Peritransplant Ruxolitinib Administration is Safe and Effective in Patients with Myelofibrosis: a Pilot Open-Label Study

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Abstract:
We report results of our prospective pilot trial (NCT02917096) evaluating safety/feasibility of peritransplant administration of ruxolitinib for myelofibrosis treatment. Primary objectives were to determine the safety and identify maximum tolerated dose (MTD) of ruxolitinib. Ruxolitinib was given at two dose levels (DL) of 5 and 10mg twice daily, with fludarabine/melphalan conditioning regimen and tacrolimus/sirolimus GVHD prophylaxis. We enrolled 6 and 12 patients in DL-1 and DL-2, respectively. Median age at transplant was 65 years (range:25-73) for all patients. Per DIPSS, 4 patients were at high and 14 were at intermediate risk. PBSCs was the graft source from a matched sibling (n=5) or unrelated (n=13) donor. At each DL one patient developed DLTs: Grade 3 cardiac and GI with Grade 4 pulmonary in DL-1 and Grade 3 kidney injury in DL-2. All patients achieved engraftment. Cumulative incidence (CI) of acute GVHD grade 2-4 and 3-4 were 17% (95% CI: 6-47) and 11% (95% CI: 3-41), respectively. CI of 1-year chronic GVHD was 42% (95% CI: 24-74). With the median follow-up of 22.6 months (range:6.2-25.8) in surviving patients the 1-year overall and progression free survival were 77% (95% CI: 50-91) and 71% (95% CI: 44-87), respectively. Causes of death (n=4) were cardiac arrest, GVHD, respiratory failure, and refractory GVHD of liver. Our results showed that peri-HCT ruxolitinib was safe and well-tolerated with the MTD determined as 10 mg BID, associated with dose-dependent PK and cytokine profile. The early efficacy data are highly promising in this group of high-risk older patients with MF.

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Short Title: Ruxolitinib given peri-HCT in MF patients

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- Peri-HCT ruxolitinib was safe and well-tolerated in patients with MF with the MTD determined as 10 mg BID.
Abstract

We report results of our prospective pilot trial (NCT02917096) evaluating safety/feasibility of peri-transplant administration of ruxolitinib for myelofibrosis treatment. Primary objectives were to determine the safety and identify maximum tolerated dose (MTD) of ruxolitinib. Ruxolitinib was given at two dose levels (DL) of 5 and 10mg twice daily, with fludarabine/melphalan conditioning regimen and tacrolimus/sirolimus GVHD prophylaxis. We enrolled 6 and 12 patients in DL-1 and DL-2, respectively. Median age at transplant was 65 years (range:25-73) for all patients. Per DIPSS, 4 patients were at high and 14 were at intermediate risk. PBSCs was the graft source from a matched sibling (n=5) or unrelated (n=13) donor. At each DL one patient developed DLTs: Grade 3 cardiac and GI with Grade 4 pulmonary in DL-1 and Grade 3 kidney injury in DL-2. All patients achieved engraftment. Cumulative incidence (CI) of acute GVHD grade 2-4 and 3-4 were 17% (95% CI: 6-47) and 11% (95% CI: 3-41), respectively. CI of 1-year chronic GVHD was 42% (95% CI: 24-74). With the median follow-up of 22.6 months (range:6.2-25.8) in surviving patients the 1-year overall and progression free survival were 77% (95% CI: 50-91) and 71% (95% CI: 44-87), respectively. Causes of death (n=4) were cardiac arrest, GVHD, respiratory failure, and refractory GVHD of liver. Our results showed that peri-HCT ruxolitinib was safe and well-tolerated with the MTD determined as 10 mg BID, associated with dose-dependent PK and cytokine profile. The early efficacy data are highly promising in this group of high-risk older patients with MF.
Introduction

Myelofibrosis (MF) is a clonal myeloproliferative neoplasm that can be classified as primary or secondary to either essential thrombocythemia or polycythemia vera, characterized by varying degrees of cytopenia, bone marrow (BM) fibrosis, and heterogeneous symptom burden/prognosis. Dysregulation of JAK-STAT pathway is the hallmark of MF and has become the therapeutic target for this disease. Ruxolitinib is a potent JAK 1/2 inhibitor that was approved in the United States in 2011 for treatment of intermediate- or high-risk MF, based on results of two Phase III trials: the double-blind COMFORT-I and the open-label COMFORT-II. While JAK inhibitors are currently the best available therapy for splenomegaly and constitutional symptoms associated with MF, treatment with these drugs has no lasting disease modifying effect. Allogeneic hematopoietic cell transplantation (HCT) remains the only potential curative therapy for MF, and is increasingly offered for patients with intermediate and high-risk disease. Unfortunately, HCT is associated with a significant risk of transplant-related morbidity and mortality, with one of the most serious complications being graft-versus-host disease (GVHD).

Ruxolitinib, through its inhibition of JAK1/2 signaling, was shown to reduce GVHD in mice by inducing durable and profound specific T-cell tolerance, and preserving regulatory T cells (Tregs). Results of a small pilot study treating steroid refractory (SR) severe acute and chronic GVHD with ruxolitinib showed excellent clinical activity accompanied by reduction in serum pro-inflammatory cytokines and increase in FoxP3+ Tregs. Multiple prospective and retrospective studies have confirmed the safety and clinical efficacy of ruxolitinib for treatment of SR-GVHD. Specifically, REACH1 and REACH2 trials led to approval of ruxolitinib for treatment of SR-acute GVHD.

At present, the optimal use of ruxolitinib in MF patients immediately before and after HCT is unknown. Pre-HCT administration of ruxolitinib has shown to have beneficial effects on spleen size and potentially engraftment. However, ruxolitinib is generally tapered off prior to start of conditioning, since sudden stoppage of the drug is often associated with a cytokine storm-like condition called “ruxolitinib withdrawal syndrome”. Results of a pilot study (n=12) by Kroger et al showed continuing ruxolitinib at 5 mg twice
daily through peri-HCT period until stable engraftment is feasible with a lower incidence of GVHD, when cyclosporine, mycophenolate mofetil, and anti-T cell globulin (ATG) were administered as the GVHD prophylaxis. Outcomes of another prospective study in which ruxolitinib was added to post-transplantation cyclophosphamide from day -7 to +100 in twenty patients with MF showed acceptable acute and chronic GVHD rates; however, ruxolitinib administration was associated with primary graft failure (n=1), death before engraftment (n=2), and, severe poor graft function (n=11).17

At City of Hope (COH), we pioneered reduced intensity conditioning (RIC) HCT using fludarabine and melphalan (Flu/Mel) as conditioning regimen combined with tacrolimus and sirolimus (Tac/Sir) as GVHD prophylaxis with excellent survival.18-20 We previously reported on the clinical outcome a large cohort (n=110) of MF patients who underwent HCT with this regimen at our center and showed excellent 5-year overall survival (OS) of 64%, with low risk of relapse (17%).21 Flu/Mel has been shown to be highly effective in myeloid malignancies, and Tac/Sir is associated with faster engraftment than commonly used Tac/methotrexate.22 Therefore, tac/Sir-based GVHD prophylaxis may limit the potential myelosuppression when combined with the JAK1/2 inhibitor, ruxolitinib.

Based on the hypothesis that continuing ruxolitinib during HCT may prevent ruxolitinib withdrawal syndrome, improve engraftment, and reduce GVHD, we conducted a pilot phase I trial (NCT02917096) evaluating the safety and efficacy of peri-HCT administration of ruxolitinib in patients with MF who were eligible for RIC HCT.

**Methods**

**Protocol**

This prospective single center open-label clinical trial was approved by the COH Institutional Review Board and registered with clinicaltrials.gov (NCT02917096). An assurance was filed with and approved by the Department of Health and Human Services. Informed consent was obtained for all study participants in compliance with the Declaration of Helsinki.
Study Design

In this pilot study with an expansion cohort, adult patients with MF received peri-HCT ruxolitinib administration (day -3 to day +30) along with our standard RIC of Flu/Mel and Tac/Sir as GVHD prophylaxis. The primary objectives were to determine the maximum tolerated dose (MTD) and recommended phase II dose (RP2D) of ruxolitinib and determine its safety. Secondary objectives were grade II-IV acute GVHD (cumulative incidence), chronic GVHD, donor cell engraftment (recovery of granulopoiesis and megakaryopoiesis), grade 3-4 infection, OS, progression-free survival (PFS), cumulative incidence of disease relapse or progression (cumulative incidence) and non-relapse mortality (NRM).

We employed a rolling 6 dose escalation design, with rules similar to a standard 3+3 phase 1 design but allowed up to 6 patients to be treated at a dose level with only 3-at-risk for dose-limiting toxicities (DLT) at any one time. Two dose levels (DL) of 5 and 10 mg twice a day (DL1 and DL2, respectively) were examined. DLT was defined as any regimen-related grade 3 or 4 toxicity per Bearman criteria, any grade 4 neutropenia with fever, or infection lasting for more than 21 days, or grade 4 neutropenia lasting for more than 28 days (engraftment failure), any other regimen-related death, and any grade 5 sepsis-related toxicity that was assigned an attribution level of at least possibly related to ruxolitinib. The MTD was based on the assessment of DLT from the start of therapy to 45 days post-hematopoietic cell infusion (15 days after last full dose of ruxolitinib).

Subjects and Treatment

Adult patients between 18 and 75 years of age with primary or secondary MF at intermediate-2 or high-risk per DIPSS criteria, who were scheduled to undergo their first allogeneic transplant from an 8/8 HLA-matched (A,B,C, DR by high resolution) were eligible for the study. Prior use of ruxolitinib was allowed.

As shown in Figure 1a, conditioning regimen consisted of fludarabine (25 mg/m^2/day, calculated on actual body weight) as a daily infusion from day -9 to day -5. Melphalan was administered at a single dose of 140mg/m^2 (optional 100 mg/m2 for patients older than 70 years old) on day -4. Patients who were on ruxolitinib prior to conditioning continued it till day -10; but ruxolitinib administration at a lower dose during conditioning was allowed on a case-by-case basis. This decision was made out of abundance of caution and to avoid ruxolitinib withdrawal syndrome and the potential interaction between ruxolitinib and conditioning.
chemotherapy. Ruxolitinib was then administered at the assigned DL after conditioning from day -3 to day +30. Thereafter, ruxolitinib was tapered as follows: 5 mg daily for 5 days then stop for DL1, and 5 mg BID for 3 days then stop for DL2.

GVHD prophylaxis consisted of tacrolimus (0.02 mg/kg/d continuous IV), beginning on day –3 and converting to oral dosing when the patient was able to tolerate and absorb oral medications. Sirolimus was administered at a 12 mg oral loading dose on day -3, followed by 4 mg orally as a single morning daily dose. Target serum levels for both tacrolimus and sirolimus are 5-10 ng/ml for each by HPLC and were adjusted by treating physicians to maintain this range. In the absence of GVHD, the immunosuppressive taper was provided as per COH standard operating procedures.\(^{19,26}\) Infection monitoring practice included CMV PCR (twice per week starting on day +7 and continued until day +100), hepatitis B/C and HIV tests at admission. EBV and HHV6 tests were done if clinically indicated.

**Statistical Analysis**

Patient demographic and baseline characteristics, including age, gender, medical history, and prior therapy, were summarized using descriptive statistics. For continuous variables, descriptive statistics [number (n), mean, standard deviation, standard error, median (range)] were provided. For categorical variables patient counts and percentages were provided. Observed toxicities were summarized in terms of type (organ affected or laboratory determination), severity, time of onset, duration, probable association with the study treatment and reversibility or outcome. The cumulative incidence of acute and chronic GVHD were calculated using the Gray method with prior death or relapse considered competing events. Survival estimates were calculated using the Kaplan-Meier method.

All the cytokines and biomarkers were measured repeatedly over time. The median and range at each time point for two does levels were displayed by box plot. The difference between the two dose levels were examined by Wilcoxon Rank Sum test.

**Endpoint definition**
Platelet engraftment was defined as the first of 7 consecutive days in which the platelet count was more than $10 \times 10^9 / \text{L}$ without transfusion support. Neutrophil engraftment was defined as absolute neutrophil count $\geq 0.5 \times 10^3 / \mu\text{L}$ achieved and sustained for 3 consecutive lab values on different days with no subsequent decline.

GVHD-free/relapse-free survival (GRFS) was a post-hoc endpoint defined as survival without grade 3-4 acute GVHD, moderate/severe chronic GVHD, relapse, progression or death (from any cause).

**Pharmacokinetics**

Blood samples for pharmacokinetics studies were collected prior to the morning ruxolitinib doses on days -2 through day +5. Additional post-dose samples were obtained following the morning dose on day +5 at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 12 hours. Blood samples were kept on ice until plasma was separated by centrifugation at 1500 x g (within 1 hour). Plasma samples were stored at $<-70^\circ\text{C}$ until analysis. Plasma concentrations of ruxolitinib was measured using an LC-MS/MS assay. The analytical method was based on a previously reported assay by Veeraraghavan S et al. that has been validated over a concentration range of 0.2-250 ng/mL from a starting plasma volume of 50 µl. Ruxolitinib concentration-versus-time data was analyzed using standard non-compartmental analysis methods according to the Rule of Linear Trapezoids. Non-compartmental pharmacokinetic parameters were derived for each patient and included trough concentrations ($C_{\text{trough}}$), maximum plasma concentration ($C_{\text{max}}$), time to maximum plasma concentration ($T_{\text{max}}$), terminal-phase elimination half-life ($t_{1/2}$), area under the plasma concentration–time curve from 0 to t ($AUC_{0-t}$), and oral clearance ($CL/F$). The individual parameters were summarized as the means or medians, along with standard deviations or ranges.

**Flow Cytometry, Plasma cytokines and GVHD biomarkers**

Peripheral-blood samples were collected on days +21, +35, and +100 post-HCT. Peripheral-blood mononuclear cell (PBMCs) were isolated and cryopreserved following standard procedures. For Treg staining, PBMCs were surface stained for CD3, CD4, CD8, CD19, CD56, CD25, and CD127 (eBioscience™) and intracellularly stained for Foxp3 (eBioscience™) using Foxp3/Transcription Factor Staining Buffer Set (eBioscience™).
Flow cytometry was performed using a BD FACSCelesta™ (BD Biosciences) and data were analyzed using Flowjo software (Tree Star). Foxp3 values were based on isotype controls.

Serum samples, obtained on days +7, +21, +28, +42, and +100, were analyzed for 30 different cytokines using “Human Cytokine Thirty-Plex Antibody Magnetic Bead Kit” (Invitrogen, Camarillo, CA) per manufacturer’s recommendations. Flexmap 3D luminex system (Luminex corp.) was used for analysis and cytokine concentrations were calculated using Bio-Plex Manager 6.2 software with a five parameter curve-fitting algorithm applied for standard curve calculations for duplicate samples.

For original data, please contact “harisali@coh.org”. Individual participant data will not be shared.

Results

Patients and Transplant Characteristics

A total of 18 patients were enrolled, of whom 12 (5 patients in DL1 and 7 patients in DL2) were on ruxolitinib treatment before conditioning regimen, and 6 were taken off ruxolitinib pre-conditioning. Ruxolitinib duration and continuation in patients who took ruxolitinib pre-conditioning is summarized in Supplementary Table 1. Patient demographics and disease/transplant characteristics are summarized in Table 1. Briefly, median age was 65 years (range: 25-73) for all patients, 53 years (range: 25-67) in DL1 (n=6) and 69 years (range: 55-73) in the DL2 (n=12). By DIPSS criteria, four patients were high-risk and the remaining 14 were intermediate-2. By MIPSS70, 14 patients were at high-, one was at intermediate-risk, and 3 patients did not have available data. By MIPSS70+, 7 patients were categorized at very high, 6 at high, and 2 at intermediate-risk. Cytogenetic abnormalities were unfavorable in 5, and favorable in 13 patients. The driver mutations were JAK2 (n=9), CALR (n=4), and MPL (n=1). High-molecular risk category (HMR) mutations were present in 8 patients of which 2 patients carried two HMR mutations. Time from diagnosis to HCT was 17.2 months (range: 3.0-74.5). All patients received mobilized peripheral blood stem cells from a matched related donor (n=5) or unrelated donor (n=13) at a median CD34+ cell dose of $6.0 \times 10^6$ /kg (range: 3.9-9.1). The median follow-up duration for surviving patients was 22.6 months (range: 6.2-25.8). Only one patient had splenomegaly after HCT for poor
counts. Ruxolitinib compliance through the intended treatment period of 34 days was 337.5 mg (range: 295-340) for DL-1, and 680 mg (range: 500-740).

Safety/Toxicity

All patients completed treatment with ruxolitinib. Common adverse events (AE), DLTs, and serious AE (SAE) are summarized in Table 2 according to the DLs. Of the first 3 patients in DL1, the first patient developed a DLT (severe mucositis resulting in airway obstruction with consequent respiratory failure and cardiac arrest due to pulseless electrical activity from which he recovered but died of recurrent respiratory failure on day 48). After observing one DLT on DL1, we enrolled five additional patients in this arm and once this dose level was deemed safe, we escalated the dose to 10 mg twice a day and enrolled 12 more patients at DL2. One patient in DL2 developed DLT of acute kidney injury on day 23 requiring hemodialysis, which was thought to be due to thrombotic microangiopathy (TMA) associated with tacrolimus. TMA was successfully treated with eculizumab. This patient completely recovered and became dialysis independent by day 103 of HCT. Overall majority of the AEs were grade 1 and 2. SAEs were reported in 8 patients (19 events), none of which was considered as related to the study intervention.

Infectious complications from Day -9 to +100 included CMV viremia (n=3), respiratory infections (n=3), and BK virus cystitis (n=1). Three patients had bacteremia with gram positive coccids, and four developed C. difficile colitis.

Engraftment

All patients achieved a neutrophil recovery at a median of 19 days (range: 13-23) in DL1 and 16 days (range: 12-22) in DL2 (Figure 1b) Platelet engraftment was achieved in 14 patients at a median of 25 days (range: 13-119) (20 days [range: 19-42] for DL1 and 28 days [range: 13-119] for DL2) (Figure 1c). Four patients did not achieve platelet transfusion-independence; contributing factors were SR-acute GVHD (n=3) and CMV infection (n=1). Engraftment analyses of the evaluable patients (n=16) demonstrated ≥95% donor chimerism for total BM cells while BM T cells were all >90%, except for one patient in DL1 whose donor chimerism was 85% at
day 30. At day 100 all patients had 99% donor chimerism for BM and BM T cells except one patient in DL1 arm with 95% chimerism. At 1 year, all patients had 100% donor chimerism either by blood or BM. None of the patients had secondary graft failure.

**GVHD**

The cumulative incidence of grade 2-4 and 3-4 acute GVHD at 100 days were 17% (95% CI: 6-47) and 11% (95% CI: 3-41), respectively, for the entire cohort. The number of cases and individual grades are summarized in Table 3. All three patients with diagnosed with acute GVHD grades II-IV developed GVHD before Day +30 and during the ruxolitinib administration period. One patient with grade II-IV GVHD underwent HCT from an HLA mismatched donor, and the other two patients could not tacrolimus as GVHD prophylaxis due to kidney dysfunction. None of the patients in our cohort developed delayed onset acute GVHD. The first-line treatments for acute GVHD was systemic corticosteroids. All patients with grades II-IV GVHD (n=3) developed steroid-refractory acute GVHD requiring second-line agents of basiliximab (n=2) or ECP/Infliximab/ruxolitinib (n=1). Chronic GVHD was developed in 50% of patients at the median onset of 6.7 months (Table 3). The cumulative incidence of the moderate/severe cGVHD at 1 year was 24% (95% CI: 10-55). For organ involvement and severity of acute and chronic GVHD see Supplementary Table 2.

**Survival Outcomes**

The 1-year OS and PFS for the entire cohort were 77% (95% CI: 50-91) and 71% (95% CI: 44-87), respectively. Cumulative incidence of relapse and NRM at 1 year were 6% (95% CI: 1-40) and 23% (95% CI: 10-54), respectively. The estimate of one-year GRFS was 52% (95% CI: 26-73). Causes of death were cardiac arrest (n=1), GVHD (n=2), and respiratory failure related to severe mucositis (n=1).

**Pharmacokinetics**

As expected, the average trough levels of ruxolitinib from day -2 to day +4 were higher in DL2 9.0 ± 2.5 nM for DL1 and 73.4 ± 24.4 nM for DL2 (p<0.01) (Figure 2a). On day 5, detailed PK samples were obtained at eleven different timepoints over 12 hours, which demonstrated Cmax of 150.8 ± 39.9 nM for DL1 and 273.4 ± 78.4 nM for DL2 (p<0.01), AUC 0-inf was 546.2 ± 186.5 nM x hr for DL1 and 990.6 ± 373.5 nM x hr for DL2.
(p<0.01) T1/2 was similar between the two DLs: 2.3 ± 0.4 in DL1 and 2.2 ± 0.9 hours in DL2 (p=0.74). CL/F was also similar between DL1 (32.8 ± 11.2 L/hr) and DL2 (36.4 ± 10.8 L/hr) (p=0.57). (Figure 2b).

**Immune Reconstitution, Plasma Cytokines and GVHD Biomarkers**

Recovery of total lymphocytes, CD4+ T cells, CD8+ T cells, NK cells, B cells, and Treg cells is depicted in Figure 3. The median lymphocyte, CD4 T cell, CD8 T cell, NK cell (CD3-CD56+), B cell (CD19+), and Treg (CD4+CD25+CD127-Foxp3+) counts (per microliter) on day 100 for the entire cohort were 0.4 (range: 0.1-0.7), 106.6 (range:15.8-323.6), 48.5 (range: 9.8-324.7), 103.9 (range: 52.4-163.3), 8.1 (range: 0.2-34.8), 2.3 (range: 0.0-8.9), respectively. On day +21, we observed a trend (p value=0.07) towards faster recovery of CD3+ T cells at DL2 (median=97.0, range: 29.2-714) compared to DL1 (median:38.5, range: 21.6-62.7). Same trend was seen on the recovery of CD8+ cells on day +21 (median=22.8, range: 2.1-317.0 at DL2 versus., median=7.2, range 3.1-12.5 at DL1, p=0.06), and CD27+ memory B cells on day +35 (median=0.7, range 0.2-2.0 at DL2 versus., median=0.3, range: 0.2-0.5 at DL1, p=0.09) (Figure 3).

When plasma cytokine levels were compared between DL1 and DL2, we observed that IL-2, IFN-γ, TNF-α were significantly lower at DL2 (unadjusted for multiple testing, p <0.05) (Supplementary Figure 1). No significant difference was detected in levels of GVHD biomarkers between DL1 and DL2 at 0.05 level. (Supplementary Figure 2) Supplementary Figure 3 shows levels of GVHD biomarkers in the three patients with higher grades of acute GVHD compared to minimum, maximum, and median levels in patients at each dose level.

**Discussion**

We previously have reported long-term outcomes of patients with MF who underwent HCT with Flu/Mel conditioning and Tac/Siro as GVHD prophylaxis with excellent outcomes and long-term remission.18-20 In the current study, we showed in our high-risk cohort of patients with MF, addition of ruxolitinib to this regimen was safe and well tolerated, associated with low rates of GVHD and promising survival. We identified the ruxolitinib dose at 10 mg twice a day as the recommended phase 2 dose, as the toxicities were acceptable with majority of them being grade 1 toxicities. The incidence of infection was similar to what is expected in HCT.
population. It is possible that our study design of limiting ruxolitinib to 30 days post-HCT might have contributed to the tolerability and lack of prolonged immunosuppression. Day +30 was selected to provide initial immune tolerance during the engraftment period. We chose not to continue ruxolitinib administration further than Day +30 to avoid prolonged cytopenia and subsequent increased risk of infection. There was no immune flare after stopping ruxolitinib in our cohort, which could be attributable to carefully planned tapering schedule and effective disease control within the first 30 days after HCT.

In MF, hematopoietic engraftment after HCT may be delayed due to the underlying bone marrow fibrosis and hepatosplenomegaly. Moreover, since cytopenia is a side effect ruxolitinib, delayed engraftment or engraftment failure is a concern when ruxolitinib is administered during the HCT. Our data showed that addition of ruxolitinib was not associated with engraftment delay compared with our historic data previously reported by our group in this patient population.\textsuperscript{21} With the use of semi-ablative Flu/Mel conditioning, all patients achieved full/near-full donor chimerism early post-transplant. In our study, mixed lymphoid and myeloid lineage was extremely rare, and with the small sample size, it was not possible to assess the impact of mixed chimerism on transplant outcomes.

We observed promising rates of acute GVHD; especially, low incidence of grade 3-4 acute GVHD in our cohort of older adult patients (median age of 65 years), with a majority of HCTs (72\%) from unrelated donor. Ruxolitinib has been shown to be effective as a therapeutic agent for established acute or chronic GVHD refractory to systemic corticosteroids.\textsuperscript{29-31} However, to date, ruxolitinib has not been prospectively evaluated as GVHD prophylaxis with sirolimus/tacrolimus in clinical trials, and our data represent the first of such data, demonstrating the safety profile, PK, and clinical outcomes with a major relevance in the evolving field of GVHD prevention and treatment. We have previously evaluated and reported outcomes of patients with myelofibrosis (n=110) who underwent allogeneic HCT with fludarabine/melphalan conditioning regimen at city of hope from 2004-2017,\textsuperscript{21} and reported that cumulative incidence (CI) of acute GVHD grades II to IV and III to IV by day 100 of 45\% and 17\%, respectively. Cumulative incidence of chronic extensive GVHD at 12 months was 45\%. In the current study, peri-HCT administration of ruxolitinib resulted in lower acute GVHD...
grades II-IV and III-IV of 17% and 11%, respectively. Ruxolitinib administration lowered the cumulative incidence of the moderate/severe cGVHD at 1 year was 24%.

Owing to the small sample size, it was not possible to definitively demonstrate the dose-dependent differences in cellular immune reconstitution or serum cytokine levels between DL1 and DL2 of ruxolitinib. Interestingly, our exploratory analyses without adjustment for multiple testing showed potential dose-relation in Th1 cytokines levels (i.e., IL-2, IFN-γ, TNF-α) consistent with the proposed mechanisms of action,\textsuperscript{32} and supporting that ruxolitinib at 10 mg twice daily is potentially more biologically active and immunologically favorable than 5mg BID. The difference in levels of some of these cytokines (IL-2, IFN-γ and TNF-α) persisted at day 100 even though ruxolitinib was stopped at day 33. This may mean that a shorter course of ruxolitinib may have lasting effects of the cytokines milieu even after it was stopped.

In summary, in this pilot phase I trial, we successfully established the safety, feasibility, and RP2D of ruxolitinib during the peri-HCT period in patients with MF undergoing HCT, associated with preventing severe acute GVHD. Future studies to optimize the duration and tapering strategies of ruxolitinib are of importance and our data support a larger phase II randomized trial to prospectively define the benefits of ruxolitinib in peri-transplant setting.

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**Authorship and Conflict-of-Interest Statements**
HA, DSS and RN contributed to study concept and design and data interpretation. NT, TS and JP were the study biostatisticians and did the statistical analysis. TS designed and performed PK studies and analysis. WT performed flow cytometry and cytokine analysis experiments. SM drafted the report. The remaining authors contributed to critical revision of the manuscript for intellectual content. Authors have no relevant conflict of interest. All authors read and approved the final version.
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Figure Legends

Figure 1. (a) Study Schema: Peri-HCT ruxolitinib administration was from day -3 pre-HCT to day +30 post-HCT. (b) Neutrophil engraftment post-HCT and (c) Platelet Engraftment post-HCT

Figure 2. Ruxolitinib Pharmacokinetics

Figure 3. Comparison of immune reconstitution between dose levels 1 (5 mg BID) and 2 (10 mg BID) on days 21, 35, and 100 post-HCT.

Tables

Table 1. Patient and HCT characteristics

|                             | Arm 1 (N=6) | Arm 2 (N=12) | All (n=18) |
|-----------------------------|------------|-------------|------------|
|                             | N (%) or  | N (%) or    | N (%) or   |
|                             | Median (Range) | Median (Range) | Median (Range) |
| Age at start of treatment   | 53 (25-67) | 69 (55-73)  | 65 (25 – 73) |
| Sex                         |           |             |            |
| Female                      | 1 (17)    | 3 (25)      | 4 (22)     |
| Male                        | 5 (83)    | 9 (75)      | 14 (78)    |
| Race                        |           |             |            |
| Caucasian                   | 4 (67)    | 12 (100)    | 16 (88)    |
| Asian                       | 1 (17)    | 0           | 1 (6)      |
| Pacific Islander            | 1 (17)    | 0           | 1 (6)      |
| Ethnicity                   |           |             |            |
| Hispanic                    | 2 (33)    | 1 (8)       | 3 (17)     |
| Non-Hispanic                | 4 (67)    | 11 (92)     | 15 (83)    |
| Age at start of treatment   | 53 (25-67)| 69 (55-73)  | 65 (25 – 73)|
| Baseline Myelofibrosis      |           |             |            |
| Mild                        | 1 (17)    | 0           | 1 (6)      |
| Moderate                    | 4 (33)    | 2 (17)      | 6 (33)     |
| Severe                      | 1 (17)    | 10 (83)     | 11 (61)    |
| Disease status at baseline  |           |             |            |
| No response/stable disease  | 6 (100)   | 11 (92)     | 17 (94)    |
| Progression from hematologic| 0         | 1 (8)       | 1 (6)      |
| HCI CI                       | N=6       | N=10*       | N=16       |
| Performance status          | 1 (0-3)   | 3 (1-5)     | 3 (0-5)    |
| 80                          | 0 (0)     | 4 (33)      | 4 (22)     |
| 90                          | 4 (67)    | 7 (59)      | 11 (61)    |
| 100                         | 2 (33)    | 1 (8)       | 3 (17)     |
| Myelofibrosis type          |           |             |            |
| Primary                     | 4 (67)    | 9 (75)      | 13 (72)    |
| Secondary                   | 2 (33)    | 3 (25)      | 5 (28)     |
| Myelofibrosis risk (DIPSS criteria) |       |             |            |
| High                        | 1 (17)    | 3 (25)      | 4 (22)     |
| Intermediate II             | 5 (83)    | 9 (75)      | 14 (78)    |
| HLA                         |           |             |            |
| 7/8                         | 1 (17)    | 0 (0)       | 1 (6)      |
| 8/8                         | 5 (83)    | 12 (100)    | 17 (94)    |
| Donor Type                  |           |             |            |
| Sibling | Unrelated |
|---------|-----------|

| Donor/Recipient CMV pre-HCT |  |  |
|-----------------------------|-------------------------|
| **Negative/Negative**       | 1 (17)                  |
| **Negative/Positive**       | 2 (33)                  |
| **Positive/Negative**       | 0 (0)                   |
| **Positive/Positive**       | 3 (50)                  |

| CD34 Dose | 6.0 (4.3 – 9.1) | 6.0 (3.9 – 8.6) | 6.0 (3.9 – 9.1) |
|-----------|-----------------|-----------------|-----------------|

| Time: Diagnosis to HCT (month) | 12.9 (3.0 – 30.8) | 27.2 (4.0 – 74.5) | 17.2 (3.0 – 74.5) |
|-------------------------------|-------------------|-------------------|-------------------|

| Time: Diagnosis to Treatment (month) | 12.6 (2.7 – 30.5) | 26.9 (3.7 – 74.2) | 17.0 (2.7 – 74.2) |
|-------------------------------------|-------------------|-------------------|-------------------|

*: 2 patients who transplanted in 2020 did not have data in CIBMTR yet.
Table 2: Toxicity Summary Per Bearman Criteria

| Organ     | DL1: 5 mg (Day +60) (n=3) | DL1: 5 mg (Day +45) (n=3) | DL2: 10 mg (Day +45) (n=12) |
|-----------|---------------------------|---------------------------|-------------------------------|
|           | Grade I I I I | Grade I I I I | Grade I I I I | Grade I I I I | Grade I I I I | Grade I I I I | Grade I I I I | Grade I I I I | Grade I I I I | Grade I I I I | Grade I I I I | Grade I I I I | Grade I I I I | Grade I I I I | Grade I I I I | Grade I I I I | Grade I I I I | Grade I I I I | Grade I I I I | Grade I I I I | Grade I I I I |
| Bladder   | 0 0 0 0 | 0 0 0 0 | 2 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| Cardiac   | 0 0 1* 0 | 0 0 0 0 | 4 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| CNS       | 0 1 0 0 | 0 0 0 0 | 2 1 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| GI        | 2 0 1* 0 | 2 0 0 0 | 9 0 0 0 | 2 0 0 0 | 2 0 0 0 | 2 0 0 0 | 2 0 0 0 | 2 0 0 0 | 2 0 0 0 | 2 0 0 0 | 2 0 0 0 | 2 0 0 0 | 2 0 0 0 | 2 0 0 0 | 2 0 0 0 | 2 0 0 0 | 2 0 0 0 | 2 0 0 0 | 2 0 0 0 | 2 0 0 0 | 2 0 0 0 |
| Hepatic   | 1 1 0 0 | 0 0 0 0 | 1 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| Pulmonary | 1 0 0 1* | 0 0 0 0 | 4 2 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| Renal     | 2 0 0 0 | 0 0 0 0 | 5 2 1* 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| Stomatitis| 3 0 0 0 | 3 0 0 0 | 6 2 0 0 | 3 0 0 0 | 3 0 0 0 | 3 0 0 0 | 3 0 0 0 | 3 0 0 0 | 3 0 0 0 | 3 0 0 0 | 3 0 0 0 | 3 0 0 0 | 3 0 0 0 | 3 0 0 0 | 3 0 0 0 | 3 0 0 0 | 3 0 0 0 | 3 0 0 0 | 3 0 0 0 | 3 0 0 0 | 3 0 0 0 |

* Dose limiting toxicity.

Note: First three patients at DL-1 were followed for DLTs until Day +60 post-transplant. The rest of the patients were followed for DLTs for 45 days after transplantation.

Table 3: GVHD Summary

|                  | Acute GVHD Max Grade | Chronic GVHD Overall Severity |
|------------------|----------------------|------------------------------|
|                  | None | I | II | III | IV | None | Mild | Moderate | Severe |
| DL1: 5 mg        | 3    | 2 | 0  | 1  | 0  | 1    | 3    | 1        | 1      |
| DL2: 10 mg       | 6    | 4 | 1  | 1  | 0  | 8    | 1    | 2        | 1      |
Figure 1

(a) Schematic overview of the study timeline and treatment regimen:
- Fludarabine 25 mg/m² daily
- Melphalan 140 mg/m²
- Stem Cell Infusion
- End of aGVHD period
- Study Follow-up
- Ruxolitinib PO QD at assigned dose
- Immunosuppressant GVHD prophylaxis
- Tacrolimus/Sirolimus (each adjusted to 5-10 ng/ml blood)

(b) Neutrophil Engraftment
- Median: 17 Days (range: 12-23)
- n=18

(c) Platelet Engraftment
- Median: 25 Days (range: 13-119)
- n=18
Figure 2

(a) Trough Levels Day -2 to Day +4

- **Ruxolitinib 5 mg bid (n=5)**
- **Ruxolitinib 10 mg bid (n=12)**

(b) Day +5

- **Ruxolitinib 5 mg bid (n=5)**
- **Ruxolitinib 10 mg bid (n=12)**

| Dose      | Average Throught Conc. (nM) |
|-----------|-----------------------------|
| 5 mg BID  | 9.0 ± 2.5                   |
| 10 mg BID | 73.4 ± 24.4                 |

| Dose Level | PK Parameter | 5 mg bid (n=5) | 10 mg bid (n=12) |
|------------|--------------|----------------|------------------|
|            | Cmax (nM)    | 150.8 ± 39.9   | 273.4 ± 78.4     |
|            | AUC 0-inf (nM × hr) | 546.2 ± 186.5 | 990.6 ± 373.5   |
|            | T1/2 (hr)    | 2.3 ± 0.4      | 2.2 ± 0.9        |
|            | CL/F (L/hr)  | 32.8 ± 11.2    | 36.4 ± 10.8      |
Figure 3

(a) CD3+ T Cells
(b) CD4+ T Cells
(c) CD8+ T Cells
(d) FoxP3+ Tregs
(e) CD27+ B Cells
(f) CD56+ NK Cells

Each graph shows the absolute cell number for different cell types over time (Day 21, Day 35, Day 100) for two different doses (DL1: 5 mg, DL2: 10 mg). The data is presented in box plots.