Posttransplantation Cyclophosphamide-based Graft versus Host Disease Prophylaxis with Non-myeloablative Conditioning for Blood or Marrow Transplantation for Myelofibrosis

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Abstract

We describe outcomes with posttransplantation cyclophosphamide and non-myeloablative conditioning based allogeneic blood or marrow transplantation for myelofibrosis using matched or mismatched, family or unrelated donors. The conditioning regimen consisted of fludarabine, cyclophosphamide and total body irradiation. Forty-two patients, with a median age of 63 years, were included, of whom 19% had intermediate-1, 60% had intermediate-2, and 21% had high-risk DIPSS-plus disease, and 60% had atleast one high-risk somatic mutation. Over 90% patients engrafted neutrophils at a median of 19.5 days and 7% had graft failure. At 1 and 3-years, respectively, the overall survival was 65% and 60%, relapse-free survival was 65% and 31%, relapse was 5% and 40%, and non-relapse mortality was 30% and 30%. Acute graft versus host disease grade 3–4 was noted in 17% at 1 year and chronic graft versus host disease requiring

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systemic therapy in 12% patients. Spleen size 17 cm or prior splenectomy was associated with inferior relapse-free survival (HR 3.50, 95% CI 1.18–10.37, P=0.02) and higher relapse rate (SDHR not calculable, P=0.01). Age > 60 years (SDHR 0.26, 95% CI: 0.08–0.80, P=0.02) and peripheral blood graft (SDHR 0.34, 95% CI 0.11–0.99, P=0.05) was associated with lower risk of relapse. In our limited sample, the presence of a high-risk mutation was not statistically significantly associated with an inferior outcome although *ASXL1* was suggestive of inferior survival (SDHR 2.36. 95% CI 0.85–6.6, P=0.09). Overall, this approach shows comparable outcomes as previously reported and underscores the importance of spleen size in evaluation of transplant candidates.

Keywords

post-transplantation cyclophosphamide; myelofibrosis; non-myeloablative conditioning

Introduction

Growing evidence supports the use of high dose posttransplantation cyclophosphamide (PTCy) to prevent graft versus host disease (GVHD) following allogeneic blood or marrow transplantation (BMT)[1–6]. PTCy is not toxic to donor stem cells as these are likely quiescent and express high level of the detoxifying enzyme aldehyde dehydrogenase that confers resistance to cyclophosphamide[7–9]. Additionally, the specific timing of PTCy, preceding calcineurin inhibitors, selectively targets proliferating alloreactive T cells and preserves regulatory T cells[9,10]. Many reports have now shown that nonmyeloablative HLA-haploidentical donor BMT, with PTCy GVHD prophylaxis is associated with low rates of both acute and chronic GVHD[6,11,12]. Moreover, relapse rates and survival are similar to those seen in matched donor BMT. Accordingly, haploidentical donor BMT has become a standard approach for patients in need of BMT but lacking a matched donor. PTCy has also been shown to be effective GVHD prophylaxis for non-myeloablative BMT[1].

These studies, however, are not informative for the use of non-myeloablative conditioning or PTCy for GVHD prophylaxis in BMT for myelofibrosis, as these patients were not included in most of the above referenced studies. BMT remains the only potential cure in myelofibrosis. However, with a median age above 60, most affected patients are too old to be considered for myeloablative conditioning[13]. Hence, a non-myeloablative conditioning regimen allows for transplantation in patients with complex comorbidities resulting from the chronic myeloid malignancy or older age. We aim to address this gap in knowledge by describing clinical outcomes of patients who underwent non-myeloablative conditioning PTCy based BMT for myelofibrosis.

Materials and Methods

Patient selection and overall plan

All patients of age 18 years who underwent non-myeloablative BMT for myelofibrosis between January 1, 2010 and August 1, 2020 using PTCy based GVHD prophylaxis and nonmyeloablative conditioning regimen at the Sidney Kimmel Comprehensive Cancer

Center at Johns Hopkins University were included in this analysis. BMT using matched or mismatched related or unrelated donors were included. A diagnosis of myelofibrosis was made using WHO criteria[14]. Patients who had progressed to acute myeloid leukemia (> 20% blasts in the peripheral blood or bone marrow) at any time prior to BMT were not included. Clinical data were obtained by conducting a retrospective chart review. The study was approved by the Johns Hopkins Institutional Review Board. Primary outcomes of interest included overall survival (OS) and relapse free survival (RFS). Additional outcomes of interest were hematopoietic recovery following BMT, cumulative incidence of relapse, non-relapse mortality (NRM), and acute as well as chronic GVHD.

Treatment regimen and supportive care

The non-myeloablative conditioning regimen consisted of cyclophosphamide 14.5 mg/kg/day intravenously (IV) on days −6 and −5, fludarabine 30 mg/m^2/day IV on days −6 through −2 with total body irradiation of 200 or 400 cGy on day −1 delivered as single fraction[4,6]. On day 0, the donor marrow or peripheral blood graft was infused. Cell dose is capped at 10×10^6/kg for patients receiving peripheral blood grafts from haploidentical donors. GVHD prophylaxis included administration of PTCy 50 mg/kg IV on days +3 and +4 along with mesna, following which mycophenolate mofetil along with tacrolimus or sirolimus was started on day +5.

Mycophenolate mofetil was stopped at day +35 in all patients. In the absence of GVHD, tacrolimus or sirolimus was continued up to day +180 until around 2018 when our center determined that the duration could be shortened to 60 days[15,16]. Granulocyte colony stimulating factor was given starting day +5 until absolute neutrophil count was greater than 1×10^9/L for 3 days. Supportive care included antimicrobial prophylaxis against *Pneumocystis jirovecii*, *Candida albicans*, and herpes zoster/simplex infections, administered according to the institutional practice guidelines. Supportive care with anti-pyretics and intravenous fluids is also used for management, as appropriate, in patients who develop cytokine release syndrome. No prophylactic therapy is used to prevent cytokine release syndrome. Ultrasonography for spleen size assessment is now routinely done prior to BMT in these patients. In patients in whom imaging was not available, measurement recorded by physical exam was used for analysis and palpable spleen size was added to a baseline normal size of 11 cm.

Definitions

Dynamic International Prognostic Scoring System (DIPSS)-plus scoring was calculated as previously described[17]. High-risk cyogenetics were defined as +8, −7/7q-, i(17q), −5/5q-, 12p-, inv(3) or 11q23 rearrangement[17]. High-risk somatic mutations were pathogenic mutations in *ASXL1, EZH2, IDH1, IDH2, SRSF2, U2AF1 Q157, TP53* based on published data on myelofibrosis[18–20]. Mutation-enhanced international prognostic scoring system Version 2.0 (MIPSS70+ v2.0) was calculated using published data[21].

Neutrophil engraftment was defined as first of the 3 consecutive days when absolute neutrophil count was 0.5×10^9/L and platelet engraftment was first of the 3 consecutive days when platelet count was 20×10^9/L, without transfusion support. Complete donor

chimerism refers to > 95% donor chimerism. Graft failure was defined as failure to engraft with donor chimerism following BMT, without evidence of relapse[22]. NRM was defined as death from any cause in the absence of disease relapse. Acute GVHD was graded based on previously published standard criteria[23,24]. The initial date for time-to-event outcomes was date of BMT. OS was defined from date of transplantation to death from any causes, or censored at the last follow-up date for alive patients. Relapse was defined as recurrence of disease related cytopenias (identified in a setting of lack of donor chimerism or marrow findings and after ruling out infections or medications as the etiology of cytopenias), and/or reappearance of molecular or genetic markers of disease. The event of RFS included relapse and death, whichever occurred first. Alive patients who never relapsed were censored at the last of follow-up date for RFS.

Somatic mutations testing

Most patients underwent molecular mutation testing using 63-gene panel testing established by Johns Hopkins Molecular Pathology laboratory, certified by the Clinical Laboratory Improvement Amendments[25]. Variants were identified and filtered using institutional algorithm as previously published[26]. Testing was done on peripheral blood or bone marrow specimens during the disease course or from the pre-BMT sample from archived DNA per institutional protocol.

Statistical analysis

Patient characteristics were summarized via descriptive statistics including median (range) for continuous variables and number (percentage) for categorical variables. Kaplan-Meier method was applied for point estimators of OS and RFS. Cumulative incidence of NRM, GVHD, and relapse were estimated accounting for the corresponding competing events. When estimating the cumulative incidence of relapse, NRM was a competing event and vice versa. The competing events for estimating cumulative incidence of GVHD included graft failure and death event without GVHD/graft failure. Associations between risk factors and outcomes were assessed via Cox proportional hazards model for OS and RFS[27] and via Fine and Gray's proportional subdistribution hazards model for relapse and NRM[28]. Specifically for spleen size, the nonlinearity association with RFS was evaluated first by constructing restricted cubic spline function of spleen size with three knots placed at 25th, 50th, and 75th percentile. For convenience of interpretability, the spleen size was then categorized based on median estimate and the estimators of log relative hazards by spleen size. Due to limited sample size, the associations were conducted univariately. P-values were two-sided with significant threshold of 0.05 for hypothesis generating. All analyses were conducted in R version 3.6.0 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Patient and disease characteristics

Forty-two consecutive patients underwent BMT with non-myeloablative conditioning and PTCy for myelofibrosis at our center between January 1, 2010 and August 1, 2020. Patient, disease and BMT related details for these patients are shown in Table 1. Twenty-six (62%) were older than 60 years and 15 (36%) were 65 years of age at the time of BMT. JAK2

mutation was the most common driver mutations in 26 (62%) patients while *CALR* was the driver mutation in 9 (21%) patients, of which 5 (56%) were type 1 *CALR* mutation. Somatic mutation testing was obtained on bone marrow sample from pre-BMT marrow biopsy in 20 (48%), prior to initiation of treatment in 14 (33%) and during treatment in 8 (19%) patients. High-risk somatic mutations were identified in 25 (60%) patients and were dominated by the presence of *ASXL1* frameshift or nonsense mutations in 20 (48%) patients. Co-occurrence of somatic mutations, including the driver mutations, is shown in Figure 1.

Thirty-four (81%) patients received prior JAK inhibitor therapy, all of whom had received ruxolitinib while one had also received pacritinib and momelotinib on respective clinical trials. A hypomethylating agent was used along with ruxolitinib in 18 (43%) patients, for immunosuppression and promoting engraftment following BMT or for spleen size reduction as previously published in chronic phase myelofibrosis[29]. All patients had <5% blasts in the bone marrow by immunohistochemistry at the time of BMT. One patient had 16% blasts on the flow cytometry of the aspirate but was included in the analysis as aspirate was hemodiluted, blasts were <5% on immunohistochemistry on marrow and never reported to be >20%. The dose of total body irradiation was 200cGy in 19(45%) and 400cGy in 22(55%) patients.

Engraftment and chimerism

Table 2 summarizes clinical outcomes following BMT. Thirty-eight (91%) patients had engraftment of neutrophils at a median of 19.5 (IQ range 17, 26.75 days) and 32 (76%) patients had platelet engraftment at a median 31 days (IQ range, 23–44 days). Four (10%) patients died prior to neutrophil engraftment and 7 (17%) died without achieving platelet engraftment. Three (10%) additional patients had primary graft failure with subsequent restoration of autologous hematopoiesis and are still alive at last follow up (994, 2134, and 2337 days post-BMT, respectively) with chronic phase disease and improvement in symptoms. Two (67%) of these three had received a peripheral blood graft while one (33%) received a marrow graft. All three received 200cGy total body irradiation. Peripheral blood chimerism of both whole blood and T cell is depicted over time in Figure 2.

OS and RFS

Median follow-up, in this study, was 2.72 years (range, 14 days – 6.99 years) after BMT based on reversed Kaplan-Meier method. The probability of OS was 65% (95%CI: 52–82%) and 60% (95%CI: 46–79%) at 1-year and 3-years respectively, while RFS was 65% (95%CI: 52–82%) and 31% (95%CI: 17–56%), respectively (Figure 3A). In the univariate analysis, spleen size of   17 cm and those who had undergone a splenectomy had an inferior RFS (HR 3.50, 95% CI 1.15–10.65, *P*=0.03 for spleen size   17cm and HR 3.53, 95%CI 0.86 – 14.33, *P*=0.08).

NRM and relapse

In the entire cohort, the cumulative incidence of NRM was 17% (95%CI: 5–28%) at day +100 and 30% (95% CI: 15–44%) at 1 year. NRM in the cohort of age   60 years was 6% (1/16) and in age > 60 years was 42% (11/26). Univariate analysis demonstrated association of age > 60 years at BMT [subdistribution hazard (SDHR) 9.46, 95%CI: 1.37–65.16,

P=0.02] or increase in age per decade (SDHR 3.18, 95%CI: 1.05–9.62, *P*=0.04) with a worse NRM. All clinical outcomes for patients for age > 60 years at BMT, who composed a major proportion of the study, are depicted in Supplementary Figure 1 in comparison with the younger cohort (age   60 years).

The cumulative incidence of relapse was 5% (95%CI: 2–12%) at 1 year and 40% (95%CI: 20–60%) at 3 years (Figure 3B). In univariate analysis, age > 60 years (SDHR 0.26, 95%CI: 0.08–0.80, *P*=0.02) and peripheral blood graft (SDHR 0.34, 95%CI 0.11–0.99, *P*=0.05) were associated with lower risk of relapse; while spleen size   17 cm or prior splenectomy (HR not calculable, *P*=0.01) and *CALR* driver mutation (HR 5.41, 95%CI 1.68–17.47, *P*=0.005) were associated with higher risk of relapse. Cumulative incidence of relapse at 3-years in patients with spleen <17 cm was 0%,   17 cm was 40%, and in those with prior splenectomy was 80%. Other patient-, disease-, and BMT-related characteristics studied were not statistically significantly associated with these clinical outcomes.

The causes of the 16 deaths were relapse in 4 (25%), GVHD in 2 (12%), infection in 7 (44%) and organ toxicity in 1 (6%) patients. One patient had diffuse large B cell lymphoma before myelofibrosis which was in remission at the time of myelofibrosis diagnosis and at BMT. This patient had a relapse of lymphoma 364 days after BMT and died of relapsed lymphoma, while myelofibrosis remained in remission at the time of death. The cause of death is unknown in one patient. Infections were bacterial in one patient, fungal in 4 patients and viral in 2 patients. No patients who died of infection had a prior splenectomy and 6/7 had received prior ruxolitinib treatment.

Acute and chronic GVHD

Any grade (grades 1–4) acute GVHD was noted in 20 (48%) patients. Skin was the most common organ involved in 15/20 (75%) patients – skin alone in 7/16 (44%), skin and gastrointestinal tract in 4/16 (25%), skin and liver in 2/16 (13%), while all 3 organs were involved in 2/16 (13%). Isolated gastrointestinal tract was noted in 2/20 (10%) while liver the only organ in 1/20 (5%) patient. Both gastrointestinal tract and liver were involved in 2/20 (10%) patients. At day +100, the incidence of grade 2–4 acute GVHD was 12% (95%CI 2–22%) and grade 3–4 acute GVHD was 10% (95%CI 9–19%%), while at 1 year, grade 2–4 acute GVHD was 32% (95%CI 17–29%) and grade 3–4 acute GVHD was 17% (95%CI 5–29%) (Figure 3C). The cumulative incidence of chronic GVHD at 1 year was 20% (95%CI 7–34%) and at 3 years was 22% (95%CI 8–36%) (Figure 3D). Chronic GVHD requiring systemic therapy was noted in 5/42 (12%) patients.

There was no statistically significant difference in grade 3–4 acute GVHD or chronic GVHD between patients who received tacrolimus versus sirolimus. However, there was a suggestion of higher grade 2–4 acute GVHD in patients who received sirolimus (Supplementary Figure 2).

Spleen size and prior splenectomy

Of patients with intact spleen (n=36), the median spleen size was 16.75 cm (range, 11 – 28 cm). Spleen size was available by imaging (CT or ultrasound) in 27 out of 36 patients with an intact spleen. With spleen as a continuous variable, an increase in spleen size beyond 11

cm was linearly associated with an increased log relative hazard of RFS up to a spleen size of 17 cm (Supplementary Figure 3). With an increase in spleen size beyond 17 cm, the log relative hazard RFS remained relatively stable.

Five patients had a prior splenectomy due to absence of response in spleen size with JAK-inhibitors or advanced disease. Another patient had a splenectomy during surgical debulking for pseudomyxoma peritonei. Patients with a prior splenectomy (n=6) underwent BMT with marrow graft in 1/6 (17%) patients and peripheral blood graft in 5/6 (83%) patients. These 6 patients engrafted neutrophils at a median of 18 (range, 15–30) days and platelets at a median 30 (range, 16–47) days. All had full donor chimerism in both T cell fraction and unsorted samples at day +60. However, 4/6 (67%%) of these relapsed at days +244, +314, +428 and +667, respectively, and 3/6 (50%) have died at the time of last follow-up from relapse disease. Three (50%) are alive at days +244, +376 and +1146 from BMT, respectively.

Pre-BMT driver and non-driver somatic mutations

The type of driver mutation (*JAK2* vs *CALR* vs *MPL*/triple negative) was not statistically significantly associated with OS, RFS or NRM while *CALR* was associated with higher relapse (SDHR 5.41, 95%CI 1.68–17.47, *P*=0.005; Table 3). The presence of high-risk somatic mutations and a higher number of high-risk mutations was also evaluated with these clinical outcomes and no statistically significant association was noted (Table 3 and Supplementary Figure 4). Since *ASXL1* was the most prevalent high-risk mutation, we analyzed outcomes with *ASXL1* separately. While not statistically significant in this relatively small sample size, patients with *ASXL1* had an inferior OS (HR 2.36, 95%CI 0.85–6.60, *P*=0.09), RFS (HR 1.85, 95% CI 0.77–4.43, *P*=0.17), and NRM (HR 1.73, 95%CI 0.57–5.3, *P*=0.34; Table 3 and Figure 5). Relapse rates were statistically similar (HR 1.09, 95%CI 0.37–3.7, *P*=0.89). To integrate genomic and non-mutational risk factors, we further analyzed by MIPSS70+ version 2.0 at the time of BMT. Patients with low/intermediate MIPSS70+ version 2.0 did not have statistically different clinical outcomes compared to patients with high/very high MIPSS70+ version 2.0 score as depicted in Table 3 and Supplementary Figure 5.

Outcomes with peripheral blood graft and TBI 400cGy

Twenty three (55%) underwent BMT with our current standard regimen using 400cGy TBI along with fludarabine and cyclophosphamide as conditioning chemotherapy and peripheral blood grafts. The median follow up is 355 days (range, 33 –764 days) from BMT based on reversed Kaplan-Meier method. Nineteen (83%) of these were done using a haploidentical donor, 2 (9%) with matched unrelated donor BMT and 1 (4%) each with matched unrelated and mismatched unrelated donor. No graft failure occurred in this cohort. Although a smaller sample size and shorter follow-up than the entire group, 6-month estimate of OS was 69% (95%CI 53%–91%) and RFS was 69% (95%CI 53%–91%), as shown in Supplementary Figure 6. Three (13%) patients have relapsed so far and have died as a result of progressive disease. Five (22%) had grade 3–4 acute GVHD and 2 (9%) had developed chronic GVHD requiring systemic immunosuppression.

Discussion

While the role of BMT as a potentially curative option is well-established in myelofibrosis, the variability in BMT platforms could influence outcomes[30,31]. Our study adds to the growing data on the use of PTCy based regimens for GVHD prophylaxis, specifically using non-myeloablative conditioning in older patients with myelofibrosis. Clinical outcomes with this platform are at least comparable to other registry and institutional data, with acceptable levels of adverse events[32–36]. Registry data from Center for International Blood and Marrow Transplant Research reported BMT outcomes of 233 myelofibrosis patients using reduced intensity conditioning and calcineurin inhibitor based GVHD prophylaxis[32]. In this study, only 38% had intermediate-2 or high risk disease per DIPSS scoring and 88% received a peripheral blood graft. At 3 years, they reported an OS of 52%, RFS 32%, NRM 22%, and relapse 47%. GVHD was grade 2–4 in 37% and grade 3–4 in 19% patients by day +100 and chronic GVHD was seen in 42% patients at 1 year. Of note, patients were much younger at BMT in this study compared to our cohort, with a median age of 55 years and only 27% patients over the age of 60 years. For older patients, which constitutes a majority of patients in our study, an EBMT study in patients of age 65 years, predominantly receiving matched unrelated donor graft and busulfan based reduced intensity conditioning demonstrated a 5-year OS of 50%, NRM 37% and incidence of relapse 25%[35]. Studies demonstrating the role of reduced intensity conditioning using fludarabine-busulfan or fludarabine-melphalan, have shown lower rates of relapse at 9–29% but this was at the cost of a higher NRM of 30–40%[37,38].

As stands out in this study and others, relapse of primary disease continues to be a challenge in this patient population and also a major cause of death (around 40% at 3 years in our study), while NRM and GVHD have improved due to superior supportive care over the years. In a larger study with a longer follow up using Human Mortality Database, relapse was reported as a major cause of death in 41% myelofibrosis patients at 2–5 years and 61% patients over 5 years from BMT[39]. Persistence of transfusion dependence and molecular evidence of disease at day +100 have previously been identified as risk factors for poorer OS and RFS in patients with myelofibrosis[40]. Using this available information, prevention of relapse following BMT in myelofibrosis, especially in patients with these high-risk features at day +100, becomes an area of unmet need and studies directed to improve relapse rates are urgently needed. Based on an early suggestion of improved disease control with peripheral blood graft and better engraftment with higher dose of TBI, we recently changed our standard practice to include peripheral blood grafts and 400cGy radiation for all patients with myelofibrosis. While follow-up on this cohort is limited, the RFS appears encouraging.

Spleen size has long been considered a poor prognostic factor in myelofibrosis although the size cut-offs have never been defined. Additionally, spleen size is not commonly included in most risk stratifications for myelofibrosis including the recently developed clinical-molecular transplant scoring system for patients undergoing BMT[17,41,42]. An Italian study established a spleen size of > 22cm as a risk factor for inferior survival along with transfusion dependence and donor other than HLA-identical sibling[43]. In another analysis from CIBMTR, enlarged spleen size was associated with delayed engraftment compared to patients with normal spleen size, while OS was similar[44]. Data from EBMT has also

shown that palpable spleen size <5cm below costal margin had statistically significantly superior OS and NRM compared to patients with 5–14 cm and 15 cm while relapse rate was statistically similar[45]. In our study, an increase in spleen size especially up to the size of 17 cm was linearly associated with increased relapses and inferior RFS. Beyond a spleen size of 17 cm, the association of spleen size with outcomes tapered off. Splenectomy did not appear to improve these outcomes in our study, suggesting that spleen size may be a marker for advanced disease rather than an independent prognostic variable; hence, suggesting the need for inclusion in prediction models for BMT in this disease.

The understanding of the role of somatic genomic alterations, both driver and other somatic mutations, in myelofibrosis prognostication has markedly improved in recent years. Mutations in JAK-STAT pathway, including *JAK2, CALR* and *MPL*, are the hallmark of MPN pathogenesis. In preclinical models, additional mutations such as *TET2, EZH2, TP53* suggest features of progression[18,46,47]. In retrospective studies, mutations in *ASXL1, EZH2, SRSF2, IDH1, IDH2* and *U2AF1 Q157* are associated with inferior prognosis[19,20]. Such an impact of genomic alterations is not well established in the context of BMT as different studies have shown conflicting results. Furthermore, no studies have reported the impact of somatic mutations on BMT outcomes with the non-myeloablative PTCy platform. Kroger *et al* demonstrated an association of lower NRM and improved RFS in patients with *CALR* mutation while *ASXL1* and *IDH2* were associated with lower RFS[48]. Subsequently a study by Tamari *et al* showed no impact of *ASXL1* or *IDH2*; however, *U2AF1* and *DNMT3A* were associated with inferior OS[49]. In yet another study by Ali et al, *CBL* mutations were associated with lower OS and higher NRM while *ASXL1, IDH* or *CALR* mutations were not associated with inferior outcomes[50]. In our study with the PTCy platform, patients with high-risk non-driver somatic mutations, particularly *ASXL1*, appeared to have an inferior overall survival but this was not statistically significant in this limited sample size. In contrast to the study by Ali et al, we did not find a difference in outcomes by MIPSS70+ version 2.0 which is likely related to a different BMT platform and a smaller sample size in our study[50]. Taken together, it is evident from the above discrepant results that the impact of specific genomic alterations is not the same in the non-BMT and BMT contexts. Such an association of somatic mutations with BMT outcomes warrants a dedicated larger study addressing this specific question.

In our study, older age (> 60 years) was associated with higher NRM which is consistent with prior reports[35,39] but was also with lower relapse. The high SDHR (9.46) for NRM in patients with age > 60 years at BMT underscores the need for careful selection of older patients for BMT. At the same time, the wide 95% confidence interval (1.37–65.16) suggests the uncertainty of this estimate which is likely due to low number of NRM events in the cohort of age 60 years (1 NRM event in age 60 years). The lower relapse incidence in patients with age > 60 years is likely in part due to the higher NRM in this cohort. Such associations may reflect on some of the limitations of this retrospective study with a small sample size and limited number of events likely contributing to these effects. Another unexpected finding is higher relapse in patients with *CALR* mutation, which is likely related to the fact that around half of these were not the type 1 mutations which are associated with better prognosis[51]. Furthermore, 3 out of 5 patients with *CALR* type 1 had a concurrent *ASXL1* mutation which in turn is known to have a poorer prognosis. This is potentially

also a consequence of a limited sample size. Nevertheless, we describe the successful use of PTCy based BMT platform in myelofibrosis for the first time to our knowledge, with a suggestion that peripheral blood grafts and 400 cGy of TBI may be beneficial for engraftment. This data aligns with the growing interest in PTCy as GVHD prophylaxis in various disease, donor and conditioning regimen settings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

Abbreviations

BMT	Allogeneic blood or marrow transplantation
DIPSS	Dynamic international prognostic scoring system
GVHD	Graft versus host disease
HLA	Human leukocyte antigen
HR	hazard ratio
IV	intravenously
MIPSS70+ v2.0	Mutation-enhanced international prognostic scoring system Version 2.0
NRM	Non-relapse mortality
OS	Overall survival
PTCy	Post-transplantation cyclophosphamide
RFS	Relapse free survival
SDHR	Subdistribution hazard ratio

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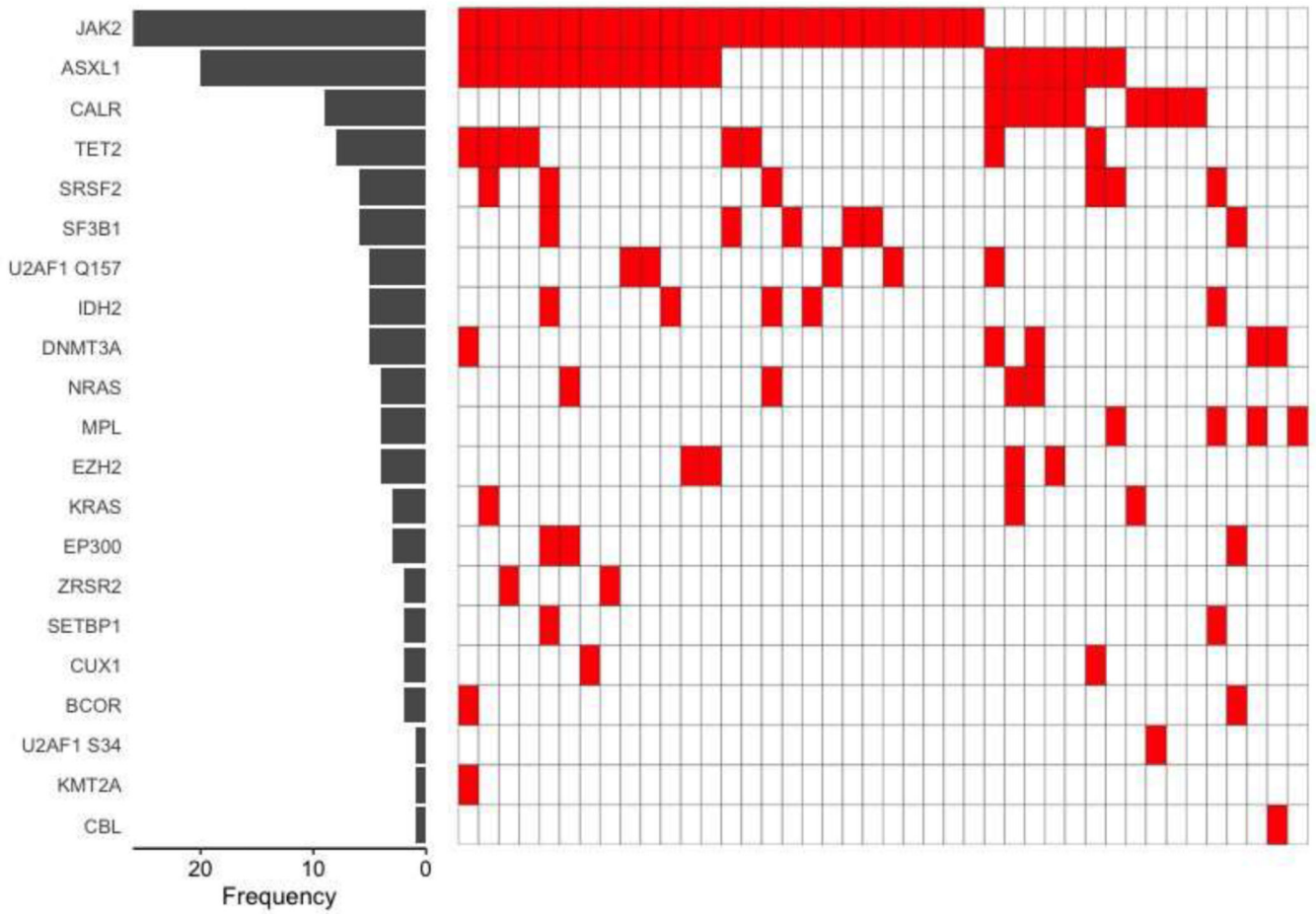
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Highlights

1. PTCy with non-myeloablative conditioning results in acceptable clinical outcomes with BMT in myelofibrosis

2. Older age had higher NRM, while increased spleen size or marrow graft had higher relapse

3. No graft failure has been noted with peripheral blood graft using 400cGy total body irradiation with fludarabine and cyclophosphamide

Figure 1:
Mutational landscape of all patients

A Chimerism T Donor

B Chimerism Unsorted

*thick horizontal bar denotes the median measurement for each time point.
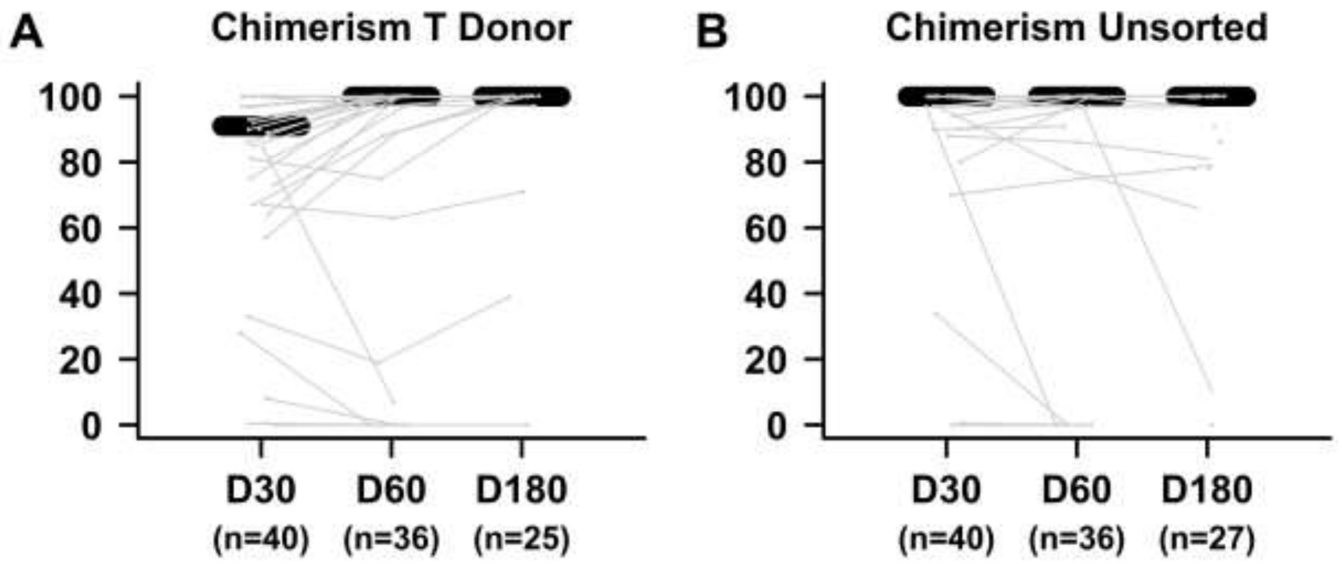
Figure 2:
Peripheral blood T cell and unsorted chimerism over time
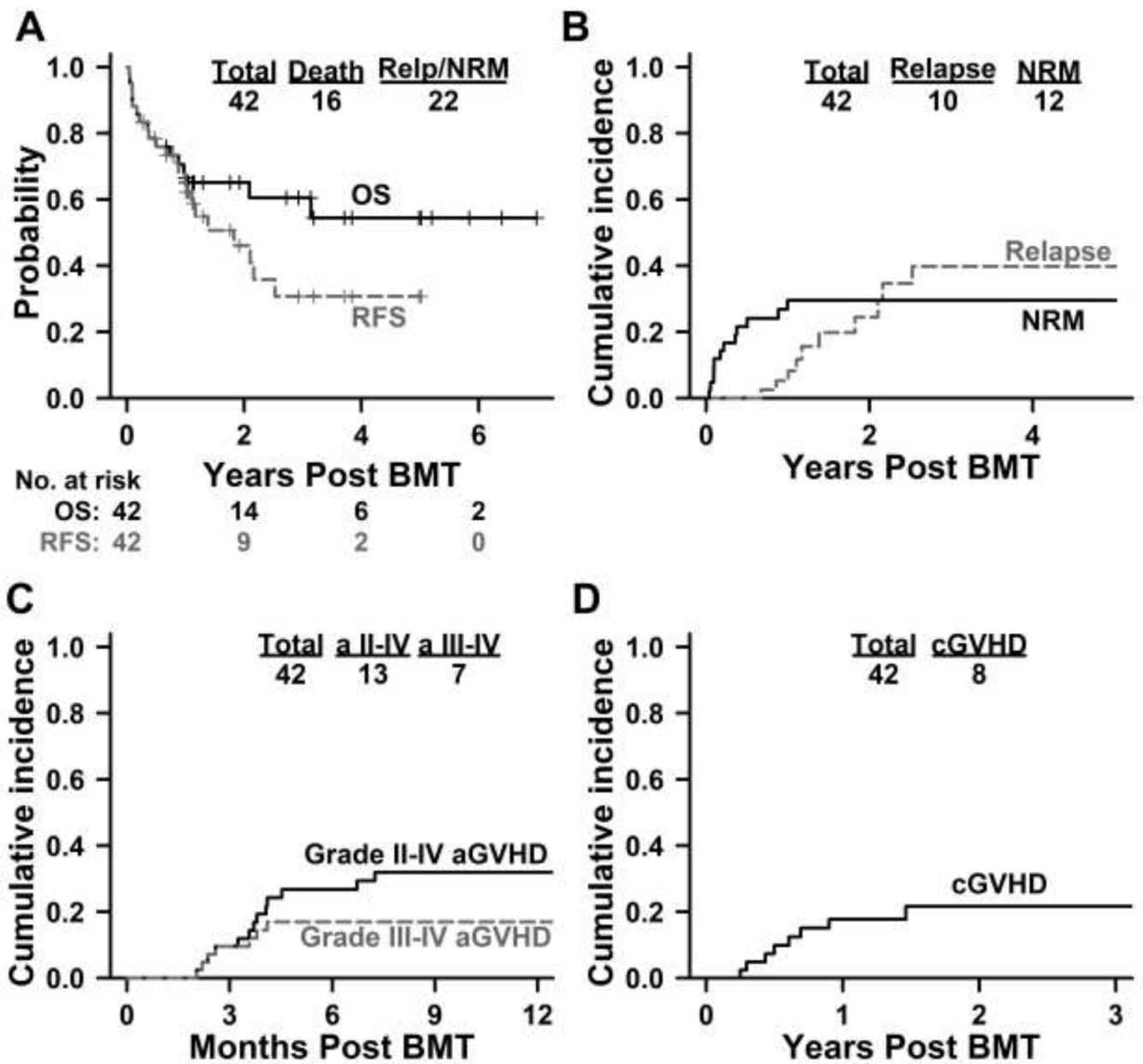
Figure 3:
Clinical outcomes with PTCy based non-myeloablative conditioning BMT in myelofibrosis
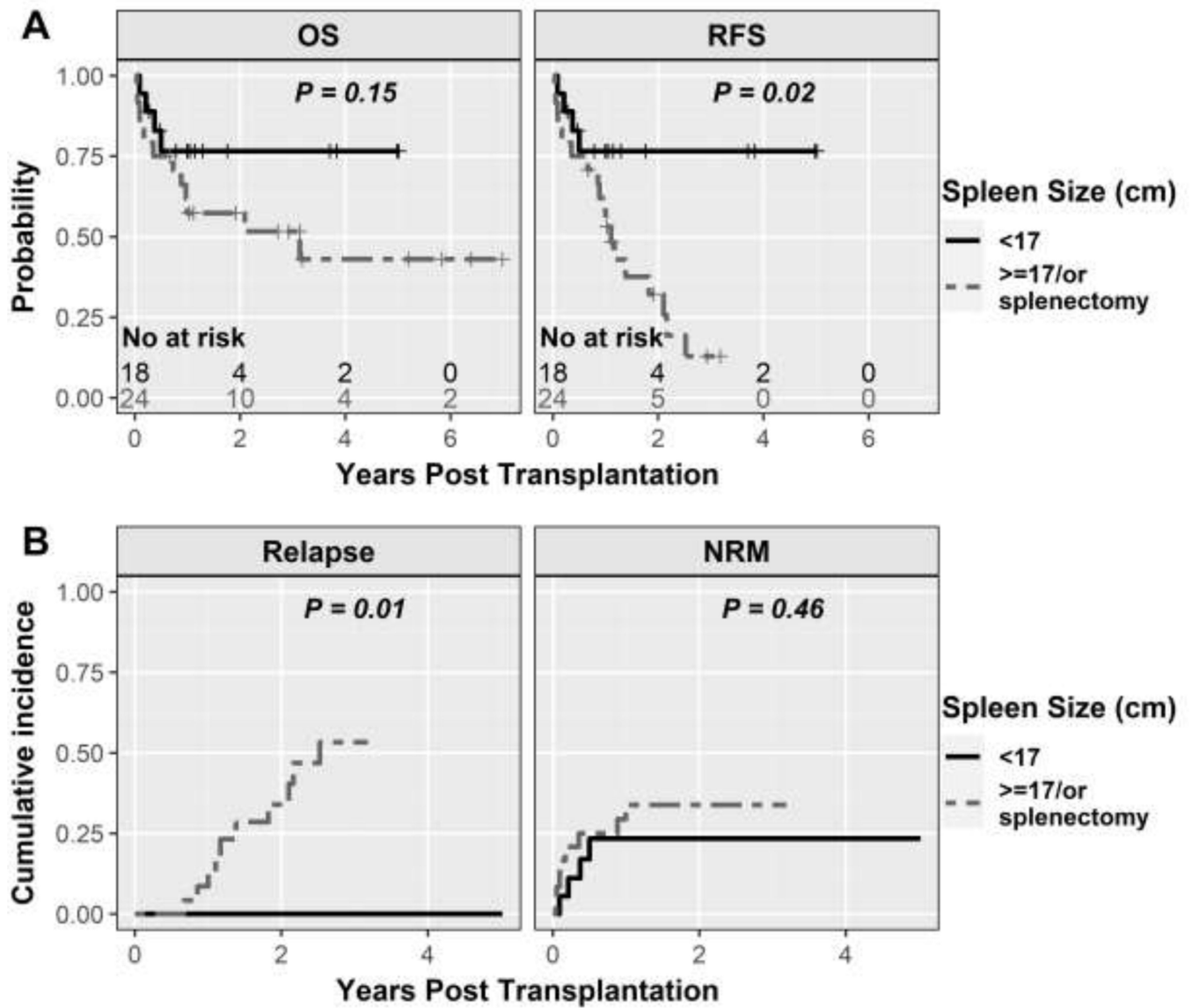
Figure 4:
Kaplan-Meier curves for clinical outcomes by spleen size category (11–16 cm versus ≥17 cm/splenectomy)
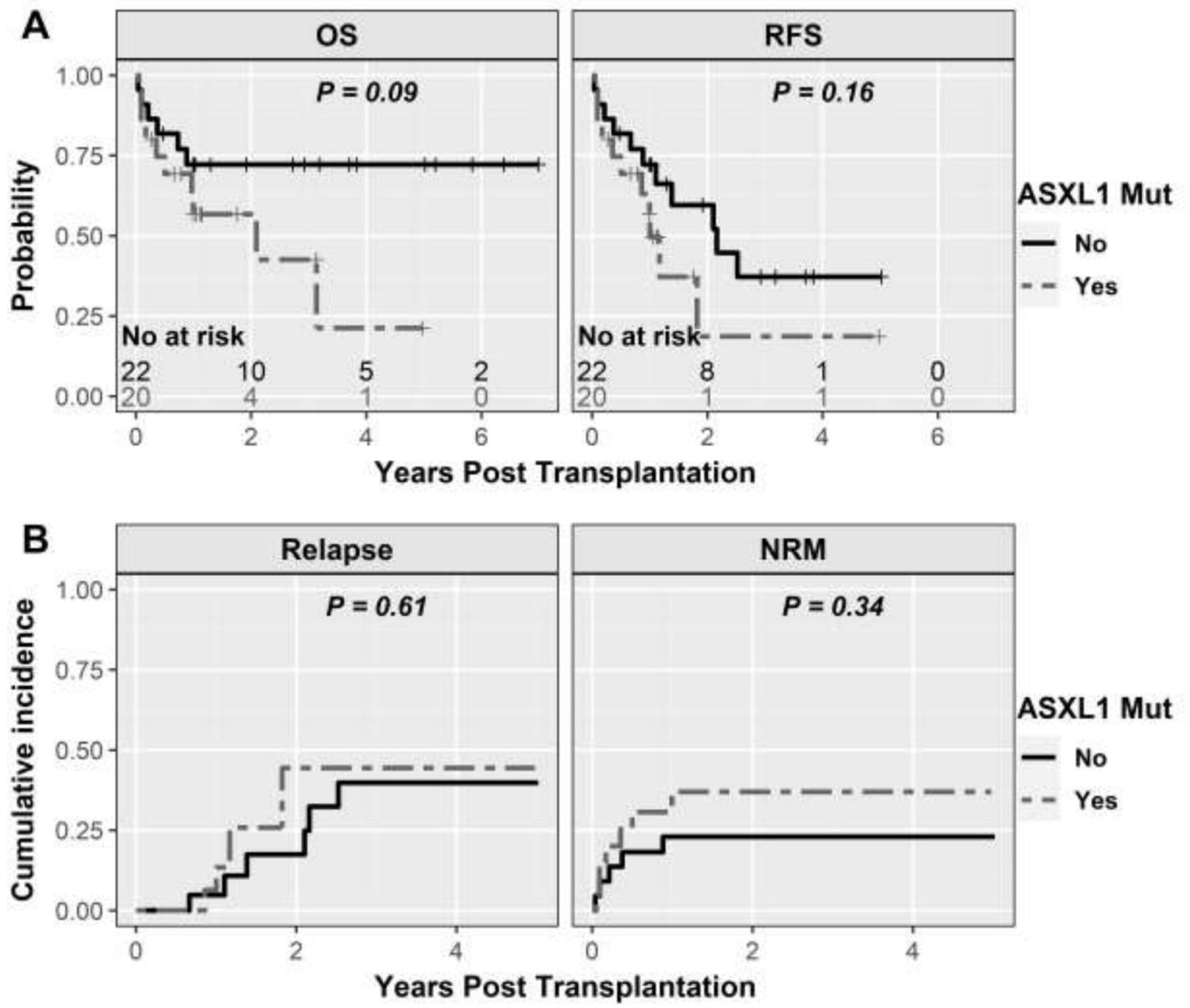
Figure 5:
Kaplan-Meier curves for clinical outcomes by presence of *ASXL1* mutation

Table 1:

Baseline patient and disease characteristics

Characteristic	N = 42
Age at BMT -median (range)	63 (32, 74)
Gender (%)	
Male	25 (60)
Female	17 (40)
BMT Year (%)	
2010–2016	14 (33)
2017–2019	28 (67)
HCT-CI (%)	
0–1	20 (48)
2	22 (52)
Diagnosis (%)	
Primary myelofibrosis	19 (45)
Post ET/PV	23 (55)
Median time (in months) from original MPN diagnosis to BMT (range)	78.8 (6.2 – 321.7)
Median time (in months) from myelofibrosis diagnosis to BMT (range)	39.8 (6.2 – 321.7)
Driver mutation (%)	
JAK2	26 (62)
CALR Type 1	5 (12)
Non-Type 1 *CALR*	4 (10)
MPL	4 (10)
Triple negative	3 (7)
DIPSS-Plus at BMT (%)	
Intermediate 1	8 (19)
Intermediate 2	25 (60)
High risk	9 (21)
MIPSS70+ v2.0 at BMT (%)	
Low	2 (5)
Intermediate	8 (19)
High	19 (45)
Very high	13 (31)
High-risk karyotype (%) [*]	7 (17)
High-risk somatic mutations [**]	25 (60)
Number of high-risk mutations:	(n=25)
One	9 (36)
Two	14 (56)
Three	2 (8)

Characteristic	N = 42
ASXL1 mutation present	20 (48)
Prior JAK inhibitor use (%)	34 (81)
Prior splenectomy (%)	6 (14)
Median spleen size at BMT in patients with intact spleen in cm, n=36 (range)	16.75 (11–28)
Donor platform (%)	
Haploidentical family	33 (79)
MRD/MUD	6 (14)
MMUD	3 (7)
Graft source (%)	
Bone marrow	7 (17)
PB	35 (83)
TBI (%)	
200 cGy	19 (45)
400 cGy	23 (55)
Median cell dose x 10^6/kg (range)	7.85 (3.2 – 14.6)
GVHD prophylaxis (%)	
Tacrolimus MMF PTCy	28 (67)
Sirolimus MMF PTCy	14 (33)
Recipient CMV serostatus (%)	
Negative	24 (57)
Positive	18 (43)

[*] High-risk karyotype includes complex karyotype or abnormalities including +8, −7/7q-, i(17q), −5/5q-, 12p-, inv(3) or 11q23 rearrangement.

[**] High-risk non-driver somatic mutations include pathogenic mutations in *ASXL1, EZH2, IDH1, IDH2, SRSF2, U2AF1 Q157, TP53*

(BMT, blood or marrow transplantation; CMV, cytomegalovirus; DIPSS, dynamic international prognostic scoring system; GVHD, graft versus host disease; HCT-CI, hematopoietic cell transplantation-comorbidity index; MMUD, mismatched unrelated donor; MRD, matched related donor; MIPSS, Mutation Enhanced International Prognostic Scoring System; MUD, matched unrelated donor; PB, peripheral blood; TBI, total body irradiation)

Table 2:

Summary of clinical outcomes

Outcomes	N = 42
Median days to neutrophil engraftment, n= 38 (IQR)	19.5 (17, 27)
Median days to platelet engraftment, n =32 (IQR)	31 (23, 44)
Graft failure (%)	3 (7)
Overall survival, probability (95% CI)	
1 year	0.65 (0.52 – 0.82)
3 year	0.60 (0.46 – 0.79)
Relapse free survival, probability (95% CI)	
1 year	0.65 (0.52 – 0.82)
3year	0.31 (0.17 – 0.56)
Cumulative incidence of NRM, probability (95% CI)	
Day +100	0.17 (0.05 – 0.28)
1 year	0.30 (0.15 – 0.44)
3 year	0.30 (0.15 – 0.44)
Cumulative incidence of relapse, probability (95% CI)	
1 year	0.05 (−0.02 – 0.12)
3 year	0.40 (0.20 – 0.60)
Cumulative incidence of acute GVHD grade 2–4, probability (95% CI)	
Day +100	0.12 (0.02 – 0.22)
1 year	0.32 (0.17 – 0.47)
Cumulative incidence of acute GVHD grade 3–4, probability (95% CI)	
Day +100	0.10 (0– 0.19)
1 year	0.17 (0.05 – 0.29)
Cumulative incidence of chronic GVHD, probability (95% CI)	
1 year	0.20 (0.07 – 0.34)
3 year	0.22 (0.08 – 0.36)

(CI, confidence interval; GVHD, graft versus host disease; IQR, interquartile range)

Table 3:

Univariate analysis for OS, RFS, NRM, and relapse

Variables	OS			RFS			NRM			Relapse		
	#evt/n	HR(95%CI)	p-value	#evt	HR(95%CI)	p-value	#evt	SDHR(95%CI)	p-value	#evt	SDHR(95%CI)	p-value
Age at BMT												
60	4/16			7			1			6		
> 60	12/26	2.58(0.83–8.04)	0.10	15	1.60(0.65–3.99)	0.31	11	9.46(1.37–65.16)	**0.02**	4	0.26(0.08–0.80)	**0.02**
Age increase per decade	16/42	1.31(0.67–2.55)	0.43	22	1.20(0.68–2.14)	0.53	12	3.18(1.05–9.62)	**0.04**	10	0.48(0.28–0.82)	0.007
Gender												
Male	10/25			14			6			8		
Female	6/17	0.98(0.35–2.69)	0.97	8	0.95(0.40–2.26)	0.90	6	1.55(0.52–4.68)	0.43	2	0.37(0.08–1.58)	0.18
BMT Year												
2010–2016	5/14			10			4			6		
2017–2020	11/28	1.36(0.46–4.05)	0.56	12	0.78(0.33–1.83)	0.57	8	0.98(0.30–3.18)	0.97	4	0.48(0.16–1.47)	0.20
Years from myelofibrosis to BMT	16/42	1.01(0.94–10.9)	0.70	22	1(0.94–1.07)	0.96	12	0.99(0.87–1.13)	0.92	10	1.02(0.95–1.09)	0.62
HCT-CI												
0–1	7/20			11			6			5		
2	9/22	1.30(0.48–3.50)	0.60	11	1.14(0.49–2.65)	0.76	6	0.96(0.32–2.87)	0.94	5	1.23(0.36–4.22)	0.74
Diagnosis												
Primary myelofibrosis	6/19			8			5			3		
Post ET/PV	10/23	1.57(0.57–4.33)	0.38	14	1.85(0.75–4.34)	0.19	7	1.31(0.43–3.98)	0.63	7	2.23(0.62–0.81)	0.22
Prior JAK inhibitor use												
No	1/8			2			1			1		
Yes	15/34	4.69(0.61–36.18)	0.14	20	3.87(0.88–16.98)	0.07	11	2.70(0.31–23.48)	0.37	9	3.01(0.35–26.10)	0.32
DIPSS-Plus at BMT												
Intermediate 1/2 risk	12/33			16			9			7		
High risk	4/9	1.25(0.40–3.90)	0.70	6	1.29(0.51–3.31)	0.59	3	1.25(0.35–4.45)	0.73	3	1.36(0.43–4.25)	0.60
MIPSS70 v2.0 at BMT												

Variables	OS #evt/n	OS HR(95%CI)	OS p-value	RFS #evt	RFS HR(95%CI)	RFS p-value	NRM #evt	NRM SDHR(95%CI)	NRM p-value	Relapse #evt	Relapse SDHR(95%CI)	Relapse p-value
Low/Intermediate	4/10			5			2			3		
High/Very high	12/32	1.10(0.35–3.42)	0.87	17	1.36(0.50–3.71)	0.54	10	1.76(0.40–7.83)	0.46	7	0.84(0.22–3.15)	0.79
Spleen size												
at BMT												
11–17cm	4/18			4			4			0		
>17cm/Prior splenectomy	12/24	2.24(0.72–6.96)	0.16	18	3.5(1.18–10.37)	**0.02**	8	1.56(0.49–4.99)	0.46	10	–	**0.01**
Driver mutation												
JAK2	10/26			13			9			4		
CALR	4/9	0.91(0.28–2.91)	0.87	6	1.28(0.48–3.43)	0.62	1	0.28(0.03–2.38)	0.24	5	5.41(1.68–17.47)	**0.005**
MPL/Triple negative	2/7	0.62(0.13–2.85)	0.54	3	0.74(0.21–2.61)	0.64	2	0.78(0.17–3.64)	0.76	1	0.85(0.09–7.92)	0.88
High-risk cytogenetics												
No	16/35			20			12			8		
Yes	0/7	Not evaluable		2	0.33(0.08–1.41)	0.14	0	Not evaluable		2	1.21(0.29–5)	0.80
High-risk somatic mutations												
No	5/17			9			4			5		
Yes	11/25	1.81(0.63–5.24)	0.27	13	1.42(0.60–3.36)	0.43	8	1.52(0.47–4.92)	0.49	5	0.93(0.29–3.02)	0.90
ASXL1												
Absent	6/22			11			5			6		
Present	10/20	2.36(0.85–6.6)	0.09	11	1.85(0.77–4.43)	0.17	7	1.73(0.57–5.3)	0.34	4	1.09(0.32–3.7)	0.89
Number of high-risk mutations												
0	5/17			9			4			5		
1	5/9	2.16(0.62–7.52)	0.23	6	1.5(0.53–4.21)	0.45	4	2.07(0.55–7.79)	0.28	2	0.73(0.14–3.7)	0.70
>=2	6/16	1.6(0.48–5.26)	0.44	7	1.35(0.49–3.72)	0.56	4	1.20(0.31–4.69)	0.79	3	1.14(0.3–4.41)	0.85
Donor platform												
Haploidentical family	13/33			19			10			9		
MRD/MUD	2/6	0.63(0.14–2.78)	0.54	2	0.39(0.09–1.69)	0.21	1	0.44(0.07–2.86)	0.39	1	0.50(0.06–4.41)	0.53

Variables	OS			RFS			NRM			Relapse		
	#evt/n	HR(95%CI)	p-value	#evt	HR(95%CI)	p-value	#evt	SDHR(95%CI)	p-value	#evt	SDHR(95%CI)	p-value
MMUD	1/3	0.62(0.08–4.78)	0.65	1	0.41(0.05–3.04)	0.38	1	0.86(0.16–4.60)	0.86	0	0(0–0)	–
Graft source												
Bone marrow	2/7			5			1			4		
PB	14/35	2.06(0.46–9.31)	0.35	17	0.92(0.34–2.50)	0.86	11	2.54(0.40–16.29)	0.33	6	0.34(0.11–0.99)	0.05
TBI												
200 cGy	6/19			12			5			7		
400 cGy	10/23	2.22(0.72–6.85)	0.17	10	1.46(0.57–3.78)	0.43	7	1.24(0.41–3.76)	0.71	3	0.78(0.24–2.53)	0.68

(BMT, blood or marrow transplantation; DIPSS, dynamic international prognostic scoring system; #evt, number of events; MIPSS, Mutation Enhanced International Prognostic Scoring System; MRD, matched related donor; MUD, matched unrelated donor; n, number of patients; NRM, non-relapse mortality; OS, overall survival; PB, peripheral blood; RFS, relapse free survival; TBI, total body irradiation)