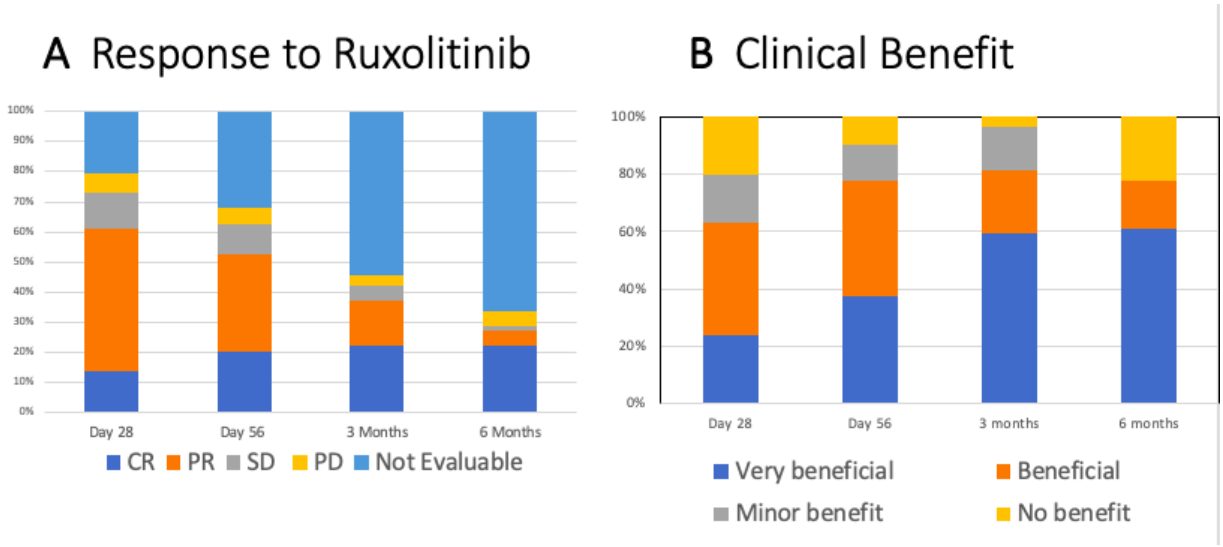
Tables and Figures

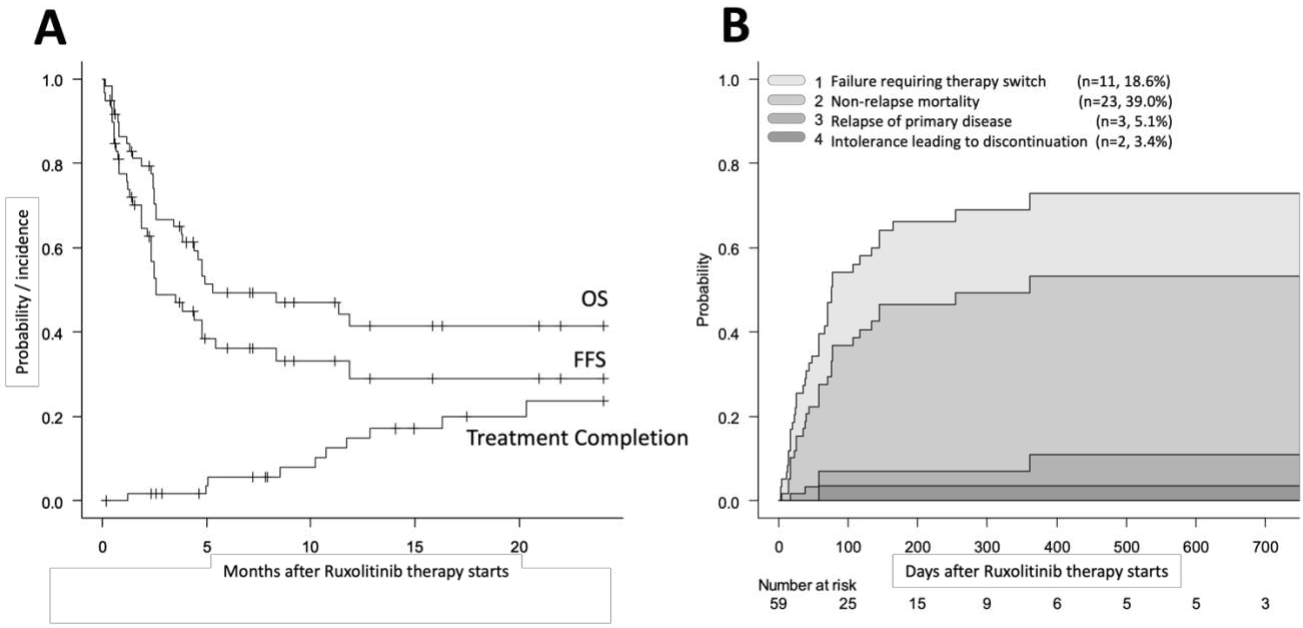
Table 1 – Demographics	
Patients (N)	59
Age (median [range])	53 [21-70]
Male Sex (N,%)	32, 54.2%
Indication for Transplant (%)	
• Acute Leukemia	29, 49.1%
• Myeloproliferative Disorder/Myelodysplastic Syndrome	16, 28.8%
• Lymphoma/Chronic Lymphocytic Leukemia	11, 18.6%
• Other (HLH, Aplastic Anemia)	3, 5.1%
Conditioning Regimen (%)	
• Myeloablative	30, 50.8%
• Reduced Intensity	29, 49.2%
Donor Type (%)	
• Matched Unrelated	26, 44.1%
• Matched Related	14, 23.7%
• Mismatched Unrelated	9, 15.3%
• Haploidentical	9, 15.3%
• Cord Blood	1, 1.7%
GVHD Prophylaxis (%)	
• ATG + CNI + (MTX or MMF)	32, 54.2%
• ATG + CNI + PTCy	13, 22.0%
• PTCy + CNI +/- MMF	5, 8.5%
• CNI + ATG	4, 6.8%
• CNI + MMF	4,6.8%
• CNI + Prednisone	1, 1.7%
GVHD onset (median days post transplant [range])	65 [15-265]
HCT-CI (median [range])	1.0 [0-5]
KPS Pre-Transplant (median [range])	90 [70-100]
KPS Pre-TKI (median [range])	80 [30-100]
Initial GVHD Stage/Grade (median [range])	
• Skin	3 [0-3]
• Liver	0 [0-3]
• GI	1 [0-4]
• Overall Grade	2 [1-4]
GVHD Stage/Grade pre TKI (median[range])	
• Skin	2 [0-4]
• Liver	1 [0-3]
• GI	2 [0-4]
• Overall Grade	3 [1-4]

Figure 1



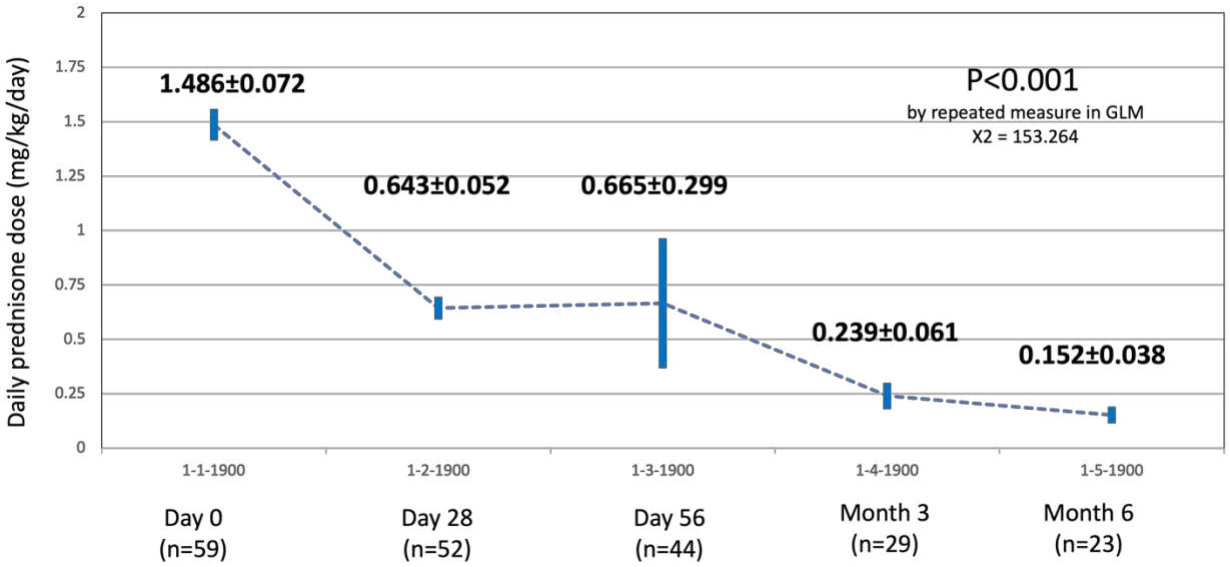
A Response to ruxolitinib therapy overtime. CR: Complete Response. PR: Partial Response. SD: Stable Disease. PD: Progressive disease. **B** Clinical benefit of ruxolitinib therapy, as adjudicated by clinicians with experience treating aGVHD. Days and months refer to time since start of ruxolitinib.

Figure 2



A. Overall and failure-free survival as well as cumulative incidence of treatment completion of ruxolitinib following its treatment for steroid refractory acute GVHD. **B.** Stacked incidence of failure according to its reason of failure among treatment resistance requiring therapy switch, non-relapse mortality, relapse of primary disease and intolerance leading to discontinuation.

Figure 3



Change in steroid dose starting at day 0 of ruxolitinib therapy. Doses reported in mg/kg/day of prednisone.

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