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Final results of a phase II study of the combination of azacitidine and ruxolitinib in patients with myelofibrosis

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All other authors declare no competing interests.

Authorship Contributions:

ND designed the clinical trial and was the principal investigator; JN provided statistical support for trial design; LZ, DS, HH, SA, LM, JS, and VC collected the data; SA and JS performed statistical analysis; PB, CD, TK, YA, MO, KT, NJ, EJ, FR, GGM, HK, NP and ND enrolled patients; SA, JS, and LM wrote the first draft of the manuscript; ND, LM and SA revised the manuscript; All authors read and approved the final manuscript.

Abstract

Monotherapy with JAK2 inhibitors reduces splenomegaly and symptom burden but has limited effect on natural progression of myelofibrosis. We conducted a phase 2 clinical trial evaluating the combination of azacitidine (AZA) and ruxolitinib (RUX) in myelofibrosis. The interim analysis with 46 patients (median follow-up 28 months) demonstrated a response rate of 72%. We now report the final results of this clinical trial. The study primary endpoint was objective response rate by the International Working Group-Myeloproliferative Neoplasms Research and Treatment (IWG-MRT) criteria. From 3/2013 to 10/2021, 61 patients were treated (median age 66 years; range, 46-87 years). By baseline blast count, 54 patients had chronic phase disease (<10% blasts) and 7 had accelerated disease (10-19% blasts). In the chronic phase cohort, responses occurred in 42 patients (78%), including symptom and spleen response in 68% and 66% respectively. Leukemic transformation occurred in 4 patients (7%) on study, and 13 patients (24%) underwent allogeneic stem cell transplantation. After a median follow-up of 95.3 months, the median overall survival was 56 months (95% CI: 38.2 - 99.3). Among the 7 patients with accelerated disease, responses occurred in 3/7 (43%), and the median overall survival was 30.8 months (95% CI: 9.2 - 34.1). The most common grade 3-5 TEAEs were anemia (33, 54%), thrombocytopenia (24, 39%), leucopenia (14, 23%), and pneumonia (9, 15%). 4 patients (7%) discontinued study due to adverse events. The AZA-RUX combination demonstrated safety and efficacy in myelofibrosis, supporting further investigation of this regimen.

Trial registration: *ClinicalTrials.gov; NCT01787487*

Introduction:

The evolution and approval of multiple Janus kinase (JAK) inhibitors (JAKi) in myelofibrosis (MF) has been an important progress in the field; however, these agents are primarily effective in reducing symptom burden and splenomegaly and have limited overall effects on the biology and natural progression of MF, including the risk of transformation to acute leukemia[1-6]. Given this, many novel agents aimed at altering the disease course in MF are under investigation in clinical trials, including combination therapy with JAKi[7]. Some of these promising recent combinatorial approaches include the use of the JAKi ruxolitinib (RUX) in conjunction with the novel BET inhibitor pelabresib[8], BCL2/xl inhibitor navitoclax[9], and XPO1 inhibitor selinexor[10]. Despite their failure to meet all their predefined endpoints in late phase 3 clinical trials, they demonstrated high response rates, especially pertaining to spleen size reduction and may have had possible additional effects on disease biology not seen with JAKi monotherapy[11-14]. Further investigation of such combinatorial strategies is of high importance to the field, with the aim of achieving deeper and more durable responses, slowing disease progression, reducing leukemic transformation, and ultimately improving long-term survival outcomes in MF.

Hypomethylating agents (HMA), like azacitidine (AZA), are a potent class of drugs approved or frequently used in myeloid malignancies like myelodysplastic syndrome (MDS), chronic myelomonocytic leukemia (CMML), and acute myeloid leukemia (AML)[15, 16]. In a phase II study using single agent AZA in MF, responses were noted in 24% of patients, and co-relative studies demonstrated AZA induced global DNA hypomethylation in treated patients[17]. Since RUX and AZA target distinct pathogenic mechanisms in MF and other myeloid disorders (aberrant JAK-STAT signaling and epigenetic dysregulation, respectively), we hypothesized that their combination could exert synergistic activity by both controlling the disease-related symptoms and targeting the underlying malignant clone. Therefore, we performed a phase 2 trial to study the safety and efficacy of AZA in combination with RUX in patients with MF and MDS/myeloproliferative neoplasms (MPN) overlap syndromes. We previously reported the results of the MF arm of this study at a median follow up of 28 months[18]. Among the 46 enrolled patients at that time, the overall response rate at 24 weeks per the International Working Group - Myeloproliferative Neoplasms Research and Treatment (IWG-MRT) criteria was 72%, with spleen and symptom responses of 62% and 54% patients, respectively.

In this update, we report the final results of this clinical trial with a total of 61 patients including long term survival outcomes (median follow up of 8 years).

Methods:

This was a phase 2 open label single institution study conducted between March 2013 to October 2021, including adult patients in two diagnostic arms: arm 1 (MF) and arm 2 (MDS/MPN). This manuscript

reports the outcomes of MF patients enrolled on arm 1 of the study; data cutoff for this analysis was December 27, 2024. This study was approved by the MD Anderson Cancer Center Institutional review board and followed the principles of the Declaration of Helsinki.

Patients

Eligible patients were adults (≥ 18 years of age) with primary or secondary MF [post-polycythemia vera (PV) or post-essential thrombocytosis (ET)] in need of therapy and with a Dynamic International Prognostic Scoring System (DIPSS) intermediate 1, intermediate-2 or high-risk disease[19]. Patients with bone marrow/peripheral blasts up to 19% (i.e., in chronic or accelerated phase) were allowed. Patients with prior exposure to RUX or AZA were excluded. Concurrent hydroxyurea to control proliferation was allowed, and previous MF directed therapy had a 14-day wash-out window. Study protocol with the full inclusion and exclusion criteria is included in the **Supplement.**

Treatment and assessment

As reported previously in detail[18], in order to mitigate the risk of overlapping myelosuppression with the combination, patients had a run-in phase that recommended single agent RUX (dosed per label) for the first 3 cycles and AZA (starting dose 25mg/m^2 on days 1-5 per 28 day cycle) be initiated in the 4th cycle. AZA could be introduced earlier in patients with proliferative disease or BM blasts >10% and the AZA dose could be increased up to 75mg/m^2 to optimize clinical benefit at the discretion of the treating physician and principal investigator. Though initially designed for 15 cycles of protocol combination therapy, subsequent amendment extended the treatment duration to up to 5 years (65-66 cycles). The total symptom score (TSS) was assessed using the MPN-symptom assessment form (SAF)[20] administered prior to each cycle for the first 6 cycles, followed by every three cycles through cycle 15, and at every protocol visit thereafter. Additional information on interval monitoring during the study, including spleen size assessment and bone marrow (BM) evaluation are in the study protocol (**Supplement**).

Statistical Methods

The primary study endpoint was the objective response rate according to the 2013 IWG-MRT criteria[21] while the key secondary endpoint was safety. Relevant exploratory endpoints included BM morphological and cytogenetic responses, molecular responses (reduction of *JAK2* allele burden), transfusion independence, response duration and survival outcomes. Adverse events (AE) were assessed as per the standardized National Cancer Institute Common Toxicity Criteria for Adverse Events, version 4.03. Using an intention-to-treat approach, all patients who received at least one dose of study therapy (either AZA or RUX) were included in the efficacy and toxicity analyses.

Duration of Response (DoR) was calculated from the date of earliest response to the date of loss of any achieved response, AML transformation or death on study, whichever occurred first, and was censored at the off-study date. Overall survival (OS) was defined as the time from study enrollment to death and was censored at the last follow up/contact. Kaplan Meier estimates and log-rank tests were used for time-to-event analysis and group comparisons. Median follow up time was estimated using the reverse Kaplan Meier method.

Results:

Patient population and baseline characteristics

61 patients were treated; 31 patients (51%) had primary MF, while 15 patients each (25%) had secondary MF from prior ET and prior PV. A total of 32 patients (53%) received prior treatments for MPNs/MF (**Supplement Table 1**). The median age was 66 years (range, 46-87 years); 32 patients (53%) were > 65 years of age. Based on baseline BM and peripheral blood blasts, the study enrolled patients in chronic phase MF (<10% blasts, n=54) and accelerated phase MF (10-19% blasts, n=7), as previously defined[22]. Since chronic phase and accelerated phase MF represent biologically and clinically distinct entities with markedly different outcomes[22, 23], the efficacy outcomes of these two groups are analyzed separately.

Baseline characteristics of all patients and separately for accelerated phase and chronic phase MF patients are summarized in **Table 1**. Aside from the expected differences in BM and peripheral blood blasts, patients with accelerated phase disease (compared with chronic phase) had significantly higher rates of transfusion dependence (at-least 6 units of packed red blood cells in the preceding 12 weeks; 43% vs 4%, p<0.01) and high-risk DIPSS disease (57% vs 17%, p=0.03). There were no significant differences in the prevalence of splenomegaly ≥ 5 cm (71% vs 76%, p=1), TSS ≥ 12 (71% vs 85%, p=0.34), MPN driver mutations (*JAK2* 71% vs 56%, p=0.69; *CALR* 25% vs 30%, p=1; *MPL* 17% vs 13%, p=1), high molecular risk mutations (HMR, including *ASXL1*, *EZH2*, *IDH1*, *IDH2*; 50% vs 33%, p=0.65). Of note, only 15 patients (25%) in the entire study cohort had *SRSF2* and *U2AF1* mutation testing performed at baseline, with 1 patient each testing positive for these mutations.

Treatment responses

Table 2 shows the responses in the full study cohort, also stratified by chronic and accelerated phase.

Chronic phase cohort (n=54)

RUX and AZA were administered on protocol for a median of 15 (range, 3 - 66) and 12 (range, 0 - 63) cycles, respectively. Five patients (9%) received RUX monotherapy only; Of these, 1 patient received fewer than 4 cycles of RUX, whereas the remaining 4 could not initiate AZA due to severe

thrombocytopenia and/or neutropenia. AZA was initiated before cycle 4 in 7 patients (13%). Up-titration of the AZA dose on study was carried out in 19 patients (35%): up to a maximum of 50 mg/m^2 in 12 patients (22%), and 75 mg/m^2 in 7 patients (13%). **Supplement Figure 1** describes the dose levels of AZA and RUX delivered on the study.

Objective responses were attained in 42 patients (78%). Among these responders, 3 patients (7%) never received AZA, while AZA was started earlier than cycle 4 in five patients (12%). Of the remaining 34 responders (81%) who received AZA at cycle 4 or beyond (after at least 3 cycles of single agent RUX), 7 of 34 responders (21%) attained their earliest response only ≥3 months after AZA initiation.

Regarding individual responses (details in **Figure 1**), CI TSS50 response was attained in 23 of 44 evaluable patients (52%) by week 24, and in 30 patients (68%) at any time on study. CI spleen response (among 41 evaluable patients per IWG-MRT criteria) was attained by week 24 in 22 patients (54%), and at any time on study in 27 patients (66%). Among those who initiated AZA at cycle 4 or beyond, 3/25 patients (12%) achieved CI TSS50 and 3/22 patients (14%) achieved CI spleen response only ≥3 months after AZA was initiated. Additionally, 34 patients were evaluable for both CI TSS50 and CI spleen responses; of these, 17 patients (50%) attained dual responses.

Additional responses (% of evaluable patients) included anemia response in 3 patients (14%), partial molecular response (≥50% decrease in *JAK2* allele burden) in 4 patients (16%) and cytogenetic complete response in 2 patients (9%). One patient (2%) met criteria for partial response.

Median DoR (all achieved responses) was 33.1 months (95% CI: 19.1 – 46.7; **Figure 2a**). Median DoR for CI TSS50 response and CI spleen response was 47.4 months (19.1 – NR; **Figure 2b**) and 46.7 months (29.3 – NR; **Figure 2c**) respectively.

Reduction in BM fibrosis was evaluated among patients with available baseline and on-study BM fibrosis grading. Fibrosis reduction by ≥1 grade anytime on study was noted in 18/51 evaluable patients (35%) in the chronic phase cohort (3 patients did not have post baseline BM fibrosis testing). Finally, among the 30 patients with baseline *JAK2* mutation, paired baseline and on-study *JAK2* variant allelic frequency (VAF) data was available for 23 patients. Among these, ≥20% reduction in *JAK2* VAF anytime on study was noted in 11 patients (48%).

Accelerated phase cohort (n=7)

Among the accelerated phase patients, RUX and AZA were administered on protocol for a median of 7 (range, 1 - 39) and 4 (range, 0 - 39) cycles, respectively. One patient (14%) did not receive AZA and received only a single cycle of RUX prior to being taken off study due to AML transformation. AZA was given before cycle 4 in 4 patients (57%). Additionally, 4 patients (57%) required up-titration of AZA dose:

up to a maximum of 50 mg/m^2 and 75 mg/m^2 in 1 patient and 3 patients, respectively (**Supplement Figure 1)**. Objective responses were attained in 3 patients (43%); CI TSS50 and CI spleen in 3/5 (60%) and 1/5 (20%) evaluable patients, respectively. Median DoR for TSS50 response was 3.5 months (95% CI: 1.8 - NR), and DoR for the lone CI spleen response was 29.4 months.

None of the patients in the accelerated phase cohort had ≥1 grade reduction in BM fibrosis or ≥20% reduction in *JAK2* VAF on study.

Study disposition

Supplement figure 2 (Consort diagram) describes the study disposition in detail.

Chronic phase cohort (n=54)

Patients were on study for a median of 13.8 months (range, 2.5 - 60.8), with 20 patients (37%) on study for ≥24 months. All patients are off study at data cutoff. Reasons for study discontinuation include treatment completed per protocol (14, 26%), transition to allogeneic hematopoietic stem cell transplant (ASCT; 13, 24%), resistant disease/disease progression (7, 13%), AML transformation (4, 7%), myelosuppression (3, 6%), death on study (2, 4%), and patient/provider preference (11, 20%).

Among the patients who had AML transformation on study (n=4, 7%), the median age was 75 years (range, 60 - 77); all had Int-2/high-risk DIPSS disease, baseline median BM blasts and peripheral blasts were 5% (range, 0 - 9) and 3% (range, 0 - 9), respectively, and 1 patient (among 3 tested) had an HMR mutation (*ASXL1*). Patients received a median of 19 cycles (range, 3 - 51) of RUX; three of these four patients received AZA, with a median of 24 cycles (range, 3 – 48), while one patient never received the combination therapy. Initial responses were attained in 2 patients (50%). The median time to AML transformation from study initiation was 17.2 months (range, 2.5 – 46.2), with 2 patients (50%) transforming within 6 months of study initiation. AML transformation rates at 96 weeks and 5-years were 3.7% and 7%, respectively.

A total of 13 patients were taken off study for ASCT in this cohort. For these patients, the median age at study initiation was 56 years (range, 48 - 71) and Int-2/high risk DIPSS disease was present in 8 patients (62%). Initial responses were attained in 11 patients (85%). At the time of ASCT, 4 patients (31%) had evidence of disease progression. Median time to ASCT from the date of study initiation and from the date of study discontinuation was 8.3 months (range, 2.9 – 61.3) and 13 days (range, 1 – 44) respectively.

Accelerated phase cohort (n=7)

These patients were on study for a median of 6.3 months (range, 0.9 - 34.1), with only 2 patients (29%) on study for ≥24 months. All patients are off study at data cut off, with reasons for study discontinuation being resistant disease/disease progression (3, 43%), AML transformation (2, 29%), death on study (1, 14%), and patient/provider preference (1, 14%).

Two patients underwent AML transformation on study: 0.9 and 2.6 months after study initiation, respectively. The first patient (age 75 years, baseline BM/peripheral blast 8%/18%) received only 1 cycle of RUX monotherapy, and the second patient (age 67 years, baseline BM/peripheral blast 15%/10%) received a total of 7 cycles of both AZA and RUX on study (this patient remained on study for an additional 4 months after transformation).

Overall survival and post-study course

All patients were followed for an extended duration even after study discontinuation to capture post-study events and overall survival through the data cutoff date of December 27, 2024 (**Supplement figure 3** using swimmer's plot illustrates the full course of each patient). The median follow-up time after study discontinuation for chronic and accelerated phase patients (excluding the two chronic phase patients and one accelerated phase patient who died on study) was 67.8 months (95% CI: 45.0 - 84.5) and 32 months (95% CI: NR - NR), respectively.

Chronic phase cohort (n=54)

Seven patients (13%) progressed to AML after study discontinuation (including one patient with mixed phenotype acute leukemia: T/myeloid). Five patients underwent ASCT in their post study follow up: 4 after subsequent salvage therapy for MF and 1 following transformation to AML.

After a median follow-up of 95.3 months (95% CI: 75.7 - 130.2) from study initiation for the 54 patients in the chronic phase cohort, 18 patients (33%) remain alive with a median OS of 56 months (95% CI: 38.2 - 99.3; **Figure 3A**). By baseline DIPSS score, the median OS for Int-1, Int-2, and high-risk patients was 100.2 months, 56 months, and 29 months, respectively (log rank for trend, p=0.003, **Supplement Figure 4**).

Chronic phase cohort patients who had ≥1 grade reduction in BM fibrosis anytime on study had a numerically higher median OS compared to those who did not (64.8 vs 45.8 months, p=0.20), though this was not statistically significant (**Supplement Figure 5**). Additionally, patients who had a ≥20% reduction in *JAK2* VAF at any time on study had a numerically higher median OS compared to those who did not, though this was also not statistically significant (71.6 months vs 44.4 months, p=0.98; **Supplement Figure 6**).

For the 13 chronic phase patients who directly underwent ASCT after coming off study, the median OS was 64.8 months (95% CI: 22.1 – NR; **Supplement Figure 7**). To further evaluate the impact of censoring at the time of ASCT on OS, we analyzed all chronic phase MF patients treated on this study who underwent ASCT at any time following study initiation (n=18). As of the last follow up, 7 patients (39%) remain alive while 11 patients have died post-transplant (cause of death described in **Supplement Table 2**); due to AML transformation in 1 patient (6%), and non-relapse mortality (NRM) in 10 patients (56%). NRM rates at 1-year and 2-year were 33% and 56%, respectively. Upon censoring at the date of ASCT, median OS for the full chronic phase cohort was 71.6 months (95% CI: 44.1 - 100.2; **Supplement Figure 8)**.

Accelerated phase cohort (n=7)

AML transformation after study discontinuation occurred in 2 patients (29%). Of these, one patient transformed after prior ASCT performed as a subsequent line of therapy for MF, while the other did not undergo transplantation. Separately, an additional patient in the accelerated phase cohort underwent ASCT following AML transformation on study. After a median follow up of 38.3 months from study initiation for the 7 patients in the accelerated phase cohort, 1 patient (14%) remains alive (s/p ASCT following AML transformation) with a median OS of 30.8 months (95% CI: 9.2 - 34.1; **Figure 3B**).

Safety/Adverse Events

Safety data was collected for the full study cohort (n=61). All 61 patients (100%) experienced at least one adverse event (AE) while on study treatment (grade ≥3 AE in 52 patients, 85%). **Supplement Table 3** presents the incidence of common AEs irrespective of treatment attribution (including all grade ≥3 AEs and AEs of any grade observed in ≥10% of patients). The most common grade 3-5 hematologic AEs were anemia (33, 54%), thrombocytopenia (24, 39%), and leucopenia/neutropenia (14, 23%), while the most common grade 3-5 non-hematologic AEs were pneumonia (9, 15%), fatigue (9, 15%), and hypertension (8, 13%). **Supplement Figure 9** illustrates the trend of mean hemoglobin, platelet and white blood cell count over time on the study. There was no significant difference in the incidence of grade ≥3 AEs between chronic phase and accelerated phase MF patients (87% vs 71%, p=0.27). The most common grade 3-4 possibly treatment-related AEs (TRAEs) were anemia (21, 34%), thrombocytopenia (18, 30%), leukopenia/neutropenia (9, 15%), fatigue (3, 5%), and weight gain (3, 5%). No grade 5 TRAEs were observed. **Table 3** presents the incidence of TRAEs in the full cohort.

AE-related dose reductions were required in 27 patients (44%), while 28 patients (46%) required AE-related dose interruptions (**Supplement Table 4**). Four patients (7%) permanently discontinued therapy due to AEs (one patient only treated with RUX monotherapy), including thrombocytopenia (n=2), neutropenia (n=1), and pneumonia (n=1). A total of 3 patients (5%) died while on study due to AEs

(pneumonia, intracranial hemorrhage, and sepsis); all of these were considered unrelated to the study regimen by treating physicians.

Discussion:

Over the past decade, research efforts have focused on improving upon the current standard of JAKi monotherapy in MF, with several clinical trials evaluating JAKi based combinatorial strategies in order to improve outcomes[24]. In this analysis, we describe the final results of our phase 2 study evaluating the AZA-RUX combination therapy in patients with MF. Similar to the data from the interim analysis[18], we report robust and durable responses with the AZA-RUX combination, especially in patients with chronic phase MF, with no new safety signals identified.

Among the patients enrolled with chronic phase MF, responses per the IWG-MRT criteria were seen in 78% of patients in a cohort that was enriched with adverse risk features (DIPSS Int-2/high in 60%, hemoglobin < 10g/dl in 39%, HMR mutations in 33%). It is important to note that the prevalence of HMR mutations in our cohort might be underestimated, since baseline testing for *SRSF2* and *U2AF1* mutations was only performed in 25% of patients. Overall, 21% of the responders achieved their first response only after ≥3 months of AZA initiation, potentially indicating the added benefit of AZA to RUX therapy. Responses were predominantly spleen and symptom improvement, occurring in 66% of patients (54% by week 24) and 68% patients (52% by week 24), respectively. Dual responses (both spleen and symptoms) at any time on study were observed in 50% of evaluable patients (with baseline TSS≥12 and splenomegaly ≥5 cm). Responses were also durable, with median DoR for both spleen and symptom improvement approaching 4 years in responders. These response rates in a high-risk chronic phase MF cohort compare favorably with prior published data using single agent RUX (CI spleen/TSS50; 30-50% at week 24)[2, 5, 25-27], and similar to other RUX based combination therapies (CI spleen/TSS50; 50-60% at week 24)[12, 13]. Additional response categories in our cohort included improvement in anemia (14%), *JAK2* molecular partial response (16%), and cytogenetic complete remission (9%). Finally, while not an individual response category per the IWG-MRT criteria but increasingly reported in contemporary MF studies, ≥1 grade reduction in BM fibrosis and ≥20% reduction in *JAK2* VAF at any time on the study was noted in 35% and 48% of evaluable patients, respectively. These findings are similar to those reported with other JAKi based combination regimens and investigational therapies in MF[13, 28, 29] , and indicate that the AZA-RUX regimen may exert effects on the underlying disease clone in MF and potentially slow disease progression. While BM fibrosis and *JAK2* VAF reduction were not associated with a statistically significant OS benefit, these analyses were likely underpowered due to small numbers. Furthermore, even patients who did not meet the IWG-MRT response criteria appeared to derive some clinical benefit with the AZA-RUX regimen, with 33% of non-responders remaining on study for >1 year. Finally, 13

patients (24%) were able to be directly bridged to ASCT after study therapy (without any intervening treatment), which remains the only potentially curative modality in MF[30].

One of the most challenging aspects of MF is the risk of AML transformation. In our chronic phase cohort, four patients (7%) had AML transformation on study (including two patients within six months of study initiation); all four patients had Int-2/high-risk DIPSS disease and one patient did not receive AZA prior to transformation. AML transformation rates in our study at 5-years (7%) may be compared with the long term follow-up results from the COMFORT-2 trial[31] (5.5% at 5-years), although these cross-trial comparisons should be interpreted cautiously given differences in risk stratification methods (DIPSS in our study vs IPSS in COMFORT-2). More recently, the 96 week follow up data from the MANIFEST-2 study[11] reported AML transformation in 5.1% and 3.7% of patients in the pelabresib-RUX and placebo-RUX arms respectively. By comparison, 3.7% of patients in our study had AML transformation at 96 weeks, despite a higher proportion of adverse risk features in our cohort (60% DIPSS Int-2/high risk vs 40% in both arms of MANIFEST-2).

The long-term OS data with the AZA-RUX combination are encouraging. After a median follow-up time of 8 years from study initiation (including subsequent off study period until the last follow up), the median OS for patients with chronic phase MF was 56 months (5-year OS 47%) without censoring at ASCT and 71.6 months (5-year OS 53%) when censoring at ASCT. A total of 18 patients underwent ASCT, majority of whom (13/18, 72%) were directly transplanted following AZA-RUX therapy. It is plausible that the OS in our cohort was adversely impacted by high rates of subsequent NRM (33% at 1-year and 56% at 2-years post ASCT). An overwhelming majority of ASCT (83%) in our cohort were performed in the 2014 – 2019 period. The field of ASCT in MF has evolved since then with the advent of better supportive care, development of fractionated busulfan based myeloablative conditioning regimens and post-transplant cyclophosphamide-based graft versus host disease prophylaxis. These advances have been associated with lower NRM and may translate into improved outcomes in more contemporary cohorts[30]. Although the median OS in our study may appear slightly lower than the reported median OS of 5.3 years in the RUX arm of the pooled analysis of COMFORT trials[32], it is difficult to draw any conclusions given inter-study differences in risk stratification scores, trial eligibility criteria, the length of follow-up (inclusion of period after study discontinuation in our survival analysis), and the likely impact of ASCT/subsequent NRM observed in our study (detailed ASCT data not reported in COMFORT[32]).

Our study also enrolled 7 patients with accelerated phase MF, a disease subgroup associated with aggressive biology, high rates of AML transformation and median OS of 1-2 years[22, 33]. Outcomes of these patients on our study compare similarly to the accelerated phase MF patients in the MPN-RC 109 trial[34]. The MPN-RC 109 phase 1 study evaluated the combination of decitabine and RUX in patients in MPN accelerated phase (n = 8) and blastic phase (≥20 blasts in the bone marrow/peripheral blood)[34]. RUX was administered at multiple dose levels, initially as monotherapy during an induction phase (for the

first 7 days of cycle 1), and decitabine 20mg/m^2 for 5 days was added starting day 8 in cycle 1, with the combination being administered from day 1 in subsequent cycles. The recommended phase 2 dose for RUX was 25 mg BID in the first cycle, and 10 mg BID in subsequent cycles. Baseline characteristics specifically for the accelerated phase patients were not reported, though in the full cohort, most frequent mutations included JAK-STAT pathway (86%), splicing factors (33%), *RUNX1* (24%), *TP53* (19%), and *TET2/IDH1/IDH2* (19%). Overall, patients with accelerated phase MPN had a response rate of 50% per the modified Cheson criteria[35], and median OS of 16 months. Similar to the above study, our accelerated phase cohort was enriched with adverse risk features, including Int-2/high risk DIPSS in 86%, hemoglobin <10g/dl in 71%, and HMR mutations in 50% of patients at enrollment, respectively. Notably, despite the fact that the median platelet count (271 x 10^9/L) was higher in these patients at baseline compared to other published datasets[33, 34] which could signify less aggressive disease with potentially more optimal administration of AZA-RUX, these patients were on study for only a short period of time (median of 6 months). None of the 7 patients could be directly bridged to ASCT. Two patients (29%, baseline blast counts of 18% and 15%) progressed to AML on study (after 0.9 and 2.6 months from study initiation, respectively), likely contributing to the overall shorter time on study as these patients were almost at the blastic phase prior to study initiation. Despite the overall short duration of treatment with AZA-RUX, the survival of accelerated phase patients in our study appears to be higher than reported in the MPN-RC 109 trial[34], with a median OS of 30.8 months. This should be interpreted with caution given the small number of patients and the use of subsequent salvage therapies and ASCT.

Long-term follow-up data did not demonstrate new safety signals with the AZA-RUX combination. While dosage reductions and temporary treatment interruptions were common, only 4 patients (7%) required permanent discontinuation of therapy due to toxicity.

Major limitations of our study include a single institution cohort and lack of a control arm. Furthermore, the starting dose of AZA used in the study (25 mg/m^2 x 5 days) is much lower compared to the standard doses used in myeloid neoplasms (75 mg/m^2 x 7 days)[36]. While up-titration of the AZA dose was allowed per protocol, increase to the full 75mg/m^2 dose was deemed necessary by the treating physician in only 13% of the chronic phase MF cohort. Although this dosing strategy was intentionally designed to mitigate early cytopenias and study discontinuation, it may have limited the therapeutic effect of AZA. Despite these limitations, our study demonstrates encouraging safety and signal for efficacy of the AZA-RUX combination in MF, in a cohort enriched with a higher proportion of adverse risk features more than what has been seen with most single agent RUX studies. In particular, we noted high response rates with durable responses for spleen and symptoms (median DoRs approximately 4 years for both), as well as improvement in anemia, along with *JAK2* allele burden and cytogenetic abnormalities in a smaller proportion of patients, indicating a deeper effect on the underlying disease biology with potential for disease modification. Overall, our findings support continued investigation of AZA-RUX and other novel HMA-JAKi combinatorial strategies, especially with oral HMAs to develop oral-oral future combinations, to

improve the deliverability of the regimen and hopefully the long-term outcomes in patients with high-risk MF.

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Table 1: Baseline characteristics of all patients, stratified by chronic and accelerated phase disease.

Parameters		**Full cohort (n=61)**	**Chronic Phase (n=54)**	**Accelerated Phase (n=7)**	**p-value**
		N (%), median [range]			
Age (years)	Age Age >65	66 [46 – 87] 32 (53)	65 [46 – 87] 26 (48)	72 [54 – 80] 6 (86)	0.10 0.11
Gender	Males	38 (62)	32 (59)	6 (86)	0.24
Race	White	52 (85)	46 (85)	6 (86)	1
Performance status	ECOG <2	59 (97)	52 (96)	7 (100)	1
Spleen size	Splenomegaly ≥ 5 cm	46 (75)	41 (76)	5 (71)	1
TSS[#]	TSS ≥ 12	49/59 (83)	44/52 (85)	5 (71)	0.34
Baseline blood/BM	Hb (g/dl) Hb<10 g/dl Transfusion dependent WBC (10^9/L) Platelet (10^9/L) LDH (IU) Peripheral blasts (%) BM blasts (%) BM blasts >5%	10.1 [7 – 16] 26 (43) 5 (8) 12.1 [2 – 61] 271 [63 – 1070] 1851 [257 – 4907] 1 [0 – 18] 2 [0 – 15] 14 (23)	10.6 [7 – 16] 21 (39) 2 (4) 12.2 [2 – 61] 271.5 [64 – 1070] 1821 [277 – 4907] 1 [0 – 9] 2 [0 – 9] 7 (13)	9.6 [8 – 12] 5 (71) 3 (43) 9.4 [4 – 36] 271 [63 – 835] 2160 [257 – 2913] 10 [0 – 18] 11 [6 – 15] 7 (100)	0.23 0.13 **<0.01** 1 0.75 0.86 **<0.01** **<0.01** **<0.01**
EUMNET fibrosis grade	MF-1 MF-2 MF-3	5 (8) 27 (44) 29 (48)	5 (9) 24 (44) 25 (46)	0 (0) 3 (43) 4 (57)	1
Cytogenetics*	Diploid Complex Others	33/59 (56) 3/59 (5) 23/59 (39)	30/53 (57) 2/53 (4) 21/53 (40)	3/6 (50) 1/6 (17) 2/6 (33)	0.33
Mutational Profile	***JAK2*** ***CALR*** ***MPL*** Triple Negative High molecular risk** *ASXL1* *EZH2* *IDH1* *IDH2* *RAS* *TET2* *TP53*	35 (57) 8/27 (30) 6/45 (13) 3 (5) 15/42 (36) 11/41 (27) 2/45 (4) 1/45 (2) 2/45 (4) 2/45 (4) 9/41 (22) 4/45 (9)	30 (56) 7/23 (30) 5/39 (13) 3 (6) 12/36 (33) 8/35 (23) 2/39 (5) 1/39 (3) 2/39 (5) 1/39 (3) 8/35 (23) 3/39 (8)	5 (71) 1/4 (25) 1/6 (17) 0 (0) 3/6 (50) 3/6 (50) 0/6 (0) 0/6 (0) 0/6 (0) 1/6 (17) 1/6 (17) 1/6 (17)	0.69 1 1 1 0.65 0.32 1 1 1 0.25 1 0.45
DIPSS	Int-1 Int-2 High	23 (38) 25 (41) 13 (21)	22 (41) 23 (43) 9 (17)	1 (14) 2 (29) 4 (57)	0.09

Abbreviations: ECOG: Eastern Cooperative Oncology Group; EUMNET: European Myelofibrosis Network; TSS: Total Symptom Score; DIPSS: Dynamic International Prognostic Scoring System;
#2 patients did not have baseline TSS available
*2 patients did not have adequate BM specimen for cytogenetics evaluation
**High molecular risk: includes *ASXL1*, *EZH2*, *IDH1*/*IDH2* mutations (*SRSF2* and *U2AF1* were not included in this category since baseline testing for these was performed in only 25% of the patients).

Table 2: Outcomes in the full cohort; A: Response parameters B: Resistant disease/AML transformation on study

A) Response Parameters				
Response type	**Eligibility criteria (at baseline)**	**Full cohort (n=61)**	**Chronic Phase (n=54)**	**Accelerated Phase (n=7)**
		N(%)		
Any response	All patients	45 (74)	42 (78)	3 (43)
CI Spleen	Spleen length ≥ 5 cm	28/46 (61)	27/41 (66)	1/5 (20)
CI TSS50	TSS score ≥ 12	33/49 (67)	30/44 (68)	3/5 (60)
CI Anemia	Hb < 10 g/dl	3/26 (12)	3/21 (14)	0/5 (0)
PR (JAK2)	JAK2 VAF≥20%	4/30 (15)	4/25 (16)	0/5 (0)
Cytogenetic CR	Abnormal karyotype	2/26 (8)	2/23 (9)	0/3 (0)
B) Resistant disease/AML transformation on study				
No objective response	All patients	16 (26)	12 (22)	4 (57)
AML on study	All patients	6 (10)	4 (7)	2 (29)

Abbreviations: CI: Clinical improvement; TSS: Total symptom score; Hb: Hemoglobin; PR: Partial response; VAF: Variant allelic frequency; CR: complete response; Acute myeloid leukemia

Table 3: All possibly treatment-related adverse events in the full cohort (n=61)

	Toxicities	**Any grade, n (%)**	**Grade 3-4*, n (%)**
Hematologic	Anemia	26 (43)	21 (34)
	Thrombocytopenia	31 (51)	18 (30)
	Leukopenia/neutropenia	15 (25)	9 (15)
	Leukocytosis	1 (2)	0 (0)
Gastrointestinal	Constipation	14 (23)	0 (0)
	Elevated ALT/AST	3 (5)	0 (0)
	Nausea/Vomiting	3 (5)	0 (0)
	Abdominal pain	2 (3)	0 (0)
	Diarrhea	2 (3)	0 (0)
	Elevated alkaline phosphatase	1 (2)	0 (0)
	Hyperbilirubinemia	1 (2)	0 (0)
	Bloating	1 (2)	0 (0)
	Mucositis	1 (2)	0 (0)
Skin disorders	Injection site reaction	5 (8)	1 (2)
	Cutaneous SCC	2 (3)	1 (2)
	Skin rash/dry skin/itching	3 (5)	0 (0)
Miscellaneous	Fatigue	6 (10)	3 (5)
	Weight gain	6 (10)	3 (5)
	Bruising	3 (5)	0 (0)
	Insomnia	2 (3)	0 (0)
	Dizziness	1 (2)	0 (0)
	Elevated creatinine	1 (2)	0 (0)
	Headache	1 (2)	0 (0)
	Night sweats	1 (2)	0 (0)
	Weight loss	1 (2)	0 (0)

Abbreviations: ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, SCC: Squamous cell carcinoma

*No grade 5 possibly treatment related adverse events were observed

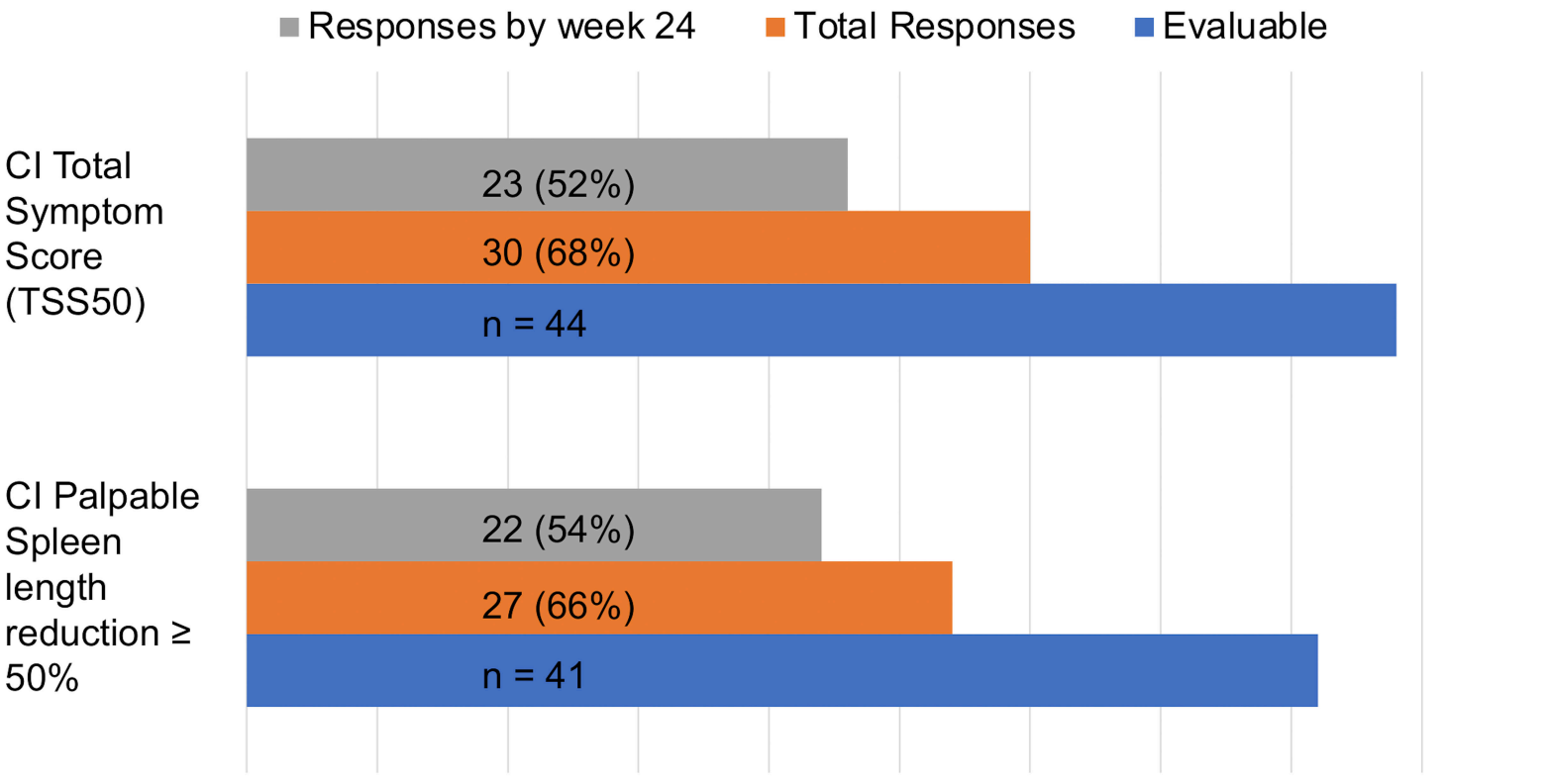
Figure Legends

Figure 1: Spleen and symptom responses in chronic phase MF cohort (n=54)

Figure 2: Duration of Response in chronic phase cohort; A) any response, B) CI TSS50 only, C) CI spleen only

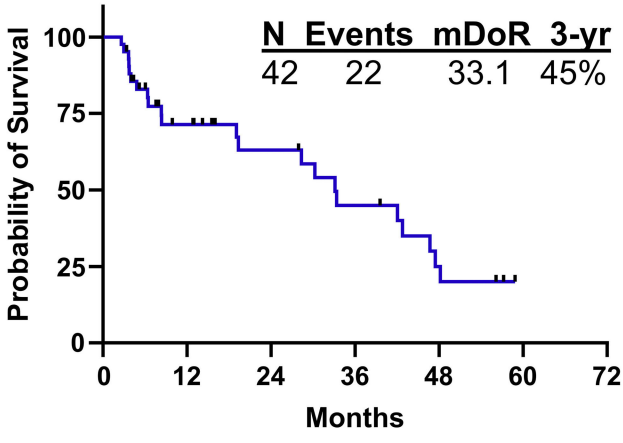
Figure 3: Overall Survival; A) chronic phase cohort, B) accelerated phase cohort

Figure 1



Only patients with baseline spleen length ≥5 cm and TSS ≥12 were evaluable for CI spleen and CI TSS50 responses respectively.

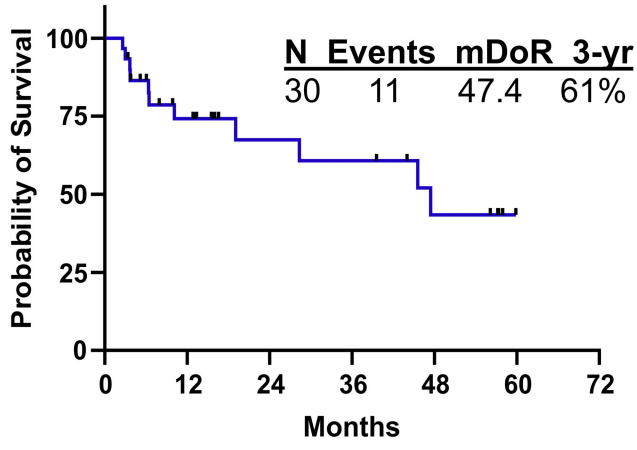
2A



Number at risk

DoR	42	23	15	10	5	0	0
	0	12	24	36	48	60	72

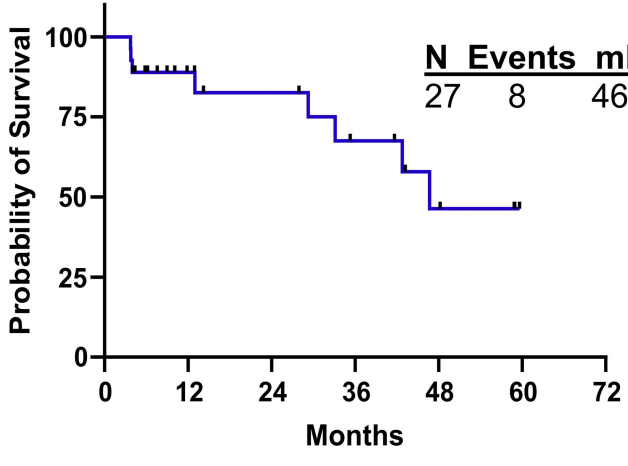
2B



Number at risk

DoR TSS50	30	17	10	9	5	0	0
	0	12	24	36	48	60	72

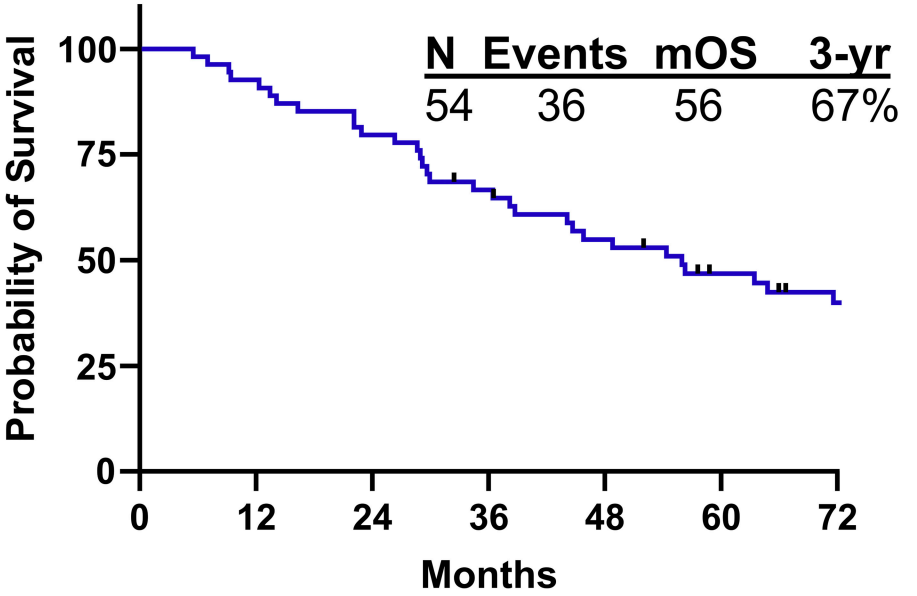
2C



Number at risk

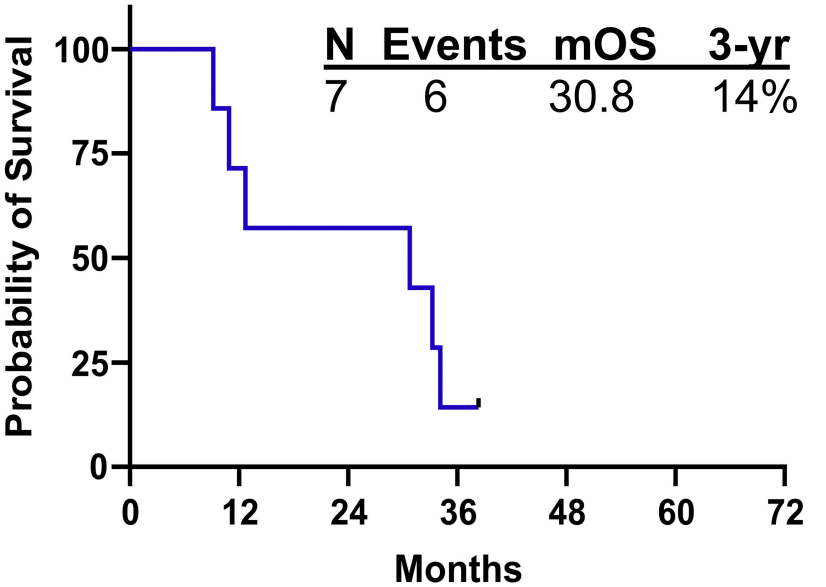
DoR Spleen	27	15	12	8	4	0	0
	0	12	24	36	48	60	72

3A



	Number at risk						
OS, chronic phase	54	50	43	35	28	21	16
	0	12	24	36	48	60	72

3B



	Number at risk						
OS, Accelerated Phase	7	5	4	1	0	0	0
	0	12	24	36	48	60	72

Supplemental Data:

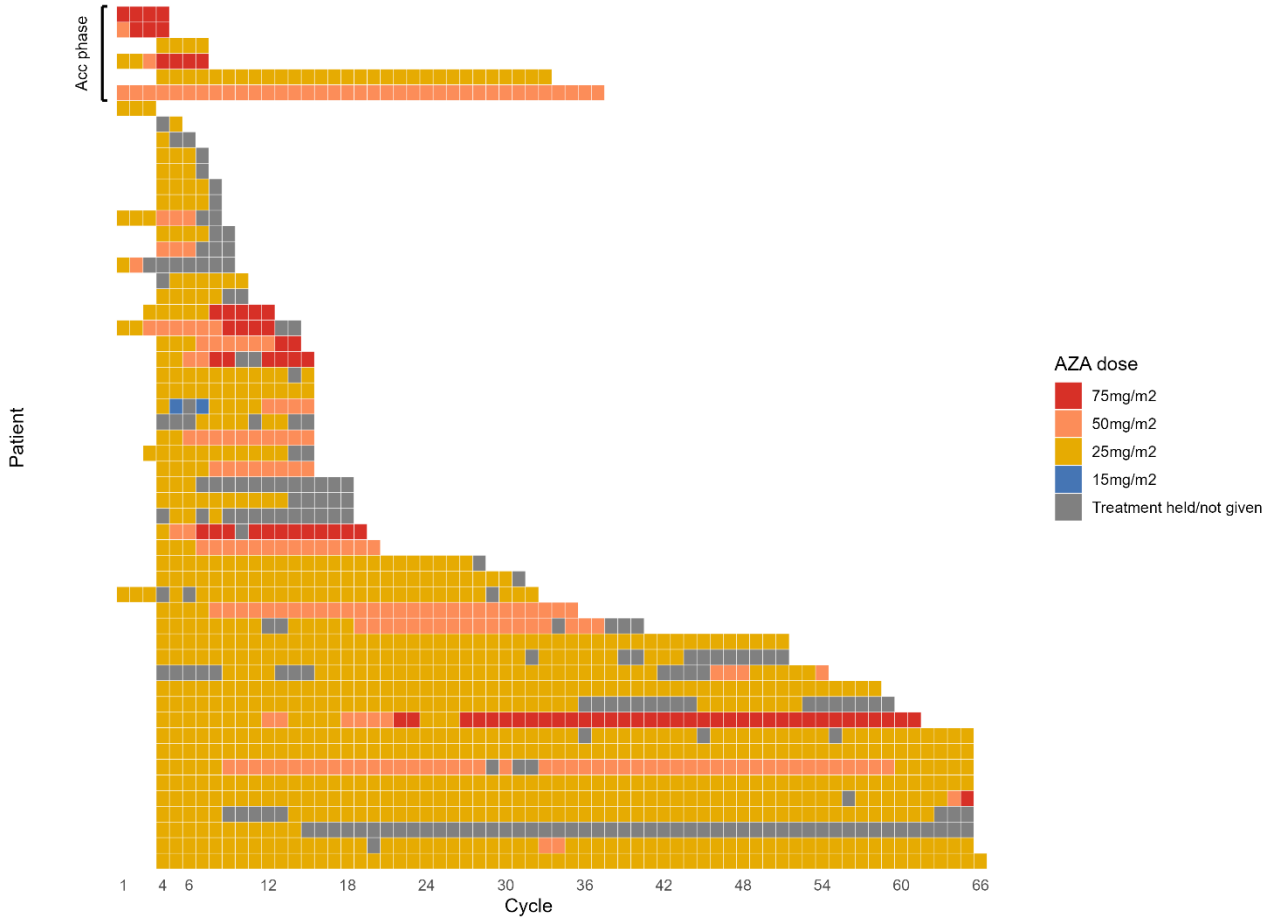
Final Results of a Phase 2 Study of the Combination of Azacitidine and Ruxolitinib in Patients with Myelofibrosis

Supplement Figures: 9

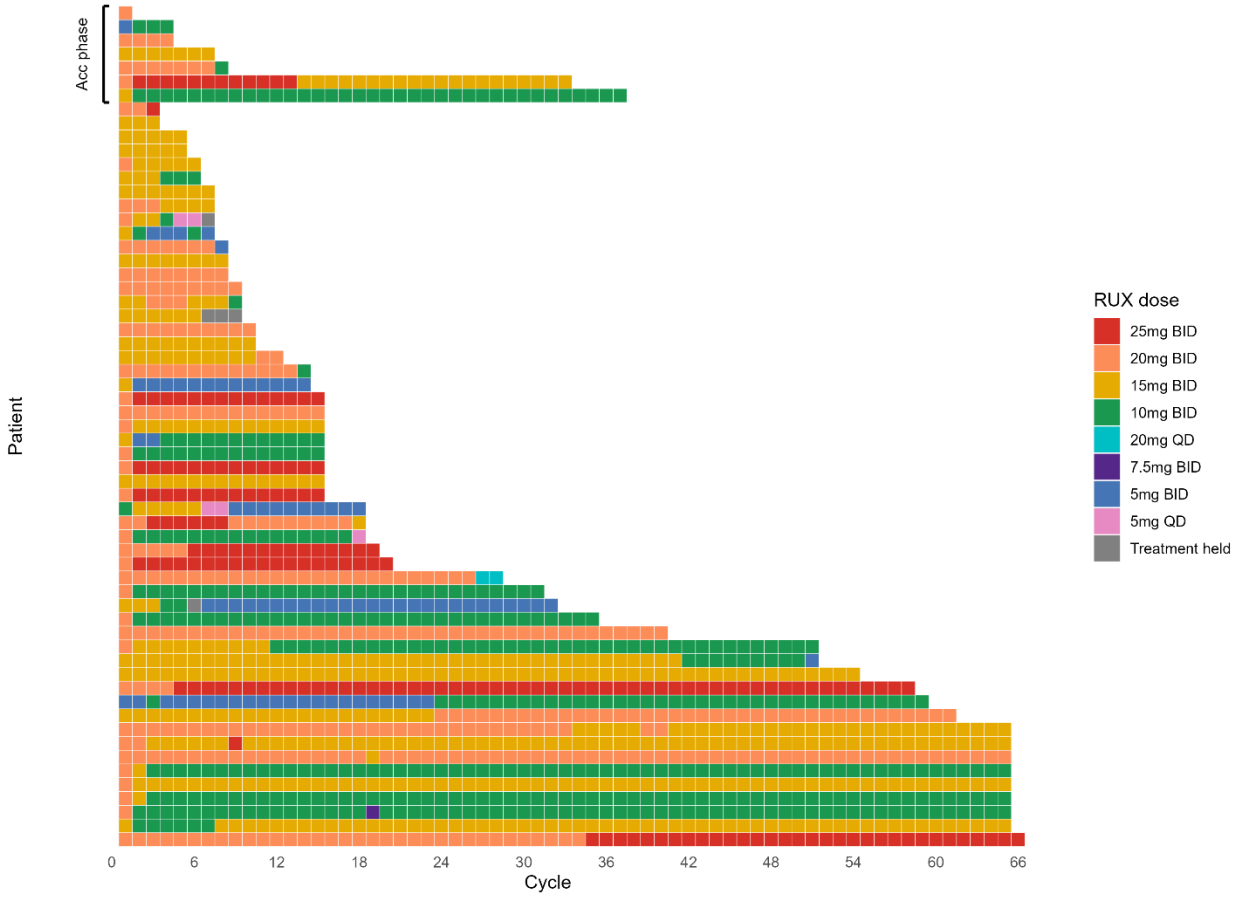
Supplement Tables: 4

Suppl. Figure 1: Heat map of different dose levels of Azacitidine and Ruxolitinib administered in the study.

1A: Azacitidine dose levels on study (n=55): including 6 patients in accelerated phase, and 49 patients in chronic phase (Six patients never received azacitidine on study). A total of 11 patients (4 in accelerated phase, 7 in chronic phase) received it prior to cycle 4.

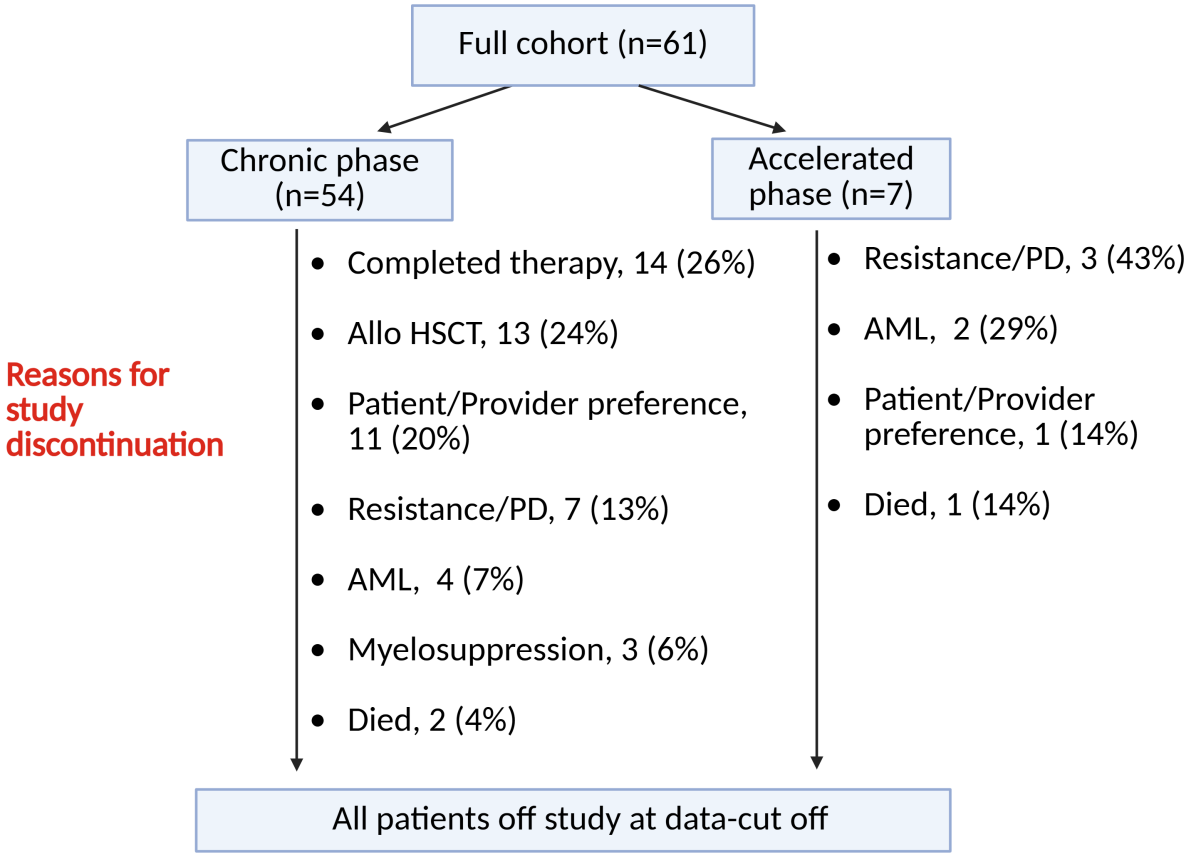


1B: Ruxolitinib dose levels on study (n=61): including 7 patients in accelerated phase, 54 patients in chronic phase.

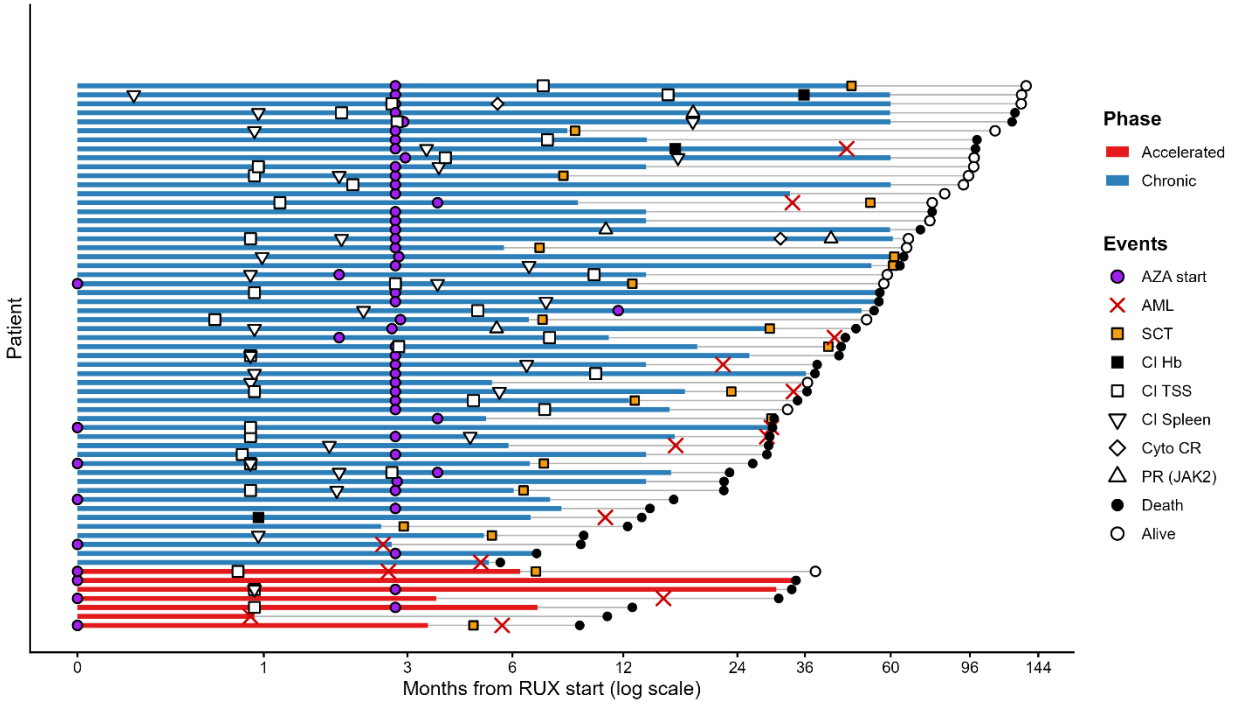


Suppl. Figure 2: Study consort diagram

(Abbreviations: - AML: Acute myeloid leukemia, Allo HSCT: allogenic hematopoietic stem cell transplantation, PD: progressive disease)

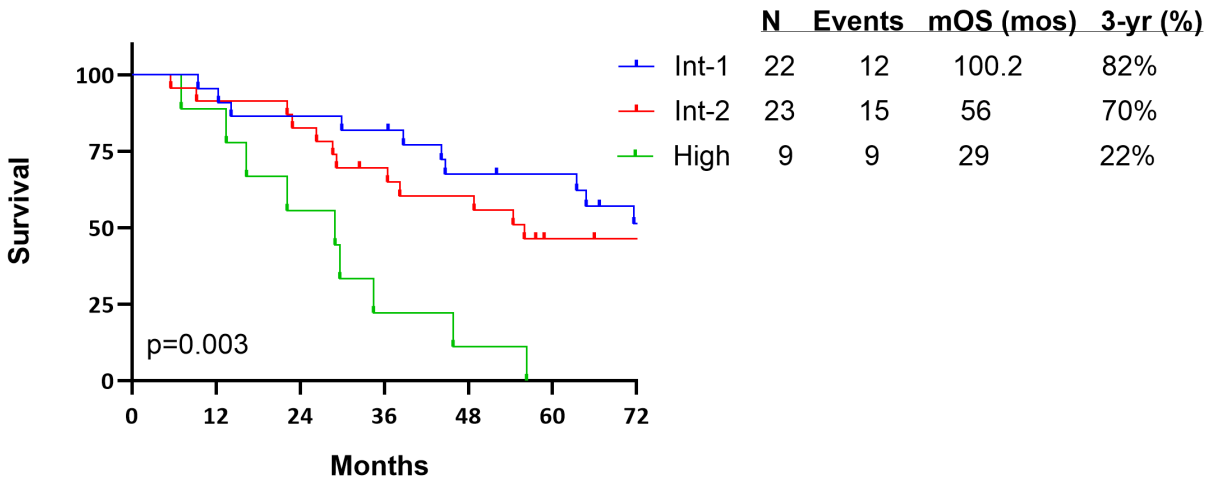


Suppl. Figure 3: Swimmer plot detailing the disease course of all study patients from the time of Ruxolitinib initiation till last follow up/death. Each horizontal bar represents an individual patient. Symbols denote key clinical events including azacitidine initiation, objective responses, AML transformation, and allogeneic stem cell transplantation. Red bars represent patients in accelerated phase at the time of trial enrollment, whereas blue bars represent those in chronic phase. The transition from colored bars to the gray line denotes the off-study date.

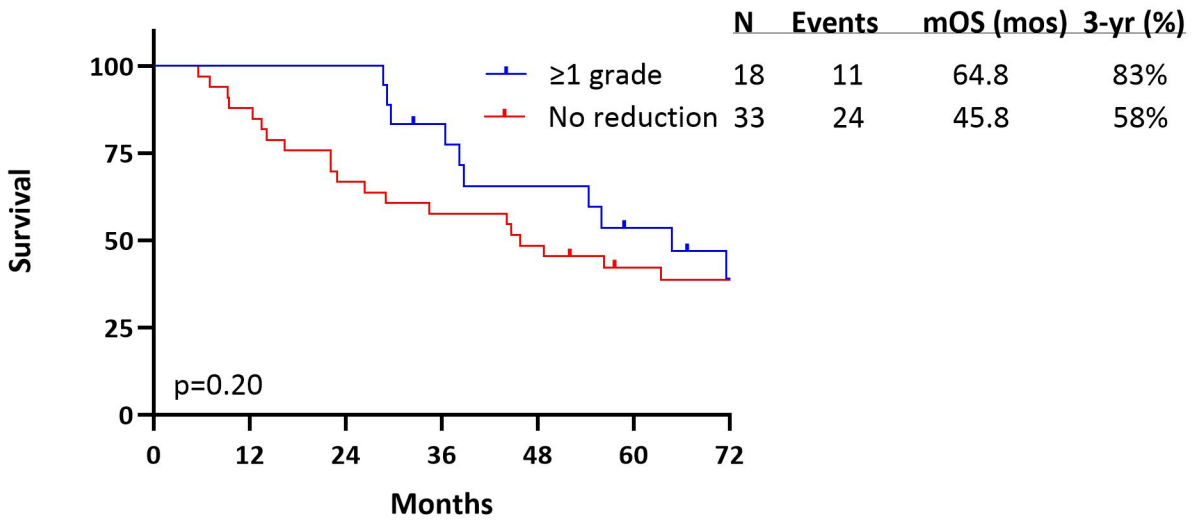


Suppl. Figure 4: Overall Survival of chronic phase patients stratified by baseline DIPSS score

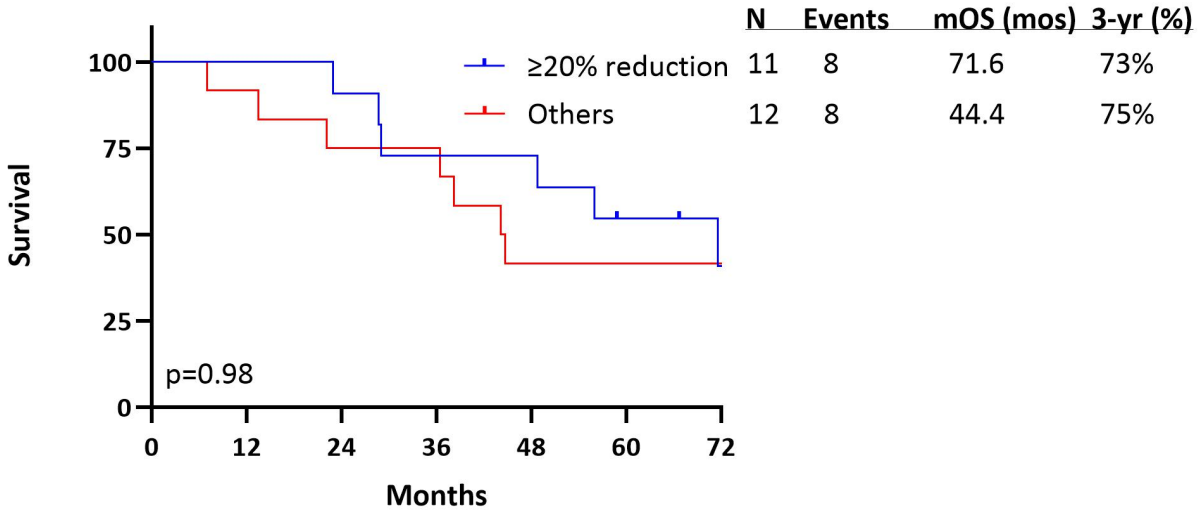
(p values – overall log rank for trend: p=0.003, Int-1 vs Int-2: p=0.46, Int-1 vs high: p<0.01, Int-2 vs high: p<0.01)



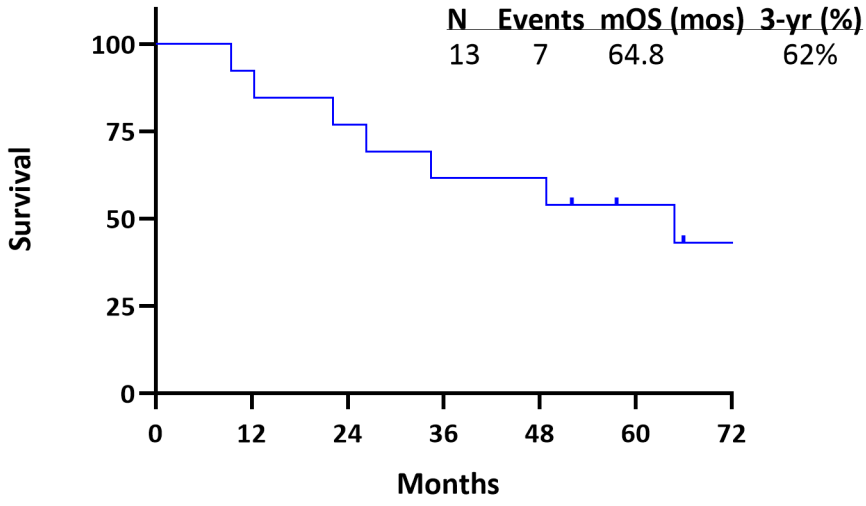
Suppl. Figure 5: Overall Survival of chronic phase patients stratified by ≥1 grade reduction in bone marrow fibrosis.



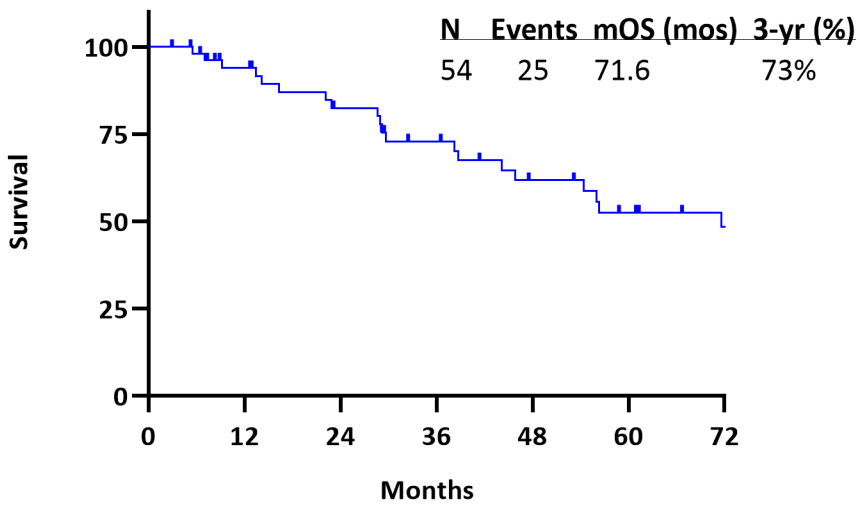
Suppl. Figure 6: Overall Survival of chronic phase patients stratified by ≥20% reduction in JAK2 variant allelic frequency.



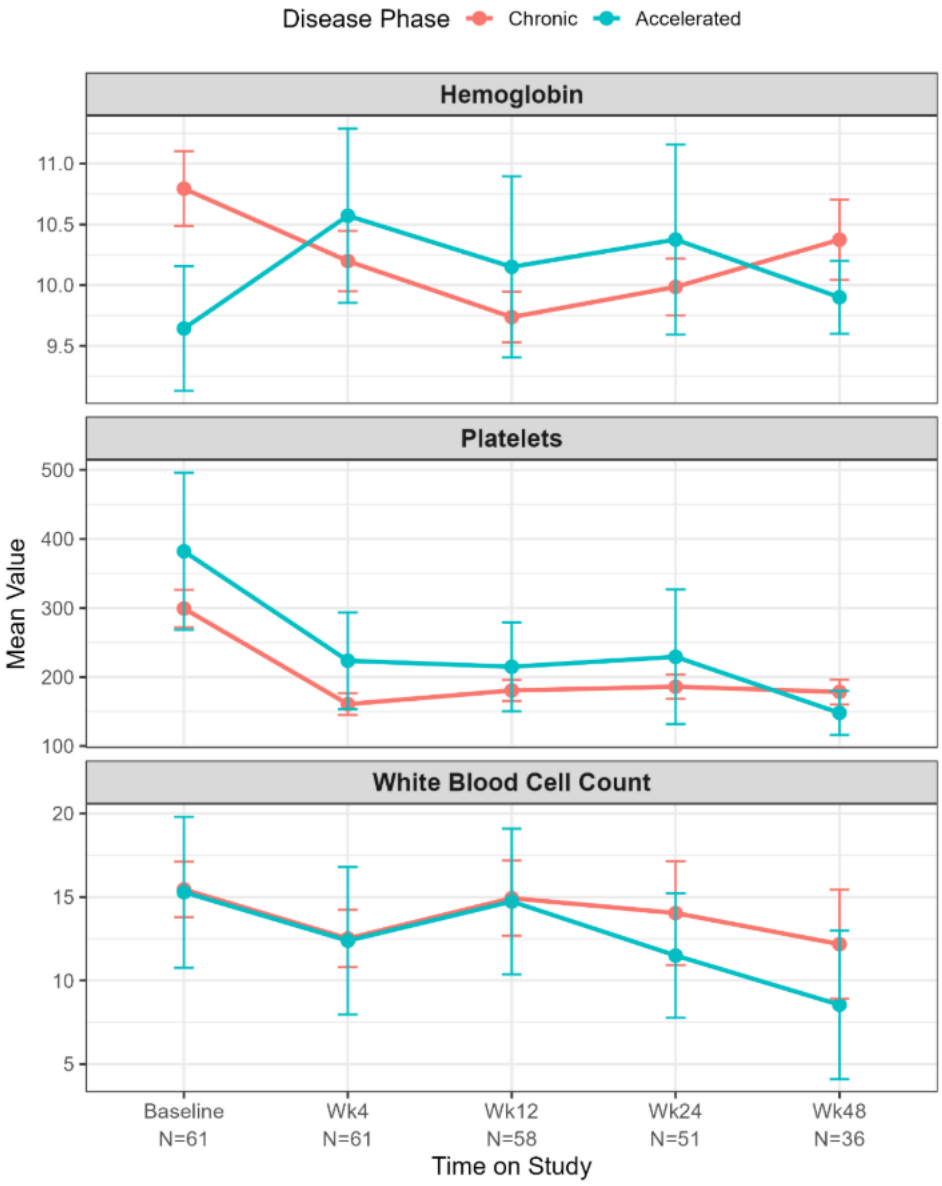
Suppl. Figure 7: Overall Survival of chronic phase patients who directly underwent allogeneic HSCT after coming off study (n=13)



Suppl. Figure 8: Overall survival of chronic phase patients upon censoring at allogeneic HSCT



Suppl. Figure 9: Graph showing trends in mean hemoglobin (g/dl), platelets (x 10^9/L) and white blood cell count (x 10^9/L) over time on study, stratified by chronic and accelerated phase (error bars represent standard deviations, N below figure represents number of patients still on study at different time points).



Suppl. Table 1: Prior treatments for MPNs/MF in the full cohort (n=61)

Prior treatment	**Number of patients (%)**
Hydroxyurea	26 (43)
Anagrelide	13 (21)
Immunomodulatory drugs (IMiDs)	7 (11)
Decitabine + Gemtuzumab ozogamicin	1 (2%)
Investigational JAK inhibitor	1 (2%)
Sotatercept	1 (2%)
Duvelisib	1 (2%)

Suppl. Table 2: Causes of post ASCT mortality in the chronic phase MF cohort (n=11)

S. No	**Cause of death**	**Time from ASCT to death (months)**	**Non-Relapse Mortality?**
1.	Infection (Pneumonia)	0.5	Yes
2.	Cardiac Arrest (unclear etiology)	21.6	Yes
3.	Unknown (was in hospice due to uncontrolled atypical CIDP), in remission at last evaluation	19.7	Likely yes
4.	Cardiac Arrest (unclear etiology)	3.5	Yes
5.	Sepsis	3.3	Yes
6.	Pneumonia	9.4	Yes
7.	Unknown (died at outside institution), in remission at last evaluation	15.7	Likely yes
8.	Unknown (died at outside institution), had developed renal failure post ASCT requiring dialysis, no relapse at last evaluation.	4.1	Likely yes
9.	Transformation to acute myeloid leukemia	13.3	No
10.	Primary graft failure	2.5	Yes
11.	Sepsis	19	Yes
Abbreviations:- ASCT: Allogeneic stem cell transplantation, CIDP: chronic inflammatory demyelinating polyneuropathy			

Suppl. Table 3: All possibly treatment-related adverse events in the full study cohort (n=61)

	Toxicities	**Any grade n (%)**	**Grade 3-4* n (%)**
Hematologic	Anemia	26 (43)	21 (34)
	Thrombocytopenia	31 (51)	18 (30)
	Leukopenia/neutropenia	15 (25)	9 (15)
	Leukocytosis	1 (2)	0 (0)
Gastrointestinal	Constipation	14 (23)	0 (0)
	Elevated ALT/AST	3 (5)	0 (0)
	Nausea/Vomiting	3 (5)	0 (0)
	Abdominal pain	2 (3)	0 (0)
	Diarrhea	2 (3)	0 (0)
	Elevated alkaline phosphatase	1 (2)	0 (0)
	Hyperbilirubinemia	1 (2)	0 (0)
	Bloating	1 (2)	0 (0)
	Mucositis	1 (2)	0 (0)
Skin disorders	Injection site reaction	5 (8)	1 (2)
	Cutaneous SCC	2 (3)	1 (2)
	Skin rash/dry skin/itching	3 (5)	0 (0)
Miscellaneous	Fatigue	6 (10)	3 (5)
	Weight gain	6 (10)	3 (5)
	Bruising	3 (5)	0 (0)
	Insomnia	2 (3)	0 (0)
	Dizziness	1 (2)	0 (0)
	Elevated creatinine	1 (2)	0 (0)
	Headache	1 (2)	0 (0)
	Night sweats	1 (2)	0 (0)
	Weight loss	1 (2)	0 (0)

Abbreviations: ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, SCC: Squamous cell carcinoma

*No grade 5 possibly treatment related adverse events were observed

Suppl. Table 4: Adverse events leading to dose reductions and interruptions in study therapy (full cohort, n=61)

Adverse Event	**Number of patients (%)**
Dose Reductions (27, 44%)	
Anemia	10 (16%)
Thrombocytopenia	8 (13%)
Both anemia and thrombocytopenia	3 (5%)
Neutropenia	2 (3%)
Fatigue	2 (3%)
Anemia, thrombocytopenia, and neutropenia	1 (2%)
Elevated alanine transaminase	1 (2%)
Dose Interruptions (28, 46%)	
Infection	10 (16%)
Thrombocytopenia	7 (12%)
Anemia	3 (5%)
Neutropenia	2 (3%)
Fatigue	2 (3%)
Both neutropenia and thrombocytopenia	1 (2%)
Nausea/vomiting	1 (2%)
Cutaneous squamous cell carcinoma	1 (2%)
Fall	1 (2%)