Myeloproliferative Disorders-Research Consortium (MPD-RC)
MPD-RC Protocol 114
Exploring the Potential of Dual Kinase JAK 1/2 Inhibitor Ruxolitinib (INC424) with Reduced Intensity Allogeneic Hematopoietic Cell Transplantation in Patients with Myelofibrosis

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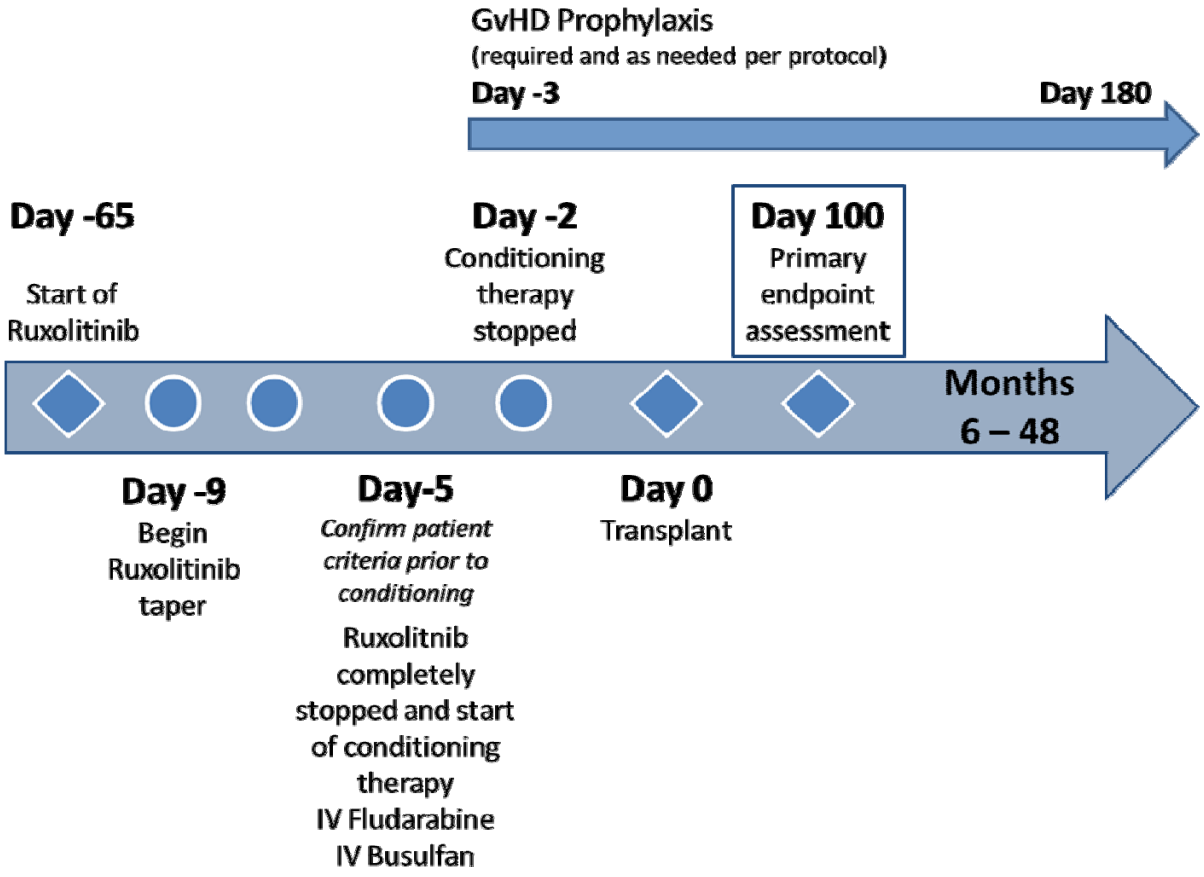
SCHEMA – TREATMENT PLAN



N.B. Schema not drawn to scale.

I. Ruxolitinib

Ruxolitinib (INC424) tablets will be started on day -65 prior to the planned infusion of stem cells. The starting dose of Ruxolitinib will be determined according to baseline platelet count (Table SCH- A Starting dose assignmentTable SCH- A) and will be modified according to platelet count (Table SCH- B) at follow-up. The drug will be given in the maximum tolerated dose as defined (Table SCH- B) in the protocol until day -10, followed by 4 days of tapering starting on day -9 (Table SCH- C). Ruxolitinib will be stopped completely on day -5, on the planned start day of conditioning therapy. The drug will be supplied as 5 mg tablets.

Table SCH- A Starting dose assignment

Baseline Platelet count	Starting dose of Ruxolitinib
>200 x 10^9/L	20 mg BID (Four 5 mg tablets twice daily)
100- 200 x 10^9/L	15 mg BID (three 5 mg tablets twice daily)
50-99 x 10^9/L	10 mg BID (two 5 mg tablets twice daily)

Platelet count will be monitored once a week, and if platelet count falls below <50 x 10^9/L, then platelet count will be monitored twice weekly. At follow-up, the dose of Ruxolitinib will be modified (increased or decreased) according to the Table SCH- B. The goal will be to continue the drug with a platelet transfusion if necessary. The drug should only be stopped if there is a concern regarding bleeding.

Table SCH- B Dose adjustment according to platelet count at follow-up (prior to start of conditioning therapy)

Platelet count at the follow-up	Dose at the time of follow-up			
	20 mg BID	15 mg BID	10 mg BID	5 mg BID
>200 x 10^9/L	No change	20 mg BID	15 mg BID	10 mg BID
100-200 x 10^9/L	15 mg BID	No change	15 mg BID	10 mg BID
50-99 x 10^9/L	10 mg BID	10 mg BID	No Change	10 mg BID
25-49 x 10^9/L	5 mg BID	5 mg BID	5 mg BID	No change
<25 x 10^9/L	5 mg BID + Platelet transfusion per investigator discretion	5 mg BID + Platelet transfusion per investigator discretion	5 mg BID + Platelet transfusion per investigator discretion	5 mg BID + Platelet transfusion per investigator discretion

Table SCH- C Tapering schedule of Ruxolitinib

Dose prior to day -9	Day -9	Day -8	Day -7	Day -6	Day -5
20 mg BID	15 mg BID	10 mg BID	5 mg BID	5 mg daily	None
15 mg BID	10 mg BID	10 mg BID	5 mg BID	5 mg daily	None
10 mg BID	10 mg BID	10 mg BID	5 mg BID	5 mg daily	None
5 mg BID	5 mg BID	5 mg BID	5 mg BID	5 mg daily	None

II. Conditioning therapy

Prior to start of conditioning, transplant physician should ensure that after completion of Ruxolitinib and prior to start of conditioning, patient meets the following criteria:

1. ECOG Performance status 0-2
2. Blasts in PB <20%
3. Adequate renal function with creatinine <1.5 x Institutional ULN
4. Adequate hepatic function with AST/ALT <2.5 x IULN and Bilirubin <1.5 x IULN
5. Absence of active uncontrolled infection or any other concurrent illness which in the Investigator's opinion puts the patient at excessive risk of transplant related toxicities.

The conditioning therapy will be:

IV Fludarabine 40 mg/m^2 IV over 30 minutes once daily x 4 days for days -5 to -2
IV Busulfan 2.0 mg/ KG of body weight IV over 2 hours once daily for 4 consecutive days for days -5 to -2.

If actual body weight is > 30% ideal body weight (IBW), use adjusted body weight (ABW) for calculating the dose of fludarabine and busulfan

Fludarabine and Antithymocyte Globulin (Rabbit) – will be dosed according to the patient's actual body weight, unless the patient weighs more than 130% of IBW, in which the dose will be based on an adjusted body weight.

IBW: Estimated ideal body weight in (kg)

Males: IBW = 50 kg + 2.3 kg for each inch over 5 feet.

Females: IBW = 45.5 kg + 2.3 kg for each inch over 5 feet.

ABW: Estimated adjusted body weight (kg)

If the actual body weight is greater than 30% of the calculated IBW, calculate the adjusted body weight

ABW = IBW + 0.4(actual weight - IBW)

III. GVHD prophylaxis

a. IV Calcineurin inhibitor [IV cyclosporine (per institutional practice) or IV tacrolimus (per institutional practice)] starting on day -2, until engraftment or when the patient is able to take PO, then switched to PO doses. Cyclosporine or tacrolimus will be given according to institutional practice. The doses should be adjusted to keep trough levels of cyclosporine between 200-400 µg/L or tacrolimus 8–12 ng/ml. Cyclosporine or tacrolimus will be continued through day 180 and subsequently in the absence of GvHD, tapered by 25% every 2 weeks thereafter. The use of cyclosporine or tacrolimus will be decision of the transplant center.

b. Methotrexate 10mg/m^2 on day +1, then 5 mg/m^2 on day +3 and day +6, Dose of methotrexate should be modified if necessary according to creatinine clearance (Table SCH- D).

Table SCH- D Methotrexate dose modification

Calculated Creatinine Clearance (mls/min)	*% Methotrexate Dose Reduction*
>70	**0%**
50-70	**25%**
30-49	**50%**
<30	**100% i.e no methotrexate**

c. Thymoglobuline (ATG). Patients undergoing unrelated donor or mismatched related donor transplantation will be given additional GvHD prophylaxis with low-dose thymoglobuline** (0.5 mg / KG of body weight on day -3, 2.0 mg/ KG of body weight on day -2, and 2.0 mg/ KG of body weight on day -1).

***** If actual body weight is > 30% ideal body weight (IBW), use adjusted body weight (ABW) for calculating the dose of ATG as detailed above.***

STUDY DESIGN

A two- stage Simon Phase II study will be conducted in each of two groups of patients: related and unrelated donor transplants. In each donor transplant group, the first stage of this design will include 11 patients evaluated for death or graft failure by 100 days post transplant. In each stratum, we will enroll additional patients (up to 20%) of stratum total to take into account exclusions due to donor failure (such as donor deemed unsuitable for stem cell donation due to medical or other reasons) only. Those patients who have toxicities related to Ruxolitinib and not been able to reach HCT due to these toxicities will be included in the estimation of overall failure rates. Only those patients who are excluded based on donor related issues without any regimen related complications will be excluded from the estimation of failure rates. However, all data on these patients will be reported.

Unrelated transplants: Based on data from the MPD-RC 101 study (31), there are 15 observed failures (as defined above) among 34 patients by day 100 (44%; of patients died or experienced graft failure by day 100) who received matched unrelated donor transplants. Based on these preliminary data, we will not pursue a regimen with a failure rate of 50% or greater in this trial.

A minimax two-stage design will be used to test the null hypothesis that the 100 day failure rate as defined above is ≥50% versus the alternative that this failure rate is ≤25%. If the regimen is actually not safe, there is a 0.04 probability of concluding that it is (the target for this value was 0.050). If the regimen is actually safe, there is a 0.19 probability of concluding that it is not (the target power is 80%). After testing the regimen in 11 patients in the first stage, the trial will be terminated if there are 6 or more failures within 100 days. If the trial goes on to the second stage, a total of 26 patients will be studied. If the total number of failures is 9 or more, the regimen will be rejected.

The unrelated arm has been temporarily on hold while the research team performed the Stage I analysis to decide whether or not the unrelated arm was deemed to be re-opened for enrollment or not. Based on the data gathered on stage I, 14 patients have been enrolled in the unrelated arm. According to the stopping rules stated on the "Study Design" section of the protocol, 6 or more

failures out of the first 11 enrollments are required for the unrelated arm to be permanently closed. A failure is defined as no engraftment or graft rejection or death before 100 days post-transplant.

The data revealed 4 failures in the first 11 patients and 7 patients were alive and engrafted at 100 days. The result of the analysis confirms that that the unrelated arm does not meet the stopping rule for the first stage.

Therefore, this new amendment is the official opening of stage 2 of MPD-RC 114, and once this amendment has gone through all the regulatory approvals. The Research team will be enrolling 12 patients in the unrelated arm in order to complete the study.

Related transplants: Based on data from the MPD-RC 101 study (31), there are 4 observed failures (as defined above) among 32 patients (12.5%) who received matched related donor transplants in this group of patients.

A minimal (and optimal) two-stage design to test the null hypothesis that the 100 day failure rate as defined above is ≥25% versus the alternative that this failure rate is ≤10%. If the regimen is actually not safe, there is a 0.04 probability of concluding that it is (If the regimen is actually safe, there is a 0.18 probability of concluding that it is not (the target power is 80%).

After testing the regimen in 11 patients in the first stage, the trial will be terminated if there are 3 or more failures within 100 days. If the trial goes on to the second stage, a total of 50 patients will be studied. If the total number of failures is 8 or more, the regimen will be rejected.

We would like to inform that the related arm has met the stopping rule stated above and that this arm will be permanently closed.

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APPENDICES

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LIST OF ABBREVIATIONS

ABW	Adjusted body weight
ACD	Acid Citrate Dextrose
AIBW	Adjusted ideal body weight
ALT	Alanine transaminase
AST	Aspartate transaminase
ATG	Antithymocyte globulin
AUC	Area under the curve
BD	Becton, Dickinson and Company
BFI	Brief Fatigue Inventory
BM	Bone marrow
BMT	Bone marrow transplant
BUN	Blood urea nitrogen
CBC	Complete blood count
CI	Clinical improvement
CIOMS	Council for International Organizations of Medical Sciences
CL	Clearance
C_{max}	Maximum plasma concentration
CMV	Cytomegalovirus
CR	Complete remission
CRA	Clinical research associate
CRF	Case report form
CRP	C-reactive protein
CTCAE	Common Terminology Criteria for Adverse Events
DIPSS	Dynamic International Prognostic Scoring System
DARF	Drug Accountability Record Form
DMA	Dimethylacetamide
DSMB	Data and Safety Monitoring Board
EBV	Epstein-Barr virus
EC	Ethics Committee
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
ESR	Erythrocyte sedimentation rate
ET	Essential thrombocythemia
EU	European Union
EWB	Emotional well-being
FACT-BMT	Functional Assessment of Cancer Therapy-Bone Marrow Transplant
FISH	Fluorescence in situ hybridization
FWB	Functional well-being
GCP	Good Clinical Practice
G-CSF	Granulocyte colony stimulating factor
GST	Glutathione S-transferase
GvHD	Graft versus host disease
Hb	Hemoglobin
Hct	Hematocrit
HCT	Hematopoietic cell transplantation
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HSV	Herpes simplex virus
IB	Investigator's Brochure
IBW	Ideal body weight
ICH	International Conference on Harmonisation
IPSS	International Prognostic Scoring System

LIST OF ABBREVIATIONS

IRB	Institutional Review Board
IWG-MRT	International Working Group for Myelofibrosis Research and Treatment
JAK1	Janus kinase 1 gene
JAK2	Janus kinase 2 gene
JAK2V617F	Mutation in JAK2 kinase
JAK3	Janus kinase 3 gene
LASA	Linear analog scale assessment
MDS	Myelodysplastic syndrome
MF	Myelofibrosis
MPD-RC	Myeloproliferative Disorders Research Consortium
MPN	Myeloproliferative Neoplasm
MPN-SAF	Myeloproliferative Neoplasm Symptom Assessment Form
MRN	Medical record number
MTD	Maximally tolerated dose
NCI	National Cancer Institute
NRM	High non-relapse mortality
NSAID	Nonsteroidal Anti-inflammatory Drugs
OS	Overall Survival
PB	Peripheral blood
PD	Progressive disease
PFS	Progression-free survival
PGIC	Patient Global Impression of Change
PIN	Patient identification number
PMF	Primary myelofibrosis
PPK	Population pharmacokinetic
PR	Partial remission
PT	Prothrombin time
PTT	Partial thromboplastin time
PV	Polycythemia Vera
PWB	Physical well-being
QC	Quality control
QOL	Quality of life
RIC	Reduced intensity conditioning
RRT	Regimen-related toxicities
SAE	Serious adverse event
SD	Stable disease
SFWB	Social/family well being
SGOT	Serum glutamic oxaloacetic transaminase
SMAC	Sequential Multiple Analyzer with Computer
SUSAR	Suspected unexpected serious adverse drug reaction
TB ID	Tissue bank ID number
TOI	Trial Outcome Index
TRM	Transplant-related mortality
TSH	Thyroid stimulating hormone
TYK2	Tyrosine kinase 2 gene
USP	United States Pharmacopeia
V	Volume of distribution
VOD	Veno-occlusive disease
WBC	White blood cell
WHO	World Health Organization

1 OBJECTIVES

1.1 Primary

To determine the feasibility of combining Ruxolitinib (INC424) with a RIC regimen likely to produce success post transplantation, success being defined as patient being alive, and without graft failure at day 100-post allogeneic stem cell transplantation in patients who receive (a) related donor transplant and in those who receive (b) an unrelated donor transplant.

1.2 Secondary

1. Neutrophil and platelet recovery
2. Non-relapse mortality (NRM) at +100 days and 1-year
3. Acute and chronic GvHD at 1-year
4. Chimerism studies at +30, +60 and +100 days post-transplant
5. Remission status according to IWG-MRT criteria (1) at day +100, and 6 and 12 months post-transplant
6. Relapse/progression (defined as per IWG-MRT criteria) (1) at 1-year post transplant
7. Progression-free survival at 1-year
8. Overall Survival at 1-year
9. Impact of allogeneic stem cell transplant on myelofibrosis associated symptoms and overall quality of life
10. Expression profiling and measurements of cytokines prior to start of Ruxolitinib, prior to start of chemotherapy for conditioning, day +30 and day +100 post-transplant
11. Association of cytokines levels with acute and chronic GvHD
12. Impact of somatic mutations such as CALR and ASXL1 on outcome of transplant

2 BACKGROUND

2.1 Myelofibrosis

Myelofibrosis is a chronic clonal myeloproliferative disorder characterized by variable degree of blood cytopenias and/or cytosis, a leucoerythroblastic blood picture, megakaryocytic hyperplasia, reactive marrow fibrosis and extramedullary hematopoiesis. Myelofibrosis is a heterogenous disease and the survival of patients can vary from 2 years to over 10 years based on various prognostic factors such as age >65 years, presence of constitutional symptoms, hemoglobin less than 10.0 gm/dL, leukocyte count >25 x 10^9/l, and circulating blast cells ≥1%. Based on International Prognostic Scoring System (IPSS) (2), presence of 0, 1, 2, and ≥3 factors are categorized as low-risk, intermediate-1 risk, intermediate-2 and high-risk disease. The median survival of patients with low or intermediate -1 risk disease is 135 and 95 months, respectively and these patients are usually treated with observation alone. The median survival of patients with intermediate-2 and high-risk disease is 48 months and 27 months, respectively, and these patients are usually considered suitable candidates for medical interventions including allogeneic transplantation or experimental drug therapy trials. IWG-MRT group further looked at the validity of above risk factor in follow-up of disease course and showed that the above risk factors are also valid at the time of follow-up and have subsequently proposed dynamic IPSS (DIPSS)(3). Further refinement of DIPSS has recently been proposed by DIPSS-plus score, which takes into account additional prognostic impact of cytogenetics, transfusion dependency and thrombocytopenia (4).

2.2 JAK inhibitors in myelofibrosis

Since the discovery of JAK2V617F mutation in patients with myeloproliferative neoplasms (MPNs), there has been considerable interest in the drug development in this area. JAK2V617F mutation is present in approximately 50% of patients with MF. Another 10-12% of patients have other mutations such as MPL515 or exon 12 mutation. Irrespective of the mutation status, a key feature of MPNs is dysregulation of JAK/STAT signaling. Data from reported and ongoing studies have shown that JAK inhibitors are effective in ameliorating the symptoms related to splenomegaly, constitutional symptoms, and improvement in HRQOL scores in patients with myelofibrosis (5, 6). These agents appear to be equally effective in patients with MF irrespective of their mutation status.

2.3 Biology and Safety of Ruxolitinib (INC424)

Several JAK inhibitors are in clinical development at present. Among the various agents in clinical development, Ruxolitinib is most advanced and has the largest experience in terms of safety and toxicity. Based on results of two phase 3 pivotal studies, (COMFORT-I and COMFORT-II), ruxolitinib has been approved by the FDA in the United States and Health Canada, in Canada.

Overview of Biological properties of Ruxolitinib

A key feature of MPNs is dysregulation of JAK/STAT signaling. The JAK/STAT pathway is involved in normal hematopoiesis, inflammation and immune function (7). The four members of the JAK family – JAK1, JAK2, JAK3 and Tyk2 are non-receptor tyrosine kinases that play a

central role in signal transduction initiated by cytokines (interleukins and interferon signaling), growth factors and hormones. Specifically:

JAK1 – role in lymphopoiesis and cytokine response; ubiquitously expressed
JAK2 – role in erythropoiesis; ubiquitously expressed
JAK3 – role in lymphocyte development and proliferation and immune response; expressed primarily in hematopoietic cells
TYK2 – role in mediation of cytokine signals; ubiquitously expressed

Ruxolitinib represents a novel, potent, and selective inhibitor of JAK1 and JAK2. Ruxolitinib potently inhibits JAK1 and JAK2 and yet it does not significantly inhibit a broad panel of 26 kinases and does not inhibit JAK3 at clinically relevant concentrations. Various clinical trials in patients with myelofibrosis have shown their efficacy in reduction of spleen size, inhibition of inflammatory cyokines and improvement in QOL scores in patients with myelofibrosis (6). Additional details as to the in vivo pharmacology of Ruxolitinib may be found in the Investigator's Brochure (IB, Edition 10, release date 29th September 2011).

Single and multiple-dose pharmacokinetic studies have been conducted in multiple species and are reported in IB. Following oral, single-dose administration of Ruxolitinib capsules, the drug is absorbed rapidly and peak plasma concentrations are reached within 1–3 hours. After attaining C_{max} at approximately 2 hours after administration, the plasma concentrations declined with a mean terminal phase distribution half-life of approximately 3–5 hours. There is no significant food effect on absorption or exposure. Ruxolitinib is metabolized in the liver by the cytochrome (CYP) P450 metabolizing enzyme system, predominantly by 3A4 isoenzyme. Thus a dose reduction of Ruxolitinib by 50% has been proposed in the clinical trials in patients who are on CYP3A4 inhibitors as concomitant medication. The pharmacokinetics of Ruxolitinib has been evaluated in patients with renal impairment (mild, moderate or severe). The pharmacokinetics and pharmacodynamics of Ruxolitinib are similar in patients with normal renal function and those with mild, moderate and severe renal impairment (IB).

Safety and efficacy in phase I/II clinical trials in patients with myelofibrosis

The results of phase I/II trial in patients with myelofibrosis were recently published (6). A total of 153 patients were enrolled in this trial. Various dosing schedules were investigated in this trial. These dosage schedules included 25 mg BID, 10 mg BID and 15 mg BID. The dose of Ruxolitinib was further adjusted according to platelet counts. This study demonstrated marked and durable reduction in spleen size. The majority of patients had a ≥25% reduction in spleen size on therapy (70% in 10 mg BID; 82% in 15 mg BID and 77% in 25 mg BID cohort). Responses occurred quickly within 1-2 months of therapy. Spleen reduction occurred regardless of presence / absence of JAK2V617F mutation status.

MPNs are usually associated with weight loss and cachexia. This is probably related to dysregulation and abnormal elevation of a variety of pro-inflammatory cytokines, which may result in weight loss and wasting seen in patients with myelofibrosis. In the reported phase I/II trial, weight gain was observed in most patients. In addition, this trial demonstrated improvement in QOL, which was rapid, with in 1 month of therapy. A specific modified myelofibrosis symptom assessment form (MFSAF) developed by Mesa and colleagues was used to investigate a range of constitutional symptoms related to splenomegaly and elevated cytokines. Symptomatic improvement was similar among the patients receiving 10 mg, 15 mg and 25 mg

BID doses, with improvements in abdominal discomfort and pain seen with accompanying reduction in spleen size. Improvement in functional status and ability to walk was observed as soon as 1 month of therapy. In summary, this study demonstrated that Ruxolitinib was associated with prompt and marked reduction in spleen size, gains in total body weight, improvements in ECOG performance scores, and improvements in constitutional symptoms, which can be debilitating in this patient population.

Tolerability and side effects of Ruxolitinib

Non-hematologic toxicities

Ruxolitinib is well tolerated and non-hematologic adverse events were uncommon. Most common side effects were diarrhea (5.9%), fatigue (4.3%), headache (3.3%), and peripheral edema (2.6%). Details of side effects are in IB. The majority of these toxicities were Grade 1/2 CTCAE toxicity. Grade 3/4 non-hematologic toxicities included fatigue (1.3%), asthenia (2%), fever (0.7%), anxiety (1.3%) and insomnia (1.3%). Further two Phase 3 trials with drug have been completed (COMFORT-1 and COMFORT-II). COMFORT-I is randomized, double blind placebo controlled trial and COMFORT-II is randomized comparison of Ruxolitinib with best supportive therapy. Data from COMFORT-I trial shows that non-hematologic toxicity profile of INC424 was very similar to placebo.

Hematologic toxicities

Hematologic toxicity consisted primarily of anemia and thrombocytopenia. Thrombocytopenia appeared to be dose related and occurring less frequently in the 15 mg BID group (3% grade 3) than the 25 mg BID group (23% grade 3; 6% grade 4).

2.4 Allogeneic transplantation in myelofibrosis and barriers to successful outcomes

HCT is the only curative treatment for patients with myelofibrosis. HCT using conventional intensity conditioning is associated with significant mortality (35–45%) (8-10). Several studies have reported encouraging results of RIC in patients with myelofibrosis with favorable impact on Transplant-related Mortality (TRM) (11-13). The main attraction of this approach is to decrease the risk of TRM and ability to offer transplant to older patients and those with co-morbidities, which otherwise would have precluded the candidacy for transplantation. Both conventional and reduced intensity transplants are associated with significant risk of graft failure and graft-versus-host disease (GvHD) in patients with myelofibrosis (14, 15).

A high mortality and morbidity in myelofibrosis patients undergoing HCT is related to multiple factors:

1. Graft failure
2. GvHD
3. Regimen-related toxicities
4. Poor performance status

Graft failure

Patients with myelofibrosis undergoing HCT have a higher risk of graft failure in the range of 10–20%. This appears very high in comparison to other hematologic malignancies, where the risk of graft failure is usually <5%. High risk of graft failure in these patients is probably related to splenomegaly, and increased risk of alloimmunization resulting from multiple blood

transfusions prior to the transplant procedure. Splenectomy prior to transplant is associated with earlier neutrophil and platelet recovery. However, this procedure is associated with significant surgery related morbidity and mortality (approximately 10%) (16, 17). As discussed above, treatment with Ruxolitinib results in prompt and marked reduction in spleen size. This property of Ruxolitinib may decrease the risk of graft failure in these patients.

GvHD

GvHD is one of the most frequent and serious complications after HCT. The incidence of acute and chronic GvHD in HCT has been reported in the range of 50–80% depending on age of patient, type of donor and graft source with a higher incidence reported for use of PBSCT. Adverse impact of active GvHD on general and mental health, functional impairments, activity limitation, and pain has been widely reported in the BMT literature (18). Patients with MPN and particularly myelofibrosis have high cytokine levels, which lead to constitutional symptoms and significant debilitation in these patients (6, 19). Several convergent lines of evidence have suggested that inflammatory cytokines act as mediators of acute GvHD. Most of the clinical manifestations of GvHD may in fact be due to the dysregulated production of cytokines by T cells and other inflammatory cells. The complex interactions among cytokines and their cellular targets suggest that individual cytokines may play an important and distinctive role in the pathophysiology of GvHD. Perturbation of the cytokine network may function as a final common pathway of target organ damage, and the rapid onset of severe, acute GvHD can be considered a 'cytokine storm'(20). Ongoing trials with JAK inhibitors such as INC424 show a rapid down regulation of cytokine levels due to anti-JAK1 mediated effect (6). This property results in rapid improvement in constitutional symptoms. Down regulation of cytokine may be of particular interest in patients undergoing HCT as this may result in decreased incidence of GvHD.

Regimen-related toxicities

Patients with myelofibrosis undergoing HCT are at significant risk of regimen-related toxicities. Of note among these toxicities are liver toxicity (8, 11). In 53 patients undergoing HCT at Princess Margaret Hospital, it was noted a ≥ Grade 2 Bearman hepatic toxicity (bilirubin level >102.6 mmol) in 44% of patients in first 6 weeks after transplantation (21) (data presented at International MPD conference in NY on November 3, 2011, manuscript submitted) and was associated with inferior survival due to high non-relapse mortality in these patients. This liver toxicity appears very high in comparison to other myeloid malignancies such as acute myeloid leukemia and myelodysplastic syndromes reported Princess Margaret Hospital (22). Although the exact mechanisms of this increased liver toxicity in myelofibrosis patients undergoing HCT are not well understood at present; a possible explanation may be involvement of the liver by the underlying MPN. Comparisons of myeloablative conditioning and RIC in patients with myelofibrosis show a reduction in mucositis and hepatic toxicity in patients with myelofibrosis undergoing RIC (14). Treatment with a JAK inhibitor prior to transplant conditioning may result in decreasing the burden of myeloproliferation in liver and may contribute towards decreasing the liver related regimen-related toxicities.

Poor performance status

Most myelofibrosis patients undergoing HCT have advanced disease at the time of HCT. Performance and functional status of these patients can be further compromised due to symptomatic splenomegaly, constitutional symptoms, and anemia. It has been shown that poor

pre-transplant performance status is an independent predictor of high TRM and poor survival in patients with myelofibrosis (15). As highlighted above, JAK inhibitors can potentially decrease the spleen size, down regulate cytokine levels resulting in improvement in constitutional symptoms and potentially can improve the performance status of the patient.

Improvement in pre-transplant performance status and possible potential of beneficial impact on GvHD make these agents attractive for exploring their potential in the transplant related strategies of myelofibrosis patients undergoing HCT.

2.5 Study rationale

Unlike BCR-ABL inhibitors, current JAK inhibitors do not lead to good partial or complete remission, and do not appear to have curative potential. HCT on the other hand has curative potential, but is associated with significant morbidity and mortality.

High non-relapse mortality (NRM) due to regimen-related toxicities (RRT), graft failure and graft versus host disease (GvHD), are major barriers to the success of allogeneic hematopoietic cell transplantation (HCT) in patients with myelofibrosis. Treatment with Ruxolitinib (INC424) a dual kinase JAK 1/2 inhibitor prior to reduced intensity conditioning (RIC) may lead to reduction of pre-transplant spleen size, improvement in performance scores, and decrease in inflammatory cytokines levels, which may have beneficial impact on TRM in patients with myelofibrosis.

2.6 Challenges of combining Ruxolitinib in a transplant strategy

As highlighted above, Ruxolitinib is associated with hematologic toxicities consisting of anemia and thrombocytopenia. Thrombocytopenia appeared to be dose related and occurring less frequently in the 15 mg BID group (3% grade 3) than 25 mg BID group (23% grade 3; 6% grade 4). These properties can result in delayed hematological recovery. These toxicities are reversible. In view of these toxicities, the ideal timing of Ruxolitinib would be prior to the transplant. The proposed timing of Ruxolitinib in this study is 60 days prior to start of conditioning. This provides a sufficient time for the impact of Ruxolitinib on reduction of spleen size, reduction in pro-inflammatory cytokines and improvement in QOL scores and performance scores. Ruxolitinib taper will start on day -9 and drug will be stopped completely at start of conditioning therapy (5 days prior to stem cell infusion). Given the short half of drug (3–5 hours), the likelihood of impact on donor cells will be very low.

3 PATIENT SELECTION

3.1 Inclusion Criteria (Patient should meet all the criteria)

1. Documented diagnosis of primary myelofibrosis according to WHO criteria or post PV myelofibrosis or post ET myelofibrosis as per IWG-MRT criteria (23)
2. Age 18–70 years
3. Intermediate-2/ high-risk disease as per Dynamic IPSS (DIPSS) criteria (3) (Appendix 1)

   OR

   Intermediate-1 risk disease with one of the following additional unfavorable features known to impact the survival adversely

   a. Red cell transfusion dependency (4)
   b. Unfavorable Karyotype (4)
   c. Platelet count <100 x 10^9/l (4)

   [See Appendix 2 and Appendix 3 for definition of transfusion dependency (24) and unfavorable karyotype (25, 26)]

4. Blasts in the PB and BM ≤ 10% prior to study enrollment
5. Availability of a suitable matched related (6/6 or 5/6) or unrelated donor (10/10 or 9/10 antigen or allele matched).
6. Able to give informed written consent
7. ECOG Performance status of 0-2.
8. Life expectancy >3 months
9. Off all MF-directed therapy including investigational agents for at least 2 weeks prior to study enrollment and recovered from all toxicities*
10. Adequate organ function
    a. Adequate renal function – creatinine <1.5 x IULN
    b. Adequate hepatic function – AST/ALT <2.5 x IULN, Total Bilirubin <1.5 x IULN
    c. Adequate hematopoietic function – Platelet ≥ 50 x 10^9/l and ANC ≥ 1.0 x 10^9/l
    d. LVEF >40% (MUGA or echocardiogram) OR Normal per Institutional standard
    e. Adequate pulmonary function with DLCO >50%

* A patient who has been on stable dose of Ruxolitinib and has received ruxolitinib ≤6 months prior to the study entry will be considered potentially eligible for the study with the caveat that there is no evidence of loss of response (>5cm increase in spleen size from the nadir).

3.2 Exclusion Criteria

1. Any previous JAK2 inhibitor treatment prior to study enrolment, with the exception of Ruxolitinib
2. Hypersensitivity to JAK inhibitor

3. Clinical or laboratory evidence of cirrhosis
4. Prior allogeneic transplant for any hematopoietic disorder
5. >20% blast in the PB or BM prior to HCT or had leukemic transformation (>20% blasts in PB or BM any time prior to HCT)
6. Syngeneic donor
7. Cord Blood transplant
8. Active uncontrolled infection
9. H/o another malignancy within 5-years of date of HCT except h/o basal cell or squamous cell carcinoma of skin or PV or ET
10. Known HIV positive
11. Pregnancy at the time of BMT
12. Any other concurrent illness which in investigator's opinion puts the patient at excessive risk of treatment related toxicities
13. Unable to give informed consent
14. Active infection with hepatitis A,B or C virus
15. Subjects who require therapy with a strong CYP3A4 inhibitor prior to enrollment to this study

3.3 Subject Recruitment and Screening

Subjects will be recruited and screened for the study from the clinical services of the MPD-RC Clinical Study Centers.

3.4 Early Withdrawal of Subjects

3.4.1 When and How to Withdraw Subjects

In accordance with the Declaration of Helsinki and the guidelines of the country of the participating MPD-RC Clinical Study Center, each subject is free to withdraw from the study at any time. An investigator also has the right to withdraw a subject from the study in the event of the patient suffering an intercurrent illness, adverse events, or other reasons concerning the health or well-being of the patient, or in the case of lack of cooperation by the patient. All serious adverse reactions need to be followed up until resolution and information returned to study coordinators.

Should a subject decide to withdraw after administration of study drug, or should the investigator decide to withdraw the subject, all efforts should be made to complete and report the observations up to the time of withdrawal as thoroughly as possible. A final evaluation, the reason for, and the date of withdrawal must be recorded on the CRF. The last visit for each subject will be defined as a study discontinuation/ end-of-study visit, which will occur 30 (±7 days) days after time of withdrawal.

Subjects with clinically significant abnormal laboratory values as determined by the investigator or who have ongoing clinically significant treatment related adverse events during their last scheduled clinical evaluation will be monitored and treated until resolution or stabilization is achieved; or, in the event that the subject's condition is not likely to improve because of disease progression, until the cause of the abnormal test result or adverse event can be determined.

3.4.2 Data Collection and Follow-up for Withdrawn Subjects

The reason for and date of withdrawal from study drug treatment; and the reason for and date of withdrawal from the study will be recorded on the case report form (CRF). If a subject withdraws consent, every attempt will be made to determine the reason. If the reason for withdrawal is an adverse event or a clinically significant abnormal laboratory test result, monitoring will continue until the event has resolved or stabilized, until the subject is referred to the care of a local health care professional, or until a determination of a cause unrelated to the study drug or study procedure is made. The specific event or test result(s) must be recorded on the CRF. All evaluations will be performed, according to the protocol, 30 days (+/- 7 days) after the last day the patient takes the study drug. A subject will be considered to be lost to follow-up if all of the following criteria have been met:

1. More than three phone calls to the subject are unanswered.
2. A next of kin is contacted and no information is available.
3. The referring physician is contacted and no information is available.
4. A telegram or certified letter is unanswered.

Subjects who experience secondary graft failure or failure to meet the primary objective will continue to be followed every 6 months for progression of disease and survival until the end of the study. The progression of disease and survival data can be collected via telephone call or through the e-medical record system. See section 4.7 for more details.

4 TREATMENT PLAN

4.1 Study Drug (Ruxolitinib), Packaging, Labelling, Supply and Storage

4.1.1 Packaging and labeling

Ruxolitinib investigational drug is provided as 5 mg tablets. Ruxolitinib 5 mg tablets are packaged in bottles. For centers in the US, the study medication is referenced as ruxolitinib 5 mg tablet on the bottle. For centers outside the US, the study medication is referenced as INC424 5 mg tablet on the bottle. Medication labels will be in the local language and comply with the legal requirements of each country. They will include storage conditions for the drug and the medication number, but no information about the subject.

4.1.2 Receiving Drug Supply, Receipt, Storage and Return

The Company will manufacture, package and label study drug and provide a central depot for study drug distribution and return to each of the MPD-RC Clinical Centers. Collaborating Institutions will request study drug based on instructions provided by the MPD-RC Consortium.

Before an MPD-RC Institution can receive study drug, protocol specific regulatory documents must be submitted to the MPD-RC Central Coordinating Office. (See section 5.1.1 regarding required regulatory documents necessary)

Ruxolitinib must be received by a designated person at the MPD-RC study site, handled and stored safely and properly, and kept in a secured location to which only the investigator and designated assistants have access. Upon receipt, the Ruxolitinib should be stored according to the instructions specified on the drug labels. An inventory must be performed and a drug receipt log filled out and signed by the person accepting the shipment. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment will be documented in the study files. The investigator must notify the study sponsor of any damaged or unusable study treatments that were supplied to the investigator's site.

Ruxolitinib supply is for Investigational use and must be used for this trial only. Under no circumstances should the investigator or any site personnel supply study drug to other investigators, subjects, clinics, or allow supplies to be used other than specified by the protocol.

Once a participating site is approved by the MPD-RC and open for recruitment, request for drug supply for one patient would be sent immediately to Incyte in the United States and Novartis outside the United States to authorize drug shipment directly to the participating site. Subsequent study drug supplies will be sent upon request/ new patient enrollment.

Table 1 Supply and Storage of study drugs

Study drugs	Supply	Storage
Ruxolitinib	Centrally supplied by Incyte (US) and Novartis (Outside the US)	*Refer to study drug label*

The study drug should be stored, under adequate security, in the pharmacy at the study center. Store Ruxolitinib at room temperature between 68°F and 77°F (20°C and 25°C).

4.1.3 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug accountability form (signed and dated). Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files. (See section 5.1, a copy of the participating study Centers drug destruction policy and procedure must be on file with the MPD-RC)

4.2 Starting Dose, Dose Adjustment and Tapering Schedule of Ruxolitinib

Ruxolitinib (INC424) tablets will be started on day -65 prior to the planned stem cell infusion. The starting dose of Ruxolitinib will be determined according to the baseline platelet count (Table 2) and will be modified according to the platelet count at follow-up (Table 3). The drug will be given in the maximum tolerated dose as defined (Table 3) in the protocol until day -10, followed by 4 days of tapering starting on day -9 (Table 4), and will be stopped completely on day –5, on the day of planned start of conditioning therapy (starting on day -5). If there is a delay in the availability of the donor or a delay in the start of the transplant conditioning regimen for any reason, contact the study PI to discuss. The dose of ruxolitinib should not be tapered in that instance until the date of the start of the conditioning regimen is known with certainty.

The drug will be supplied as 5 mg tablets.

Table 2 Starting dose assignment

Baseline Platelet count	Starting dose of Ruxolitinib
>200 x 10^9/L	20 mg BID (Four 5 mg tablets twice daily)
100- 200 x 10^9/L	15 mg BID (three 5 mg tablets twice daily)
50-99 x 10^9/L	10 mg BID (two 5 mg tablets twice daily)

Platelet count will be monitored once a week, and if platelet count falls below 50 x 10^9/L, then platelet count will be monitored twice weekly. At follow-up, the dose of Ruxolitinib will be modified (increased or decreased) according to Table 3. The drug should only be stopped if there is a concern regarding bleeding.

Table 3 Dose adjustment according to platelet count at follow-up (prior to start of conditioning therapy)

Platelet count at the follow-up	Dose at the time of follow-up			
	20 mg BID	**15 mg BID**	**10 mg BID**	**5 mg BID**
>200 x 10^9/L	No change	20 mg BID	15 mg BID	10 mg BID
100-200 x 10^9/L	15 mg BID	No change	15 mg BID	10 mg BID
50-99 x 10^9/L	10 mg BID	10 mg BID	No Change	10 mg BID
25-49 x 10^9/L	5 mg BID	5 mg BID	5 mg BID	No change
<25 x 10^9/L	5 mg BID + Platelet transfusion per investigator discretion	5 mg BID + Platelet transfusion per investigator discretion	5 mg BID + Platelet transfusion per investigator discretion	5 mg BID + Platelet transfusion per investigator discretion

Table 4 Tapering schedule of Ruxolitinib

Dose prior to Day -9	Day -9	Day -8	Day -7	Day -6	Day -5
20 mg BID	15 mg BID	10 mg BID	5 mg BID	5 mg OD	None
15 mg BID	10 mg BID	10 mg BID	5 mg BID	5 mg OD	None
10 mg BID	10 mg BID	10 mg BID	5 mg BID	5 mg OD	None
5 mg BID	5 mg BID	5 mg BID	5 mg BID	5 mg OD	None

Rationale for using a lower platelet count threshold

In the published phase 1/2 study with INC424 (6) and recently completed two phase 3 studies (Registration trials, COMFORT-I and COMFORT-II), eligibility criteria is platelet count ≥100 x 10^9/l. In this study, we have chosen baseline platelet count ≥50 x 10^9/l as eligibility criteria so as to give the benefit of this treatment to majority of patients with myelofibrosis. The patients undergoing allogeneic transplantation are usually higher risk patients and it is estimated that approximately 25% of patients undergoing allogeneic transplant will have baseline platelet count <100 x 10^9/l (31).

Rationale for not withholding study drug for grade 3–4 thrombocytopenia

In the COMFORT-1 and COMFORT-2 trials, Ruxolitinib was withheld at platelet count <50 x 10^9/l. In this study, study drug will not be withheld for grade 3-4 thrombocytopenia unless there

is a serious bleeding complication. In the drug trials patients are on study medication for a very long period of time, which can vary from several months to years. In comparison, in the transplant trial, patients are on study medication for 56 days and then medication will be tapered. To get the maximum benefit of this strategy, it will beneficial to modify the dosage and continue the study medication with additional platelet transfusions if necessary.

Management of patients with ruxolitinib withdrawal syndrome

Severe reactions have been reported to occur uncommonly following abrupt ruxolitinib withdrawal. The dose tapering strategy in this protocol is designed to minimize the risk of such reactions. Patients considered to be at high risk of ruxolitinib withdrawal syndrome, such as those with highly proliferative disease, should be closely monitored during the tapering period prior to commencement of conditioning therapy. Short-term courses of corticosteroids have been used to moderate the withdrawal of ruxolitinib and may be considered as part of a tapering strategy at the investigators discretion. In the unlikely event that patients develop a withdrawal syndrome requiring conditioning to be deferred, this will be classified as a protocol failure.

4.3 Conditioning Therapy

If HCT is delayed because of donor related issues, patient will still be considered eligible for HCT. These patients should continue on Ruxolitinib as per protocol, and proceed to transplant once donor issues are resolved. Prior to start of conditioning therapy, transplant physician should ensure that the patient meets the following criteria:

a. ECOG Performance status 0-2
b. Blasts in PB <20%
c. Adequate renal function with creatinine <1.5 x Institutional ULN
d. Adequate hepatic function with AST/ALT <2.5 x IULN and Bilirubin <1.5 x IULN
e. Absence of active uncontrolled infection or any other concurrent illness which in the Investigator's opinion puts the patient at excessive risk of transplant related toxicities.

A patient not meeting the above criteria will be counted as failure; and treating physician should discuss this patient with one of the study co-chairs.

Conditioning therapy will be as follows:

Initiate anticonvulsant prophylactic therapy prior to busulfan treatment as per institutional practice.

IV Fludarabine 40 mg/m^2 IV over 30 minutes once daily x 4 days for days -5 to -2.
IV Busulfan 2.0 mg/ KG of body weight IV over 2 hours once daily for 4 consecutive days for days -5 to -2.

****If actual body weight is > 30% ideal body weight (IBW), use adjusted body weight (ABW) for calculating the dose of fludarabine and busulfan***

Fludarabine and Antithymocyte Globulin (Rabbit) – will be dosed according to the patient's actual body weight, unless the patient weighs more than 130% of IBW, in which the dose will be based on an adjusted body weight.

IBW: Estimated ideal body weight in (kg)

Males: IBW = 50 kg + 2.3 kg for each inch over 5 feet.

Females: IBW = 45.5 kg + 2.3 kg for each inch over 5 feet.

ABW: Estimated adjusted body weight (kg)

If the actual body weight is greater than 30% of the calculated IBW, calculate the adjusted body weight

ABW = IBW + 0.4(actual weight - IBW)

Table 5 Conditioning therapy schedule

Treatment	Day -5	Day -4	Day -3	Day–2	Day–1	Day 0
Fludarabine	X	X	X	X		
Busulfan	X	X	X	X		
ATG (only if unrelated donor)			X	X	X	

4.4 GvHD Prophylaxis

a. IV Calcineurin inhibitor [IV cyclosporine (per institutional practice) or IV tacrolimus (per institutional practice)] starting on day -2, until engraftment or when the patient is able to take PO, then switched to PO doses. Cyclosporine or tacrolimus will be given per institutional practice. The doses should be adjusted to keep trough levels of cyclosporine between 200-400 µg/L or tacrolimus 8-12 ng/ml. Cyclosporine or tacrolimus will be continued through day 180 and subsequently in the absence of GvHD, tapered by 25% every 2 weeks thereafter. The use of cyclosporine or tacrolimus is according to the choice of the transplant center.

b. Methotrexate 10 mg/m^2 on day +1, then 5 mg/m^2 on day +3 and day +6, dose of methotrexate should be modified if necessary according to creatinine clearance (Table 6 Methotrexate dose modification)

Table 6 Methotrexate dose modification

Calculated Creatinine Clearance (mls/min)	*% Methotrexate Dose Reduction*
>70	**0%**
50-70	**25%**
30-49	**50%**
<30	**100% (no methotrexate)**

c. Thymoglobuline (ATG) - Antithymocyte Globulin (Rabbit). Patients undergoing unrelated donor or mismatched related donor transplantation will be given additional GvHD prophylaxis with low-dose thymoglobuline** (0.5 mg / KG of body weight on day -3, 2.0 mg/ KG of body weight on day -2, and 2.0 mg/ KG of body weight on

day -1). Patients will be premedicated with acetaminophen, antihistamine, and corticosteroids as per institutional practice.

***** If actual body weight is > 30% ideal body weight (IBW), use adjusted body weight (ABW) for calculating the dose of ATG as detailed above.***

4.5 Graft Source

Peripheral blood (PB) will be the recommended graft source; however, bone marrow (BM) will be acceptable depending on the donor or registry choice.

The recommended doses for the graft are:

PB graft: 4 x 10^6 CD34+ cells/ Kilogram of body weight of recipient;

BM graft: 3 x 10^8 Total nucleated cells (TNC)/ Kilogram of body weight of recipient

If the PB graft dose is <2 x 10^6 CD34+ cells / Kilogram of body weight of recipient or BM graft is <1 x 10^8 TNC / Kilogram of body weight of recipient; the graft will be treated as non-conforming product.

Each transplant center will follow the guidelines of their respective country regarding safe use of cellular therapy products.

A graft which is considered nonconforming nature due to either low cell dose or any other reason will be released after signing a form by the transplant physician confirming the nature of non-conformance. A sample of non-conformance letter is attached in the Appendix 11.

In case of nonconforming product, the transplant physician will report to MPD-RC about the nature of non-conformance; and this information will be collected in the study database.

4.6 Supportive Care

Each transplant center will follow local policies for supportive care for decisions regarding use of antibacterial, antifungal and antiviral prophylaxis. **It is mandatory that if a center uses anti-fungal prophylaxis with an agent which is strong inhibitor of CYP3A4 (drugs such as voriconazole or posaconazole), these should not be started before day -2 as there is drug interaction with Ruxolitinib and these drugs are likely to increase the levels of Ruxolitinib.** In case there is a clinical need to give these medications along with Ruxolitinib, then dose of Ruxolitinib should be reduced by 50%.

4.7 Study follow-up

All the patients enrolled in the study will continue to be monitored as per study schedule until last enrolled patient has reached a follow up of 2 years. There will be an annual follow-up for survival to a maximum of 4 years total from study start to finish.

Treatment in this trial will be defined as any point after enrollment whether actively receiving ruxolitinib or post stem cell infusion. Subjects who discontinue study treatment due to toxicity or require additional intervention for protocol defined graft failure or relapse/disease progression will have a 30 day follow up visit and then will continue to be followed every 6 months from the EOT (end of treatment) for survival and progression of disease until the end of the trial (after last

enrolled patient completes 2 years of follow up). The survival and progression of disease data can be collected via telephone call or medical record.

5 REGISTRATION PROCEDURES

5.1 Registration requirements

Only MPD-RC Approved Transplant Centers may participate and register patients to this study.

5.1.1 Site Registration with MPD-RC

Before an MPD-RC Institution can enroll patients, protocol specific regulatory documents must be submitted to the MPD-RC Central Coordinating Office via E-mail (PDF file preferred) or fax:

MPD-RC Central Coordinating Office
Alicia Orellana
Research Protocol Manager
Phone: (212) 241-0481
Fax: (212) 876-5276
E-mail: alicia.orellana@mssm.edu

Required Protocol Specific Regulatory Documents (E-mailed by PDF format preferred):

1. Submit a copy of IRB approval for MPD-RC 114
2. Submit a copy of the IRB approved consent form for MPD-RC 114
3. Submit a copy of the CV and medical license of the Research Pharmacist
4. Submit a copy of the site's drug destruction policy
5. Submit a copy of a signed and dated CV and medical license of the Principal Investigator and Co-Is
6. Submit a copy of Form FDA 1572 as completed by the Principal Investigator
7. Submit a copy of the Investigational Drug Order Form (will be provided to the site by the MPD-RC once we receive items 1-6)

BEFORE Study Drug Shipment
Before Study Drug can be shipped to the MPD-RC Center to begin accruing patients, the above regulatory documents will be required and forwarded to the MPD-RC Central Office to the attention of the Regulatory Coordinator, as noted above.

5.1.2 Correlative Biomarker Study (MPD-RC 107)

There is a correlative science biomarker study for which participation is mandatory (MPD-RC protocol 107). The patient must be enrolled in the mandatory companion biomarker study in order to participate in this treatment study (MPD-RC 114).

5.2 Informed Consent

The patient must be aware of the neoplastic nature of his/her disease, as well as of the expected life expectancy at his/her stage of disease (according to DIPSS Scoring System, Appendix 1). The patient must willingly consent after being informed of the procedures to be followed, the experimental nature of the therapy, alternatives, potential benefits, side effects, risks, and discomforts. Human protection committee approval of this protocol and of its consent form is required.

5.3 MPD-RC registration procedures

This study uses the MPD-RC on-line Patient Registration system (www.mpd-rc.org). Registration will be accepted only through users of the MPD-RC database. Registration must occur prior to the initiation of therapy.

Confirm eligibility criteria (Section 3) and complete the on-line Registration Worksheet. Access the online Patient Registration system via the patient registration icon on the MPD-RC Information Systems IS Application main menu. **If the registering clinical research associate (CRA) requires assistance he/she may consult the on-line help file located under the Help menu of the MPD-RC application.**

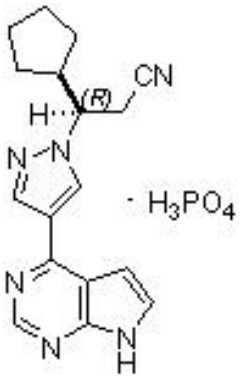
When the patient is registered to MPD-RC tissue bank protocol #107 and then onto #114, a patient identification number will be generated. Registration will not be completed if eligibility requirements are not met for the selected trial.

The registering institution will receive a Confirmation of Registration email from the database that should be printed and included in the patient chart. If a registering institution needs to correct the registration form, contact the MPD-RC Data Management Center: E-mail: mpdtrials@mpdrc.org

6 PHARMACEUTICAL INFORMATION

6.1 Study Drug-Ruxolitinib (INC424)

Therapeutic Classification:
Ruxolitinib phosphate is a kinase inhibitor with the chemical name (R)-3-(4-(7H-pyrrolo [2, 3-d] pyrimidin-4-yl)-1H-pyrazol-1-yl)-3-cyclopentylpropanenitrile phosphate and a molecular weight of 404.36. Ruxolitinib phosphate has the following structural formula:



Ruxolitinib phosphate is a white to off-white to light pink powder and is soluble in aqueous buffers across a pH range of 1 to 8.

Ruxolitinib Tablets are for oral administration. Each tablet contains ruxolitinib phosphate equivalent to 5 mg, 10 mg, 15 mg, 20 mg and 25 mg of ruxolitinib free base together with microcrystalline cellulose, lactose monohydrate, magnesium stearate, colloidal silicon dioxide, sodium starch glycolate, povidone, and hydroxypropyl cellulose.

Dosing Information
Recommended Starting Dose – (See section 4.2, Table 2)

Dose Adjustment with Concomitant Strong CYP3A4 Inhibitors
On the basis of pharmacokinetic studies in healthy volunteers, when administering Ruxolitinib with strong CYP3A4 inhibitors (such as but not limited to boceprevir, clarithromycin, conivaptan, grapefruit juice, indinavir, itraconazole, ketoconazole, lopinavir/ritonavir, mibefradil, nefazodone, nelfinavir, posaconazole, ritonavir, saquinavir, telaprevir, telithromycin, voriconazole) these CYP3A4 inhibitors should be avoided during the 62 days of ruxolitinib administration.

Method of Administration
Ruxolitinib is dosed orally and can be administered with or without food. The dose should be taken at the same time each day with a window of ±3 hours. If a dose is missed, the patient should not take an additional dose, but should take the next usual prescribed dose.

When discontinuing Ruxolitinib therapy for reasons other than thrombocytopenia, gradual tapering of the dose of Ruxolitinib may be considered, for example by 5 mg twice daily each week.

For patients unable to ingest tablets, Ruxolitinib can be administered through a nasogastric tube (8 French or greater) as follows:

- Suspend one tablet in approximately 40 mL of water with stirring for approximately 10 minutes.
- Within 6 hours after the tablet has dispersed, the suspension can be administered through a nasogastric tube using an appropriate syringe.

The tube should be rinsed with approximately 75 mL of water. The effect of tube feeding preparations on Ruxolitinib exposure during administration through a nasogastric tube has not been evaluated.

Pharmaceutical Data
Ruxolitinib, a kinase inhibitor, inhibits Janus Associated Kinases (JAKs) JAK1 and JAK2, which mediate the signaling of a number of cytokines and growth factors that are important for hematopoiesis and immune function. JAK signaling involves recruitment of STATs (signal transducers and activators of transcription) to cytokine receptors, activation and subsequent localization of STATs to the nucleus leading to modulation of gene expression.

Side Effects:
Adverse reactions occurring in patients on Ruxolitinib in the double-blind, placebo-controlled study during randomized treatment includes contusion, ecchymosis, hematoma, injection site hematoma, periorbital hematoma, vessel puncture site hematoma, increased tendency to bruise, petechiae, purpura, dizziness, postural dizziness, vertigo, balance disorder, Meniere's Disease, labyrinthitis, urinary tract infection, cystitis, urosepsis, urinary tract infection bacterial, kidney infection, pyuria, bacteria urine, bacteria urine identified, nitrite urine present, weight increased, abnormal weight gain, herpes zoster and post-herpetic neuralgia. Rare case reports of progressive multifocal encephalopathy have been reported.

Description of Selected Adverse Drug Reactions

Anemia
In the two Phase 3 clinical studies, median time to onset of first CTCAE Grade 2 or higher anemia was approximately 6 weeks. One patient (0.3%) discontinued treatment because of anemia. In patients receiving Ruxolitinib, mean decreases in hemoglobin reached a nadir of approximately 1.5 to 2.0 g/dL below baseline after 8 to 12 weeks of therapy and then gradually recovered to reach a new steady state that was approximately 1.0 g/dL below baseline. This

pattern was observed in patients regardless of whether they had received transfusions during therapy.

In the randomized, placebo-controlled study, 60% of patients treated with Ruxolitinib and 38% of patients receiving placebo received red blood cell transfusions during randomized treatment. Among transfused patients, the median number of units transfused per month was 1.2 in patients treated with Ruxolitinib and 1.7 in placebo treated patients.

Thrombocytopenia
In the two Phase 3 clinical studies, in patients who developed Grade 3 or 4 thrombocytopenia, the median time to onset was approximately 8 weeks. Thrombocytopenia was generally reversible with dose reduction or dose interruption. The median time to recovery of platelet counts above 50 X 10^9/L was 14 days. Platelet transfusions were administered to 4.7% of patients receiving Ruxolitinib and to 4.0% of patients receiving control regimens. Discontinuation of treatment because of thrombocytopenia occurred in 0.7% of patients receiving Ruxolitinib and 0.9% of patients receiving control regimens. Patients with a platelet count of 100 X 10^9/L to 200 X 10^9/L before starting Ruxolitinib had a higher frequency of Grade 3 or 4 thrombocytopenia compared to patients with a platelet count greater than 200 X 10^9/L (16.5% versus 7.2%).

Neutropenia
In the two Phase 3 clinical studies, 1.0% of patients reduced or stopped Ruxolitinib because of neutropenia.

Additional Data from the Placebo-controlled Study
25.2% of patients treated with Ruxolitinib and 7.3% of patients treated with placebo developed newly occurring or worsening Grade 1 abnormalities in alanine transaminase (ALT). The incidence of greater than or equal to grade 3 elevations in ALT was observed in 1.3% of ruxolitinib treated patients and 0% in placebo treated patients

Ruxolitinib with 1.3% Grade 3 and no Grade 4 ALT elevations.
17.4% of patients treated with Ruxolitinib and 6.0% of patients treated with placebo developed newly occurring or worsening Grade 1 abnormalities in aspartate transaminase (AST). The incidence of Grade 2 AST elevations was 0.6%.

Ruxolitinib with no Grade 3 or 4 AST elevations.
16.8% of patients treated with Ruxolitinib and 0.7% of patients treated with placebo developed newly occurring or worsening Grade 1 elevations in cholesterol. The incidence of Grade 2 cholesterol elevations was 0.6% for Ruxolitinib with no Grade 3 or 4 cholesterol elevations.

Clinical Applications:

FDA approved indication: Ruxolitinib is indicated for treatment of patients with intermediate or high-risk myelofibrosis, including primary myelofibrosis, post-polycythemia vera myelofibrosis and post-essential thrombocythemia myelofibrosis.

Health Canada approved indication: Ruxolitinib is indicated for the treatment of splenomegaly and/or its associated symptoms in adult patients with primary myelofibrosis (also known as chronic idiopathic myelofibrosis), post-polycythemia vera myelofibrosis or post-essential thrombocythemia myelofibrosis.

6.2 Fludarabine

Therapeutic Classification
Fludarabine is the 2-fluoro, 5-phosphate derivative of vidarabine.

Dosing Information:
Doses of 25 mg/m^2/day (30-minute infusion) for 5 days every 4 weeks has been effective previously treated patients with chronic lymphocytic leukemia; in non-Hodgkin's lymphoma, a loading dose of 20 mg/m^2 intravenously followed by a continuous intravenous infusion of 30 mg/m^2/24 hours for 48 hours, has been effective; dose reductions are suggested in renal insufficiency.

Pharmaceutical Data
Following intravenous administration, fludarabine phosphate is rapidly dephosphorylated to 2-fluoro-vidarabine, which subsequently enters tumor cells and is phosphorylated to the active triphosphate derivative; peak plasma levels of 2-fluoro-vidarabine have ranged from 0.3 to 0.9 mcg/mL following a short infusion of 25 mg/m^2 fludarabine; 24% of a dose of fludarabine is recovered in the urine as 2-fluoro-vidarabine; the elimination half-life of 2-fluoro-vidarabine is 9 hours.

Side effects
Myelosuppression, particularly neutropenia, is the predominant adverse effect; a severe neurotoxicity has been observed, mainly with higher doses; other adverse effects include nausea, vomiting, diarrhea, fever, ulcers, loss of appetite, swelling or water retention, muscle aches, nervousness, hearing loss, fatigue, stomatitis, skin rash, and somnolence; pneumonitis has been reported in 1 patient.

Clinical Applications
Intravenous fludarabine has been highly effective as a single agent in heavily pretreated patients with chronic lymphocytic leukemia; the drug has also produced responses in patients with non-Hodgkin's lymphoma and acute leukemia when combined with other chemotherapeutic agents; however, neurotoxicity has been a major concern, especially when used at high doses.

6.3 Busulfan (Busulfex®)

Therapeutic Classification
Busulfan is a bifunctional alkylating agent known chemically as 1, 4-butanediol, dimethanesulfonate. Busulfex® (busulfan) Injection is intended for intravenous administration. It is supplied as a clear, colorless, sterile, solution in 10 mL single use vials. Each vial of Busulfex contains 60 mg (6 mg/mL) of busulfan, the active ingredient, a white crystalline powder with a molecular formula of $CH_3SO_2O(CH_2)_4OSO_2CH_3$ and a molecular weight of 246 g/mole. Busulfan is dissolved in N,N-dimethylacetamide (DMA) 33% vol/vol and Polyethylene Glycol 400, 67% vol/vol. The solubility of busulfan in water is 0.1 g/L and the pH of Busulfex diluted to approximately 0.5 mg/mL busulfan in 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP as recommended for infusion reflects the pH of the diluent used and ranges from 3.4 to 3.9.

Busulfex is intended for dilution with 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP prior to intravenous infusion.

Dosing

Based on the results of this 24-patient clinical trial, a suggested dosing regimen of Busulfex in pediatric patients is shown in the following dosing nomogram:

Table 7 Busulfex Dosing Nomogram

Patient's Actual Body Weight (ABW)	Busulfex Dosage
≤12 kgs	1.1 (mg/kg)
>12 kgs	0.8 (mg/kg)

The usual adult dose is 0.8 mg/kg of ideal body weight or actual body weight, whichever is lower, administered every six hours for four days (a total of 16 doses). For obese, or severely obese patients, Busulfex should be administered based on adjusted ideal body weight. Ideal body weight (IBW) should be calculated as follows (height in cm, and weight in kg): IBW (kg; men)=50+0.91× (height in cm -152); IBW (kg; women)=45+0.91× (height in cm -152). Adjusted ideal body weight (AIBW) should be calculated as follows: AIBW= IBW +0.25× (actual weight -IBW). Cyclophosphamide is given on each of two days as a one-hour infusion at a dose of 60 mg/kg beginning on BMT day –3, no sooner than six hours following the 16th dose of Busulfex.

Busulfex clearance is best predicted when the Busulfex dose is administered based on adjusted ideal body weight. Dosing Busulfex based on actual body weight, ideal body weight or other factors can produce significant differences in Busulfex (busulfan) Injection clearance among lean, normal and obese patients.

Busulfex should be administered intravenously via a central venous catheter as a two-hour infusion every six hours for four consecutive days for a total of 16 doses. All patients should be premedicated with phenytoin as busulfan is known to cross the blood brain barrier and induce seizures. Phenytoin reduces busulfan plasma AUC by 15%. Use of other anticonvulsants may result in higher busulfan plasma AUCs, and an increased risk of VOD or seizures. In cases where other anticonvulsants must be used, plasma busulfan exposure should be monitored. Antiemetics should be administered prior to the first dose of Busulfex and continued on a fixed schedule through administration of Busulfex. Where available, pharmacokinetic monitoring may be considered to further optimize therapeutic targeting.

Pharmaceutical Data

The pharmacokinetics of Busulfex was studied in 59 patients participating in a prospective trial of a Busulfex-cyclophosphamide preparatory regimen prior to allogeneic hematopoietic progenitor stem cell transplantation. Patients received 0.8 mg/kg Busulfex every six hours, for a total of 16 doses over four days. Fifty-five of fifty-nine patients (93%) administered Busulfex maintained AUC values below the target value (<1500 μM·min).

Table 8 Steady State Pharmacokinetic Parameters Following Busulfex® (busulfan) Infusion (0.8 mg/kg; N=59)

	Mean	CV (%)	Range
C_{max} (ng/mL)	1222	18	496-1684
AUC (μM·min)	1167	20	556-1673
CL (mL/min/kg)*	2.52	25	1.49-4.31

*Clearance normalized to actual body weight for all patients.

Busulfex pharmacokinetics showed consistency between dose 9 and dose 13 as demonstrated by reproducibility of steady state C_{max} and a low coefficient of variation for this parameter.

In a pharmacokinetic study of Busulfex in 24 pediatric patients, the population pharmacokinetic (PPK) estimates of Busulfex for clearance (CL) and volume of distribution (V) were determined. For actual body weight, PPK estimates of CL and V were 4.04 L/hr/20 kg (3.37 mL/min/kg; interpatient variability 23%); and 12.8 L/20 kg (0.64 L/kg; interpatient variability 11%).

Distribution, Metabolism, Excretion

Studies of distribution, metabolism, and elimination of Busulfex have not been done; however, the literature on oral busulfan is relevant.

Distribution

Busulfan achieves concentrations in the cerebrospinal fluid approximately equal to those in plasma. Irreversible binding to plasma elements, primarily albumin, has been estimated to be 32.4±2.2%, which is consistent with the reactive electrophilic properties of busulfan.

Metabolism

Busulfan is predominantly metabolized by conjugation with glutathione, both spontaneously and by glutathione S-transferase (GST) catalysis. This conjugate undergoes further extensive oxidative metabolism in the liver.

Excretion

Following administration of 14C-labeled busulfan to humans, approximately 30% of the radioactivity was excreted into the urine over 48 hours; negligible amounts were recovered in feces. The incomplete recovery of radioactivity may be due to the formation of long-lived metabolites or due to nonspecific alkylation of macromolecules.

Side Effects

Dimethylacetamide (DMA), the solvent used in the Busulfex formulation, was studied in 1962 as a potential cancer chemotherapy drug. In a Phase 1 trial, the maximum tolerated dose (MTD) was 14.8 g/m2/d for four days. The daily recommended dose of Busulfex contains DMA equivalent to 42% of the MTD on a mg/m2 basis. The dose-limiting toxicities in the Phase 1 study were hepatotoxicity as evidenced by increased liver transaminase (SGOT) levels and neurological symptoms as evidenced by hallucinations. The hallucinations had a pattern of onset at one day post completion of DMA administration and were associated with EEG changes. The lowest dose at which hallucinations were recognized was equivalent to 1.9 times that delivered in a conditioning regimen utilizing Busulfex 0.8 mg/kg every 6 hours ×16 doses. Other neurological toxicities included somnolence, lethargy, and confusion. The relative contribution

of DMA and/or other concomitant medications to neurologic and hepatic toxicities observed with Busulfex is difficult to ascertain.

Treatment with Busulfex at the recommended dose and schedule will result in profound myelosuppression in 100% of patients, including granulocytopenia, thrombocytopenia, anemia, or a combined loss of formed elements of the blood.

Adverse reaction information is primarily derived from the clinical study (N=61) of Busulfex and the data obtained for high-dose oral busulfan conditioning in the setting of randomized, controlled trials identified through a literature review.

Busulfex Clinical Trials

In the Busulfex (busulfan) Injection allogeneic stem cell transplantation clinical trial, all patients were treated with Busulfex 0.8 mg/kg as a two-hour infusion every six hours for 16 doses over four days, combined with cyclophosphamide 60 mg/kg ×2 days. Ninety-three percent (93%) of evaluable patients receiving this dose of Busulfex maintained an AUC less than 1,500 μM·min for dose 9, which has generally been considered the level that minimizes the risk of HVOD.

Table 9 Summary of the Incidence (≥20%) of Non-Hematologic Adverse Events through BMT Day +28 in Patients who Received Busulfex Prior to Allogeneic Hematopoietic Progenitor Cell Transplantation

Non-Hematological Adverse Events*	Percent Incidence (%)
BODY AS A WHOLE	
Fever	80
Headache	69
Asthenia	51
Chills	46
Pain	44
Edema General	28
Allergic Reaction	26
Chest Pain	26
Inflammation at Injection Site	25
Back Pain	23
CARDIOVASCULAR SYSTEM	
Tachycardia	44
Hypertension	36
Thrombosis	33
Vasodilation	25
DIGESTIVE SYSTEM	
Nausea	98
Stomatitis (Mucositis)	97
Vomiting	95
Anorexia	85
Diarrhea	84
Abdominal Pain	72
Dyspepsia	44

Non-Hematological Adverse Events*	Percent Incidence (%)
Constipation	38
Dry Mouth	26
Rectal Disorder	25
Abdominal Enlargement	23
METABOLIC AND NUTRITIONAL SYSTEM	
Hypomagnesemia	77
Hyperglycemia	66
Hypokalemia	64
Hypocalcemia	49
Hyperbilirubinemia	49
Edema	36
SGPT Elevation	31
Creatinine Increased	21
NERVOUS SYSTEM	
Insomnia	84
Anxiety	72
Dizziness	30
Depression	23
RESPIRATORY SYSTEM	
Rhinitis	44
Lung Disorder	34
Cough	28
Epistaxis	25
Dyspnea	25
SKIN AND APPENDAGES	
Rash	57
Pruritus	28

*Includes all reported adverse events regardless of severity (toxicity grades 1-4)

The following sections describe clinically significant events occurring in the Busulfex clinical trials, regardless of drug attribution.

Hematologic
At the indicated dose and schedule, Busulfex produced profound myelosuppression in 100% of patients. Following hematopoietic progenitor cell infusion, recovery of neutrophil counts to ≥500 cells/mm3 occurred at median day 13 when prophylactic G-CSF was administered to the majority of participants on the study. The median number of platelet transfusions per patient on study was 6, and the median number of red blood cell transfusions on study was 4. Prolonged prothrombin time was reported in one patient (2%).

Gastrointestinal
Gastrointestinal toxicities were frequent and generally considered to be related to the drug. Few were categorized as serious. Mild or moderate nausea occurred in 92% of patients in the allogeneic clinical trial, and mild or moderate vomiting occurred in 95% through BMT Day +28;

nausea was severe in 7%. The incidence of vomiting during Busulfex administration (BMT Day –7 to –4) was 43% in the allogeneic clinical trial. Grade 3-4 stomatitis developed in 26% of the participants, and Grade 3 esophagitis developed in 2%. Grade 3-4 diarrhea was reported in 5% of the allogeneic study participants, while mild or moderate diarrhea occurred in 75%. Mild or moderate constipation occurred in 38% of patients; ileus developed in 8% and was severe in 2%. Forty-four percent (44%) of patients reported mild or moderate dyspepsia. Two percent (2%) of patients experienced mild hematemesis. Pancreatitis developed in 2% of patients. Mild or moderate rectal discomfort occurred in 24% of patients. Severe anorexia occurred in 21% of patients and was mild/moderate in 64%.

Hepatic
Hyperbilirubinemia occurred in 49% of patients in the allogeneic BMT trial. Grade 3/4 hyperbilirubinemia occurred in 30% of patients within 28 days of transplantation and was considered life-threatening in 5% of these patients. Hyperbilirubinemia was associated with graft-versus-host disease in six patients and with hepatic veno-occlusive disease in 5 patients. Grade 3/4 SGPT elevations occurred in 7% of patients. Alkaline phosphatase increases were mild or moderate in 15% of patients. Mild or moderate jaundice developed in 12% of patients, and mild or moderate hepatomegaly developed in 6%.

Hepatic veno-occlusive disease
Hepatic veno-occlusive disease (HVOD) is a recognized potential complication of conditioning therapy prior to transplant. Based on clinical examination and laboratory findings, hepatic veno-occlusive disease was diagnosed in 8% (5/61) of patients treated with Busulfex in the setting of allogeneic transplantation, was fatal in 2/5 cases (40%), and yielded an overall mortality from HVOD in the entire study population of 2/61 (3%). Three of the five patients diagnosed with HVOD were retrospectively found to meet the Jones' criteria.

Graft-versus-host disease
Graft-versus-host disease developed in 18% of patients (11/61) receiving allogeneic transplants; it was severe in 3%, and mild or moderate in 15%. There were 3 deaths (5%) attributed to GVHD.

Edema
Patients receiving allogeneic transplant exhibited some form of edema (79%), hypervolemia, or documented weight increase (8%); all events were reported as mild or moderate.

Infection/Fever
Fifty-one percent (51%) of patients experienced one or more episodes of infection. Pneumonia was fatal in one patient (2%) and life-threatening in 3% of patients. Fever was reported in 80% of patients; it was mild or moderate in 78% and severe in 3%. Forty-six percent (46%) of patients experienced chills.

Cardiovascular
Mild or moderate tachycardia was reported in 44% of patients. In 7 patients (11%) it was first reported during Busulfex administration. Other rhythm abnormalities, which were all mild or moderate, included arrhythmia (5%), atrial fibrillation (2%), ventricular extrasystoles (2%), and third degree heart block (2%). Mild or moderate thrombosis occurred in 33% of patients, and all episodes were associated with the central venous catheter. Hypertension was reported in 36% of

patients and was Grade 3/4 in 7%. Hypotension occurred in 11% of patients and was Grade 3/4 in 3%. Mild vasodilation (flushing and hot flashes) was reported in 25% of patients. Other cardiovascular events included cardiomegaly (5%), mild ECG abnormality (2%), Grade 3/4 left-sided heart failure in one patient (2%), and moderate pericardial effusion (2%). These events were reported primarily in the post-cyclophosphamide phase.

Pulmonary
Mild or moderate dyspnea occurred in 25% of patients and was severe in 2%. One patient (2%) experienced severe hyperventilation; and in 2 (3%) additional patients it was mild or moderate. Mild rhinitis and mild or moderate cough were reported in 44% and 28% of patients, respectively. Mild epistaxis events were reported in 25%. Three patients (5%) on the allogeneic study developed documented alveolar hemorrhage. All required mechanical ventilatory support and all died. Non-specific interstitial fibrosis was found on wedge biopsies performed with video assisted thoracoscopy in one patient on the allogeneic study who subsequently died from respiratory failure on BMT Day +98. Other pulmonary events reported as mild or moderate, included pharyngitis (18%), hiccup (18%), asthma (8%), atelectasis (2%), pleural effusion (3%), hypoxia (2%), hemoptysis (3%), and sinusitis (3%).

Neurologic
The most commonly reported adverse events of the central nervous system were insomnia (84%), anxiety (75%), dizziness (30%), and depression (23%). Severity was mild or moderate except for one patient (1%) who experienced severe insomnia. One patient (1%) developed a life-threatening cerebral hemorrhage and a coma as a terminal event following multi-organ failure after HVOD. Other events considered severe included delirium (2%), agitation (2%), and encephalopathy (2%). The overall incidence of confusion was 11%, and 5% of patients were reported to have experienced hallucinations. The patient who developed delirium and hallucination on the allogeneic study had onset of confusion at the completion of Busulfex (busulfan) Injection. The overall incidence of lethargy in the allogeneic Busulfex clinical trial was 7%, and somnolence was reported in 2%. One patient (2%) treated in an autologous transplantation study experienced a seizure while receiving cyclophosphamide, despite prophylactic treatment with phenytoin.

Renal
Creatinine was mildly or moderately elevated in 21% of patients. BUN was increased in 3% of patients and to a Grade 3/4 level in 2%. Seven percent of patients experienced dysuria, 15% oliguria, and 8% hematuria. There were 4 (7%) Grade 3/4 cases of hemorrhagic cystitis in the allogeneic clinical trial.

Skin
Rash (57%) and pruritus (28%) were reported; both conditions were predominantly mild. Alopecia was mild in 15% of patients and moderate in 2%. Mild vesicular rash was reported in 10% of patients and mild or moderate maculopapular rash in 8%. Vesiculo-bullous rash was reported in 10%, and exfoliative dermatitis in 5%. Erythema nodosum was reported in 2%, acne in 7%, and skin discoloration in 8%.

Metabolic
Hyperglycemia was observed in 67% of patients and Grade 3/4 hyperglycemia was reported in 15%. Hypomagnesemia was mild or moderate in 77% of patients; hypokalemia was mild or moderate in 62% and severe in 2%; hypocalcemia was mild or moderate in 46% and severe in 3%; hypophosphatemia was mild or moderate in 17%; and hyponatremia was reported in 2%.

Other
Other reported events included headache (mild or moderate 64%, severe 5%), abdominal pain (mild or moderate 69%, severe 3%), asthenia (mild or moderate 49%, severe 2%), unspecified pain (mild or moderate 43%, severe 2%), allergic reaction (mild or moderate 24%, severe 2%), injection site inflammation (mild or moderate 25%), injection site pain (mild or moderate 15%), chest pain (mild or moderate 26%), back pain (mild or moderate 23%), myalgia (mild or moderate 16%), arthralgia (mild or moderate 13%), and ear disorder in 3%.

Deaths
There were two deaths through BMT Day +28 in the allogeneic transplant setting. There were an additional six deaths BMT Day +29 through BMT Day +100 in the allogeneic transplant setting.

Post-Marketing Experience
The following adverse reactions (reported as MedRA terms) have been identified during post-approval use of Busulfex (busulfan) Injection: febrile neutropenia; tumor lysis syndrome; thrombotic micro-angiopathy (TMA); severe bacterial, viral (e.g., cytomegalovirus viraemia) and fungal infections; and sepsis. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to establish a causal relationship to drug exposure.

Clinical Applications
Busulfex® (busulfan) Injection is indicated for use in combination with cyclophosphamide as a conditioning regimen prior to allogeneic hematopoietic progenitor cell transplantation for chronic myelogenous leukemia.

7 CORRELATIVE BIOMARKER STUDIES

The correlative biomarker study (MPD-RC 107) will evaluate a series of biomarkers at baseline (at study entry), day -37 (pre-transplant), day +100 (post-transplant), after months 6, 12, 24 (post-transplant) and either at the time of relapse/progression or at termination of study (that is off study drug for any reason).

7.1 Central Morphological Review of Bone Marrow Aspirate Smears and Biopsies for Patients Entering MPD-RC Treatment Studies

7.1.1 Bone Marrow and Peripheral Blood Histopathology

(See MPD-RC protocol 107, for more detailed information)

At baseline (entry into the trial), at day +100 (post-transplant), and at the end of 6, 12, 24 months (post-transplant), and at termination of study for any reason the following bone marrow aspiration and biopsy specimens must be obtained and submitted for central review:

- three (3) air-dried, unstained bone marrow aspirate slides (if available)
- four (4) unstained peripheral blood films (thin smears)
- two (2) air dried unstained bone marrow biopsy touch preps for patients with no aspirate sample (these are made with 8 touches per slide – 4 horizontal touches in 2 rows on the slide)

Additionally:

- three (3) unstained paraffin-fixed bone marrow biopsy slides each containing at least 2 sections
- one (1) stained H & E biopsy slide
- one (1) biopsy slide stained for iron
- one (1) biopsy slide stained with the silver for reticulin fibrosis stain should be submitted for confirmatory cytologic and cytochemical studies. These should be submitted together with the final institutional pathology, cytochemistry, and immuno-phenotyping reports (if possible).

Bone marrow studies should be repeated at day 100, and at the end of 6, 12 and 24 months following treatment and aspirate and biopsy specimens as described above again submitted for central review. They should also be repeated if relapse occurs or transformation to MDS, myelofibrosis or acute leukemia is suspected.
The histopathology slides may be batch shipped every 3 Months to Dr. Weinberg in North America or Dr. Salmoiraghi in Europe.

There must be an adequate amount of marrow biopsy specimen in each smear. The above requirements are considered minimal. Contact the MPD-RC Study Chair with any questions.

Identify each slide with the patient's MPD-RC patient ID number, protocol number, time point, and type of sample. Pack carefully in protective slide cartons (not cardboard folders), and mail to:

(North America)
Rona Singer Weinberg, Ph. D
New York Blood Center
310 East 67th Street, Room 2-44
New York, New York 10065
Phone: (212) 570-3488
Fax: (212) 570-3495
rweinberg@nybloodcenter.org
Laboratory Phone: (212) 570-3412
Laboratory Fax: (212) 570-3495
MPDLab@nybloodcenter.org

Office hours: Monday through Friday 9AM to 5PM EST. Accepting samples Monday through Friday (up until 12 noon on Fridays only). **For shipments to be received at other times, contact Dr. Weinberg in advance of collection of samples and shipment.**

Or

(Europe)
Dr. Silvia Salmoiraghi
MPD-RC Tissue Bank
Azienda Ospedaliera Papa Giovanni XXIII
Laboratorio Paolo Belli USC Ematologia
Torre 7, Ingresso 55, piano 1
Piazza OMS- Organizzazione Mondiale della Sanità,1
24127 Bergamo
Phone: +39 035-2673769
lab.paolobelli@hpg23.it

Office hours: Monday through Friday 9AM to 5PM. Accepting samples Monday through Friday (up until 12 noon on Fridays only). **For shipments to be received at other times, contact the. Office in advance of collection of samples and shipment.**

Send a de-identified copy of the institutional bone marrow aspiration and biopsy report generated at your institution as soon as it is complete to the MPD-RC central laboratory in New York or Bergamo, Italy. This report must include differential cell counts on the marrow aspirate, if performed.

7.1.2 Cytogenetics and FISH Cytogenetic and FISH Requirements

Diagnostic and follow up cytogenetics and or FISH will be performed by local institutional laboratories. All karyotypes will be centrally reviewed (see below). To ensure uniformity of cytogenetic preparations all participating local laboratories must adhere to the following requirements established by the central karyotype review committee.

General Inclusion Criteria for Conventional Cytogenetics

1. Banding level 300 or more and structural aberrations are accepted at 400 or higher banding level or confirmed by FISH.
2. Minimum requirements for baseline assessments: From each patient, bone marrow (PV, ET) and or unstimulated PB (PMF) specimen must be set up into two, preferably three, different cultures (direct, 24 hrs with and without marrow max media). Every effort should be made to obtain the growth of cells that are cytogenetically abnormal.
3. 20 metaphase evaluated from two or more cell cultures or from marrow max culture only.
4. Only G-banding of chromosomes is acceptable. Other banding methods are not acceptable.
5. Analysis of cell at 300 band resolution, or low quality banding, irrespective of the findings, to be complemented with FISH screening for subtle aberrations, including del(20)(q11q13) with D20S108 probe.
6. ISCN 2009 must be used to describe the karyotype.
7. Aberrations classified as clonal should be present in at least 2 cells by G-banding or confirmed by FISH.
8. Unusual or novel findings should be fully characterized by FISH using appropriate probes.
9. Three karyotypes (and metaphase cells) from each patient must be uploaded electronically into the MPD-RC database to document the stated cytogenetic diagnosis. The karyotypes are uploaded either as TIFF or JPEG files.

FISH studies, IF POSSIBLE and recommended

10. Case normal by G banding, if possible, ought to be screened for cryptic changes by interphase FISH using a MPN panel of 12 probes: CEP1(1q12)/1q21, 5p15.2/5q31(*EGR1*), CEP7/7q31, CEP8, CEP9/9p21, *RB1* at 13q14, P53/CEP17, 20q12/*D20S108*.
11. FISH studies are performed on BM or PB on interphase cells processed directly (no culture) using Abbott Molecular FISH probes. A minimum of 200 cells at baseline should be scored by two individuals. Image of FISH results should be uploaded as JPEG or TIFF file into the MPD-RC database.

Central Karyotype Review Committee consists of Vesna Najfeld, Ph.D., Director, Tumor Cytogenetics, The Mount Sinai Medical Center, NY (chair) Elisabeth Nacheva, MD, Ph.D, FRC Path, Director of Cytogenetics, UCL Medical School, Royal Free Campus, London, UK, Cristina Mecucci, MD, PhD, Director of Cytogenetic laboratory, University of Perugia, Perugia, Italy and Ursula Giussani, Ph.D., Director of Laboratorio di Genetica Medica, Bergamo, Italy. Central karyotype review committee will have biannual teleconferences for central karyotype review and will meet annually or to resolve difficult karyotypes, perform QC, discuss correlative studies with hematological response and other biomarkers. Karyotypes that were not uploaded or faxed pictures of karyotypes cannot be reviewed.

One of the **QC indicators** is a proper entry and submission of cytogenetic forms and karyotypes by the institutional cytogenetic laboratories. If the local laboratory fails two consecutive specimens (poor quality of chromosome banding, missed abnormality, wrong ISCN nomenclature) or lack of entering cytogenetic results into MPD- RC, the laboratory will be informed, and the third failure will result in placing the local laboratory on probation and the institutional PI will be informed. If required, either the central lab in NY or other MPD-RC approved cytogenetic lab will perform the studies from the institution whose cytogenetic lab is placed on probation. Each institution in the RC will receive quarterly information regarding the cytogenetic entry results on the MPD-RC database.

For any questions please call Joe Tripodi MS, Cytogenetic Research Coordinator, Mount Sinai Medical Center: **212 241-8801** or Dr. Vesna Najfeld at **212 241-8801.**

7.2 Schedule of Samples for Correlative Studies

7.2.1 Samples to be collected

The following samples noted in Table 10 are to be collected at baseline (entry onto trial), day -65 (pre-transplant), day -37 (pre-transplant), day -5 (pre-transplant), day +30 (post-transplant) at day +100 (post-transplant), the end of month 6 (post-transplant), end of month 12 (post-transplant), end of month 24 (post-transplant), and at relapse or off treatment for any reason.

Table 10 Correlative Studies Sample Collection Schedule

	Sample		Baseline	Day -65	Day -37	Day -5	Day +30	Day +100	End of 6 Months	End of 12 months	End of 24 Months	Relapse/ off treatment[4]
Bone Marrow	**Aspirate** Histopathology[1]	Three (3) air dried unstained slides	X					X	X	X	X	X
	Aspirate Cytogenetics[2]	At local institution, upload data and karyotypes to the website[1]. If no aspirate, provide peripheral blood karyotypes. Karyotype at follow up only need to be repeated if abnormal prior to transplant	X					X	X	X	X	X
	Aspirate Biomarkers[3]	2 to 5 mL of bone marrow aspirate in a green top, heparinized tube (BD catalog #366480)	X					X	X	X	X	X
	Biopsy Histopathology[1]	One (1) H & E stain	X					X	X	X	X	X
		One (1) Iron stain	X					X	X	X	X	X
		One (1) Silver impregnated reticulin stain	X					X	X	X	X	X
		Three (3) Unstained paraffin-fixed slides each with at least 2 sections	X					X	X	X	X	X
		Three (3) air-dried, unstained bone marrow aspirate slides (if available)	X					X	X	X	X	X
		If no aspirate, two (2) air dried unstained touch preps, 8 touches/slide, 2 horizontal	X					X	X	X	X	X

	Sample		Baseline	Day -65	Day -37	Day -5	Day +30	Day +100	End of 6 Months	End of 12 months	End of 24 Months	Relapse/ off treatment[4]
Peripheral Blood	Histopathology[1]	Four (4) Unstained peripheral blood films, thin smears	X					X	X	X	X	X
	Cytogenetics[2]	If no BM aspirate karyotypic analysis[1]	X					X	X	X	X	X
	Biomarkers[3]	Hematocrit ≤ 32, 37 mL:										
		Three (3) ACD: yellow (BD #364606; 8.5 mL)	X					X	X	X	X	X
		One(1) EDTA: lavender (BD #366450; 8.5 mL)	X					X	X	X	X	X
		One (1) sodium citrate: light blue (BD #366415; 4.5 mL)	X					X	X	X	X	X
		Hematocrit > 32, 67 mL:										
		Five(5) ACD: yellow (BD #364606; 8.5 mL)	X					X	X	X	X	X
		One(1) EDTA: lavender (BD #366450; 8.5 mL)	X					X	X	X	X	X
		Four (4) sodium citrate: light blue (BD #366415; 4.5 mL)	X					X	X	X	X	X
	Cytokines	Two (2) ACD: yellow (BD #364606; 8.5 mL)		X[5]	X	X	X	X				

	Sample		Baseline	Day -65	Day -37	Day -5	Day +30	Day +100	End of 6 Months	End of 12 months	End of 24 Months	Relapse/ off treatment[4]
Other	Nails[3]	2 clippings (pre-treatment, at enrollment only)	X									

1 **All slides must be registered via the MPC-RC website (consult the MPD-RC website under the tissue bank section for diagram on labeling slides) the day they are collected and prior to shipment.** All slides MUST BE LABELED properly. Labels are to include the patient's tissue bank ID number (TB ID), protocol number (e.g., Protocol 114), time of study (e.g., baseline, 6 months), type of specimen (e.g., BM biopsy, BM aspirate, blood), type of stain (e.g., Iron, H& E). For unstained slides, use a pencil to label the slides. All slides are to be shipped to the tissue bank laboratory in a labeled slide box. The label on the box is to include the patient's TB ID, protocol number, and time of study. Slides may be shipped in batches together with the local institution's histopathology report. All slides are shipped to Dr. Rona Singer Weinberg (North America) or Dr. Silvia Salmoiraghi (Europe). **See MPD-RC 107, Section 5.0 Procurement for additional labeling and shipping information.**

2 Cytogenetic analyses are performed as a standard of care at the institution where the patient is being treated. Karyotypic analysis of unstimulated bone marrow after direct 24 and 48 hours, if available, or peripheral blood shall be performed. Karyotypic analysis using G-banding technology is required. Data and karyotypes are uploaded to the website. See MPD-RC 107, Section 5.0 Procurement for additional reporting information.

3 **All tissue samples must be registered via the MPC-RC website the day they are collected and prior to shipment.** All tubes, and envelopes (nails) must be labeled with patient patient's tissue bank ID number (TB ID), protocol number (e.g., Protocol 114), sample ID number (provided by the data bank at the time of registration), time of study (e.g., baseline, 6 months), type of specimen (e.g., BM biopsy, BM aspirate, blood) and date of collection.

4 At relapse/progression or off treatment as defined in section 4.7. If the subject experiences secondary graft failure or fails to meet the primary objective after transplant, the patient will be followed every 6 months for survival until the end of the study. The survival data can be collected via telephone call.

5 Cytokines collected on Day -65 must be collected **BEFORE** start of study drug treatment

Fresh blood and bone marrow are to be shipped at ambient temperature and must be shipped the day that they are collected.

Nails are to be shipped at ambient temperature and may be shipped together with blood and bone marrow samples or subsequently.

All slides are to be shipped to the tissue bank laboratory in a labeled slide box. The label on the box is to include the patient's TB ID, protocol number, and time point of study. Slides may be shipped in batches together with the local institution's histopathology report. All slides are shipped to Dr. Rona Singer Weinberg (North America) or Dr. Silvia Salmoiraghi (Europe). For additional labeling and shipping information (see Protocol 107 section 5.0 Procurement).

7.2.2 Collection of Biomarker Samples

Please see Table 10 for various time points for collection of samples (also see Protocol 107, Appendix 10.3 for the time points to obtain samples). The samples will be separated as follows.

Bone Marrow

Bone marrow aspirate samples will be collected for histopathology, biomarkers, and cytogenetics/FISH. For biomarkers and cytogenetics, obtain a total of 3-7 mL of aspirated bone marrow. Samples are to be divided as follows:

- 2-5 mL of bone marrow aspirate in a green top, heparinized tube (BD catalog number 366480) to be sent to tissue bank
- 1-2 mL of bone marrow aspirate for Cytogenetics to be done locally

Peripheral Blood: (use sterile technique)

Up to 67 mL of blood:

- For hematocrit ≤ 32, 37 mL of blood: three ACD (yellow top) tubes (BD catalogue number 364606; 8.5 mL tubes), one EDTA (lavender top) tube (BD catalog number 366450), and one sodium citrate (light blue top) tube (BD catalogue number 366415, 4.5 mL).
- For hematocrit > 32, 67 mL of blood: Five ACD (yellow top) tubes (BD catalogue number 364606; 8.5 mL tubes), one EDTA (lavender top) tube (BD catalog number 366450), and four sodium citrate (light blue top) tubes (BD catalogue number 366415, 4.5 mL).

Nail Clippings:

Two nail clippings are to be obtained from each patient during the initial entry onto the protocol. Clippings are to be placed in a paper envelope and the envelope is to be sealed. The sealed envelope may be stored and shipped at room temperature.

7.3 Shipping Samples for Correlative Studies

All shipments must be sent by overnight courier for delivery before 10 AM or morning delivery the next day. For Friday shipments (to arrive on Saturday), notify the laboratory prior to collection of tissue and shipment. For shipments sent on Friday for

Saturday delivery be certain to request Saturday AM delivery directly to the lab. It is not possible for lab personnel to go to the courier's office for pickup. See section 7.1 for shipping address.

7.4 Clonality

Applicable to informative females = probable 50 – 60% of patients. Sample amount for this test is already included in the samples being collected. No further sample collection needs to be done for this test.

7.5 MPN Associate Symptoms and Quality of Life

We will measure myelofibrosis associated symptoms, general QOL, treatment-relevant QOL and related symptoms prior to start of conditioning therapy (days -65 and -9, pre-transplant); from start of conditioning and post BMT follow-up (days -5, +30, and +100; months +6, +12, and yearly afterwards); and at time of relapse/progression and study termination for any reason. See sections Table 12 and Table 13 for schedules.

The following instruments will be used to measure QOL:

MPN-SAF:
The Myeloproliferative Neoplasm Symptom Assessment Form (MPN-SAF) was developed as a simple, concise set of 18 questions to assess constitutional symptoms and QOL in myeloproliferative neoplasms including MF, PV and ET (Appendix 5). Administered along with the 10- item Brief Fatigue Inventory (BFI) (27) it has been validated against the EORTC QLQ-C30 and available in multiple languages (English, Italian, Swedish, French, German, Dutch and Spanish). Additionally, this measure has become the standard uniform symptom assessment tool for MPN patients participating in clinical trials and aids in guiding clinical decisions, particularly in situations where objective and subjective patient complaints are discrepant. The assessment evaluates the following parameters on a linear analog self-assessment scale (LASA 0-10 scale): fatigue (from the BFI), catabolic/proliferative symptoms (night-sweats, itching, bone pain, fever, weight-loss), pulmonary symptoms (dyspnea, cough, insomnia), splenomegaly, pain mobility, and gastrointestinal effects. All of the symptoms are assessed on a clear linear scale, which has been proven to be valid in measuring symptoms in cancer patients with single item scales for fatigue, pain, stress, and anorexia (28).

EORTC QLQ-C30:
The EORTC-QLQ-C30 version 3.0 (Aaronson*, et al* 1993) (Appendix 6) is included as an instrument for co-validation on these trials, and redundant capture of impact of information for impact of protocol therapy on patient's symptoms, mood, and quality of life. The initial EORTC QLQ-C36 questionnaire, later shortened and validated with 30 questions, has the following advantages namely being a) cancer specific b) multidimensional in structure c) appropriate for self- administration d) applicable across a range of cultural settings. This instrument has been thoroughly validated for serial use in cancer clinical trial setting, and is available in standard and validated translations for Italian, French, Swedish, and German (all languages required for the enrolling centers on these trials). This instrument although highly valid for a portion of the symptom changes we wish to capture with this trial, nevertheless is not as comprehensive for MPN specific symptoms and hence the need for both instruments to be administered.

FACT-BMT:

The Functional Assessment of Cancer Therapy-Bone Marrow Transplant (FACT-BMT) is a 50-item validated self-report questionnaire specifically designed to test the quality of life in BMT patients (Appendix 7) (29). The FACT-BMT is composed of five subscales including physical well-being (PWB), social/family well-being (SFWB), emotional well-being (EWB), functional well-being (FWB), and additional concerns specific to patients undergoing BMT. Each question has response categories of "Not at all", "A little bit", "Somewhat", "Quite a bit", and "Very much". Higher scores identify better quality of life. It is able to be completed in 5-10 min and may be performed in interview format or self-administered. Question composition assesses how the patient feels today and over the previous seven days. The questionnaire will be scored according to the published scoring algorithm and the following scales and subscales will be utilized in the current study: FACT-BMT (PWB + SFWB + EWB + FWB + Additional concerns), FACT-G (PWB + SFWB + EWB + FWB), Trial Outcome Index (TOI; PWB + FWB + Additional concerns), PWB, SFWB, EWB, FWB, and Additional concerns.

Global Assessment of Change:

The Patient Global Impression of Change (PGIC) is a 7-point scale used by patients to rate the change in their emotional or physical condition or overall quality of life (Appendix 8) (30). It assesses symptom by gauging whether the patient feels (1) Very much improved, (2) Much improved, (3) Minimally improved, (4) No change, (5) Minimally worse, (6) Much worse, (7) Very much worse. Categorization of patients into these three groups will use the patient's response during the second administration to the physical condition item of the Global Impression of Change. Worsening will include "Very much worse", "Moderately worse", and "A little worse".

The same battery of QOL questionnaire will be administered at all the time points as mentioned above.

7.6 Biomarker Evaluations

The biomarkers that will be monitored in this trial at baseline (at trial entry), day -37 (pre-transplant), day +100 (post-transplant), after months 6, 12, 24 (post-transplant), and at time of relapse/progression or at the termination of the patients receiving study medication are listed in Table 10**Error! Reference source not found.**. In each disease stratum the proportions of patients on each treatment arm who are abnormal at baseline will be estimated and changes over time will be examined. The JAK2V617F allele burden will be monitored at baseline (at trial entry), day +100 (post-transplant), and at the end of 6, 12, 24 months (post-transplant) and at relapse/progression or at the termination of the patients receiving study medication. The cytogenetics and bone marrow histopathology will be performed at baseline (at trial entry), day +100 (post-transplant), and at the end of 6, 12, 24 months (post-transplant) and at time of relapse/progression or at the termination of the patients receiving study medication. The hematopoietic cell clonality will be performed in select female patients who are informatives at baseline (at trial entry), remission, progression/relapse, 12 and 24 months (post-transplant) and termination of treatment. The anticipated effects of the treatments provided on the various biomarkers are summarized in Table 11.

Table 11 Biomarker Evaluations

Test	**Result**	**Modulation**
Clonality	Clonal	Polyclonal JAK2V617F
Allele	High	Low or absent
Cytogenetics	Abnormal	Normal
Bone Marrow Histopatholgy	Abnormal Morphology	Normalization

8 STUDY CALENDAR

8.1 Screening and Enrollment

Baseline Data

All patients should be assessed for their eligibility in the study. At this time, the following procedures should be performed and appropriately recorded into the source notes:

- Patient Informed Consent
- History, physical examination, and transfusion history
- Vital signs
- Body Surface area
- ECOG performance status
- ECG
- Splenic size by clinical examination and maximum length by ultrasound examination.
- CBC (Complete Blood Count / Differential)
- Biochemistry screen (AST, ALT, T Bili, D Bili, alk. phosphatase, albumin, total protein, Ca+2, Phosphorous, LDH, creatinine, BUN, chloride, potassium, sodium, glucose, uric acid, CO2 total)
- PT/PTT and INR
- Thyroid function tests T3, T4, TSH
- Negative serum pregnancy test
- Bone marrow aspirate and biopsy
- Cytogenetics
- Biomarker blood samples for MPD-RC 107
- Blood sample for JAK2V617F
- Concomitant medication
- Nail Clippings
- MUGA / ECHO scan
- Pulmonary Function test (PFT)
- Urinalysis
- CD34+ (peripheral blood)
- Class I and II antigens (molecular typing)
- PB Chimerism studies
- ABO and Rh typing
- Infectious disease screen:
    - Serum CMV, HSV, EBV
    - Hepatitis A, B, and C tests

Study requirements must be performed within 14 days prior to registration except for the following:

- **bone marrow, cytogenetics, and infectious disease screen which must be done within 28 days prior to registration**
- **MUGA/ECHO scan and pulmonary function test which must be done within 2 months prior to registration**
- **JAK2V617F; Class I and II antigens (molecular typing); PB Chimerism studies, and ABO and Rh typing do not have to be repeated if already performed**

8.2 Donor Assessment

Donor work up will be done according to the institution's standard of care, and the following information will be captured in the eCRFs:

- Donor's age
- Donor's sex
- If donor is female, number of pregnancies
- Donor type (related, unrelated)
- Donor blood group
- Donor rhesus factor
- Donor HLA typing
- Donor Antibodies and Antigens
- Donor Cytokines (if administered or not)

8.3 Assessments prior to start of conditioning therapy (Day-65 to Day -5)

Day -65

Subjects will be seen by the Study Doctor and the following procedures will be performed:

- A physical examination including a review of current medical conditions, transfusion history, spleen palpation, concomitant medications, measurement of weight, blood pressure, heart rate
- ECOG status
- Completion of brief surveys (Symptom assessment package) about symptoms
- CBC, Biochemistry
- Cytokine profile (must be collected before start of study drug treatment)

Days -63, -55, -48, -41, -34, -27, -20, -13

These visits are only required if platelet count drops to <50,000/uL or <50 x 109/L. The following test will be performed:

- CBC

Days -58, -51, -44, -30, -23, & -16

During these study visits, the following tests and procedures will be performed:

- CBC, Biochemistry
- An assessment for any signs of harmful effects the drug or any additional problems caused by the medical treatment
- Patients' condition will be assessed to determine if dose of Ruxolitinib needs to be adjusted

Day -37

During this study visit, study subjects will meet with the study doctor and the following tests and procedures will be performed:

- CBC, Biochemistry
- Cytokine profile
- An assessment for any signs of harmful effects the drug or any additional problems caused by the medical treatment
- Condition will be assessed to determine if dose of Ruxolitinib needs to be adjusted, and if are having a positive response to Ruxolitinib
- A physical examination including a review of current medical conditions, transfusion history, spleen palpation, medications you are taking, measurement of your weight, blood pressure, heart rate
- ECOG status
- Review of the Ruxolitinib medication to ensure patients have been compliant with taking the medication
- Evaluation of patients' condition to determine if they are responding well to the study drug treatment

Day -9 (Ruxolitinib tapering)

Day -9 will begin the start of the 4 days of tapering (reducing the amount) of the Ruxolitinib drug until it is stopped completely on day -5, at the start of the conditioning therapy prior to the stem cell transplant.

During the Day -9 study visit, the following tests and procedures will be performed:

- CBC, Biochemistry
- A physical examination including a review of current medical conditions, transfusion history, spleen palpation, medications you are taking, measurement of your weight, blood pressure, heart rate
- ECOG status
- Completion of brief surveys (Symptom assessment package) about symptoms
- Pregnancy test - Serum HCG: for women of childbearing potential. Women will not be allowed to participate if they are pregnant or nursing. Both men and women who are able to have children must agree to use an effective form of birth control (hormonal or barrier method of birth control, or abstinence) from the time of screening and throughout their participation in the study.
- Review and instruct patients on the tapering schedule of the Ruxolitinib study drug.

If study subjects begin taking Ruxolitinib, and then need to discontinue taking the medication due to harmful effects it is causing and don't proceed with receiving a bone marrow transplant for any reason (i.e. donor drops out) OR have disease progression, they will have an end of study visit, 30 days after having taken the last dose of study medication. During this study visit the following tests and procedures will be performed:

- A physical examination including a review of current medical conditions, transfusion history, spleen palpation, medications you are taking, measurement of your weight, blood pressure, heart rate
- ECOG status
- CBC, Biochemistry
- The harmful effects of the drug that patients are experiencing caused by the medical treatment will be assessed.

If study subjects begin taking Ruxolitinib, and then need to discontinue taking the medication due to harmful effects it is causing, BUT proceed with receiving a bone marrow transplant, they will continue to be followed in the study.

Should study subjects complete the Ruxolitinib portion of the therapy, they will be required to be hospitalized for approximately a month for the Reduced Intensity Conditioning and Bone Marrow Transplant. In the first five days they will receive the reduced intensity conditioning regimen, the purpose of which is to improve their myeloproliferative disorder and to suppress immunity so that the stem cell transplant is not rejected by their immune system. Depending on whether the study subjects will receive stem cells from a related or unrelated donor, they will be assigned to one of the two following study treatment conditioning regimens: Fludarabine with busulfan OR fludarabine with busulfan and ATG. ATG is only given to subjects undergoing unrelated donor transplantation.

Fludarabine will be given by IV infusion (through a needle placed in the vein) daily for 4 days (Days -5 to -2). Busulfan will then be given by IV infusion into a vein, daily for 4 days (Days -5 to -2). To allow convenient administration of these two study drugs, study subjects will receive these drugs through a central line. See section 4.3 and Table 5 for conditioning therapy instructions.

If stem cells are received from an unrelated or mismatched donor, study subjects will be given an additional study drug named “anti-thymocyte globulin” or ATG, on 3 consecutive days before the stem cells are given (Days -3 to -1). This drug further weakens the immune system to reduce the chances of rejection of the transplant (graft), as well as reducing the chance that the graft cells attack the body’s cells, a condition known as graft-versus-host disease. ATG is only given to subjects undergoing unrelated donor transplantation. See section 4.4 and Table 5 for further details.

Table 12 Schedule of assessments prior to start of conditioning therapy

All study visits after Day -65 and before Day-9 have a window of ± 2 days.

Assessments	Baseline [a]	Day -65 (Start of RUX)	Day -63, -55, -48, -41, -34, -27, -20, -13 [b]	Day -58	Day -52	Day -44	Day -37	Day -30	Day -23	Day -16	Day -9 (RUX tapering)	End of Treatment visit [k]	30 Day Follow-up visit [l]	Off-treatment follow-up [m]
Informed consent/eligibility	X													
Medical History	X						X				X	X	X	
Concomitant Medications	X	X					X				X	X	X	
Transfusion History [c]	X	X					X				X	X	X	
Physical Examination	X	X					X				X	X	X	
Palpable Spleen Size	X	X					X				X	X	X	
Co-morbidity evaluation [d]	X													
ECOG status	X	X					X				X	X	X	
MUGA scan / ECHO	X [a]													
PFT	X [a]													
EKG	X													
Symptom assessment package & QOL Instruments		X [e]									X			
Samples for Cytokines [f]		X [f]					X [f]							
Serum Pregnancy test (non-menopausal only)	X										X			
Urinalysis	X													
CBC (Complete Blood Count/ Differential)	X	X	X	X	X	X	X	X	X	X	X	X	X	
Biochemistry [g]	X	X		X	X	X	X	X	X	X	X	X	X	
Infectious disease screen [h]	X													
CD34+ (peripheral blood)	X													
PT/PTT	X													
Thyroid function tests (T3, T4, TSH)	X													
Class I and II antigens (molecular typing)	X [a]													
ABO and Rh typing	X [a]													
Bone Marrow Aspirate and Biopsy [i]	X [a]													
Cytogenetics	X [a]													
JAK 2 mutation	X [a]													
PB Chimerism studies	X [a]													
Hematological toxicities		X	X	X	X	X	X	X	X	X		X	X	
Non-hematological toxicities		X		X	X	X	X	X	X	X		X	X	
Infectious Complications		X		X	X	X	X	X	X	X				
Study Drug accountability							X					X		
Dose adjustment of INC424 (if required)				X	X	X	X	X	X	X				
Tapering schedule of INC424											X			
Research samples for MPD-RC tissue bank [j]	X [j]											X [j]		
IWG response							X							
Survival and Progression of disease														Every 6 months from treatment d/c

a Study requirements must be performed within 14 days prior to registration except for the following:
- bone marrow, cytogenetics, and infectious disease screen which must be done within 28 days prior to registration
- MUGA/ECHO scan and pulmonary function test which must be done within 2 months prior to registration
- JAK2V617F; Class I and II antigens (molecular typing); PB Chimerism studies and ABO and Rh typing do not have to be repeated if already performed

b only required if platelet count drops to <50,000/uL or <50 x 10^9/L
c Blood and platelet transfusions in the preceding 3 months
d Co-morbidity evaluation by HCI-CI criteria as described by Sorror et al.
e Symptom assessment package and QOL instruments to be done before start of study drug treatment.
f Sample needs to be shipped to reference laboratory, please see Table 10
g AST, ALT. T Bili, D Bili, alkaline phosphatase, albumin, total protein, Ca+2, Phosphorous, LDH, PT/PTT, CO2 total, creatinine, BUN, potassium, sodium, glucose, uric acid level
h infectious disease screen includes: Serum CMV, HSV, EBV; Hepatitis A, B, and C
i Sample needs to be shipped to reference laboratory, please see Table 10
j MPD-RC research samples specified in Table 10. Samples need to be shipped to reference laboratory, please see Table 10
k End of treatment as defined in section 4.7
l this visit occurs 30 days (±7 days) after end of treatment. Treatment defined in section 4.7.
m please see as described in section 4.7

8.4 Assessments from start of conditioning (Day -5) to post BMT follow-up (+48 months)

Day -5 study requirements

During Day -5, when study subjects will begin the additional chemotherapy regimen, they will have the following additional tests and procedures performed:

- A physical examination including a review of current medical conditions, transfusion history, spleen palpation, medications they are taking, measurement of their weight, blood pressure, heart rate
- ECOG status
- Completion brief surveys (Symptom assessment package) about symptoms
- CBC, Biochemistry
- Cytokine profile
- Research samples for MPD-RC Tissue Bank
- Study subjects will be assessed for any signs of harmful effects from the study drug or any additional problems caused by the medical treatment
- Evaluation of the study subjects' condition to determine if they responded well to the study drug treatment

Reduced Intensity Transplant (Standard of Care)

Two days after the last dose of fludarabine and busulfan, on the day of transplant (Day 0), the donor stem cells will be infused (through the central IV line).

Day 0 study requirements (Day of Transplant)

During Day 0, study subjects will have the following additional tests and procedures performed before the transplant:

- A physical examination
- CBC, Biochemistry
- They will be assessed for any signs of harmful effects the drug or any additional problems caused by the medical treatment

Post-Transplant (Standard of Care)

Immediately after the Reduced Intensity transplant, study subjects will receive two FDA approved standard drugs to reduce the risks of graft versus host disease - tacrolimus/cyclosporine and methotrexate. Beginning the 2 days before the transplant (day -2) and continuing for at least 180 days after the transplant, study subjects will take the drug known as tacrolimus to reduce the activity of the immune system and to lower the risk of transplant rejection. Tacrolimus will be given by an intravenous infusion only for the first 3 days. Then, for the remaining 180 days, study subjects will take tacrolimus orally as a tablet. They will take tacrolimus tablets twice a day (the number of tablets taken depends on their weight) or IV cyclosporine per institutional practice. These drugs will help prevent the body from rejecting the cells received from the donor. Methotrexate will be given intravenously on days 1, 3 and 6 after transplant. This drug will help prevent the body from rejecting the cells received from the donor. If a study subject's disease were to return or persist after reduced intensity transplant, or if their body does not successfully "take" (accept) the transplanted donor cells, the study subject may receive additional infusions of stem cells from their donor at two-month intervals without any study treatment conditioning regimen.

If the transplant is an effective treatment of the underlying myeloproliferative neoplasm, it will result in production of normal blood. It usually takes approximately two to three weeks for the donor stem cells to produce blood. During this time, study subjects will be dependent on transfusion of blood and platelets and will need antibiotics to prevent and/or treat infections. They will remain in the hospital until the blood counts are fully recovered and they are in good clinical condition. Usually this means a hospital stay of at least three or four weeks after the transplantation.

After the transplantation, they will be seen regularly by the study doctor to have frequent blood tests, similar to the ones in the Ruxolitinib phase.

Post-transplant study requirements (Day +30 to Month +48)

After the stem cell transplant, and after treatment in the hospital, study subjects will be seen by the study doctor and study team at specific intervals: Day +30, +60, +100, 6 months, 12 months, 18 months, 24 months, 36 months, and 48 months. At all of these study visits, the following tests and procedures will be performed:

- A physical examination including a review of current medical conditions, transfusion history, spleen palpation, medications, measurement of weight, blood pressure, heart rate
- ECOG status
- CBC, Biochemistry
- They will be assessed for any signs of harmful effects of the drug or any additional problems caused by the medical treatment
- They will be assessed to see whether or not their body is experiencing Graft Versus Host Disease (GVHD), a side effect of bone marrow or stem cell transplantation. In cases of GVHD, the new donor cells treat the body as "foreign" and launch an attack against it.

Additionally, on these specific study visits, the following additional tests and procedures will be done:

Day +30
- Cytokine profile
- Symptom assessment package
- ABO and Rh typing
- PB Chimerism studies

Day +60
- ABO and Rh typing
- PB Chimerism studies

Day +100
- Symptom assessment package
- Cytokine profile
- Bone marrow aspiration/biopsy/cytogenetics/clonality
- Research samples for the MPD-RC Tissue Bank
- PB Chimerism studies
- Evaluation of their condition to determine if they are responding well to the study drug treatment

+6 months and +12 months
- Symptom assessment package
- JAK2 V617F blood test
- Research samples for the MPD-RC tissue bank
- Bone marrow aspiration/biopsy/cytogenetics/clonality
- PB Chimerism studies
- Evaluation of their condition to determine if they are responding well to the study drug treatment

+18 months
- Evaluation of their condition to determine if they are responding well to the study drug treatment

+24 months
- Symptom assessment package
- JAK2 V617F blood test
- Research samples for the MPD-RC tissue bank
- Bone marrow aspiration/biopsy/cytogenetics/clonality
- PB Chimerism studies
- Evaluation of their condition to determine if they are responding well to the study drug treatment

+36 months and +48 months
- Symptom assessment package
- JAK2 V617F blood test
- PB Chimerism studies
- Evaluation of their condition to determine if they are responding well to the study drug treatment

Table 13 Schedule of Assessments from start of conditioning to post BMT follow-up

All study visits after Day 0 have a window of ±2 days.

Assessments	Day -5	Day 0[a]	Day +30	Day +60	Day +100	After +6 months	After +12 months	After +18 months	After +24 months	After +36 months	After +48 months	End of Treatment visit[j]	30 Day Follow-up visit[k]	Off-treatment follow-up[l]
Limited Re-assessment of Eligibility criteria[b]	X													
Physical Examination	X	X	X	X	X	X	X	X	X	X	X	X	X	
Co-morbidity evaluation	X		X	X	X	X	X	X	X	X	X	X	X	
ECOG status	X		X	X	X	X	X	X	X	X	X	X	X	
Palpable Spleen Size	X		X	X	X	X	X	X	X	X	X	X	X	
Transfusion history	X		X	X	X	X	X	X	X	X	X	X	X	
Symptom assessment package & QOL Instruments	X[c]		X		X	X	X		X	X	X			
Samples for Cytokines[d]	X[d]		X[d]		X[d]									
CBC (complete blood count / differential)	X	X	X	X	X	X	X	X	X	X	X	X	X	
Biochemistry[e]	X	X	X	X	X	X	X	X	X	X	X	X	X	
ABO and Rh typing			X	X										
JAK 2 mutation						X	X		X	X	X			
Research samples for MPD-RC tissue bank[f]					X[f]	X[f]	X[f]		X[f]			X[f]		
Bone Marrow Aspirate and Biopsy[g]					X[g]	X[g]	X[g]		X[g]			X[g]		
Cytogenetics[h]					X[h]	X[h]	X[h]		X[h]			X[h]		
Hematological toxicities	X											X	X	
Non-hematological toxicities												X	X	
Regimen-related toxicities (RRT)[i] (see Appendix 9)		X	X	X										
Infectious Complications	X		X	X	X	X	X	X	X	X	X			
Engraftment and graft failure			X	X	X	X	X	X						
PB Chimerism studies			X	X	X	X	X		X	X	X			
GvHD assessment (See Appendix 10)			X	X	X	X	X	X	X	X	X			
IWG response assessment	X				X	X	X	X	X	X	X			
Survival and Progression of disease														Every 6 months from treatment d/c

a Day 0 is counted as day of stem cell infusion
b See protocol section 4.3
c Symptom assessment package & QOL Instruments to be done before start of condition therapy
d Sample needs to be shipped to reference laboratory, please see Table 10
e AST, ALT. T Bili, D Bili, alkaline phosphatase, albumin, total protein, Ca+2, Phosphorous, LDH, PT/PTT, CO2 total, creatinine, BUN, potassium, sodium, glucose, uric acid level
f MPD-RC research samples specified in Table 10. Samples need to be shipped to reference laboratory, please see Table 10
g Sample needs to be shipped to reference laboratory, please see Table 10
h Repeat cytogenetics only if there is an abnormality at baseline
i RRT assessed according to Bearman Criteria
j End of treatment as defined in section 4.7
k this visit occurs 30 days (±7 days) after end of treatment. Treatment defined in section 4.7.
l please see as described in section 4.7.

8.5 Off-treatment Assessments

See definition of off-treatment in section 4.7.

During the off-treatment visit, the following tests and procedures will be performed:

- A physical examination including a review of current medical conditions, transfusion history, spleen palpation, concomitant medications, weight, blood pressure, heart rate
- ECOG status
- Bone marrow aspiration/biopsy/cytogenetics/clonality
- Research samples for the MPD-RC Tissue Bank
- CBC, Biochemistry
- They will be assessed for any signs of harmful effects of the drug or any additional problems caused by the medical treatment

30 days (±7 days) after the off-treatment visit, the following tests will be performed:

- A physical examination including a review of current medical conditions, transfusion history, spleen palpation, concomitant medications, weight, blood pressure, heart rate
- ECOG status
- CBC, Biochemistry
- They will be assessed for any signs of harmful effects of the drug or any additional problems caused by the medical treatment

Subjects who come off treatment will be followed every 6 months for survival and disease progression data via telephone call or review of medical records. This data will be collected from the end of treatment to the end of the trial.

9 MEASUREMENT OF EFFECT

Hematological recovery: Neutrophil recovery will be defined as first of the three consecutive days with neutrophil count ≥0.5 x 10^9/l. Platelet recovery will be defined as first of the 7 days with platelet count ≥20 x 10^9/l, without platelet transfusion support and both maintained for 30 days without transfusion support or myeloid cytokine support.

Graft failure: Graft failure will be defined as failure to achieve hematological recovery (primary) or a decline in peripheral blood counts after achieving a sustained hematological recovery to below threshold levels defined as neutrophil count ≥0.5 x 10^9/l and platelets ≥20 x 10^9/l in the absence of platelet transfusion or lack of sustained neutrophil count and platelets at the above range in the absence of a reversible cause of drop in blood counts (e.g treatment of CMV infection with ganciclovir or other active infection).

NRM: will be defined as death in first 30 days due to any cause, and subsequently death due to any cause without the recurrence or progression of myelofibrosis. Cumulative incidence of NRM will be calculated taking relapse/progression as competing event.

Acute and chronic GvHD: will be defined according to standard criteria. Cumulative incidence of acute and chronic GvHD will be calculated and death due to any cause will be considered as competing event.

Remission status: will be defined according to IWG-MRT criteria and will be categorized as complete remission (CR), partial remission (PR), clinical improvement (CI), progressive disease (PD), and stable disease (SD) (Appendix 4).

Relapse: Only the patients who achieve CR or PR will be eligible for analysis of relapse. A patient with CR or PR is considered to have undergone relapse when he or she no longer fulfills the criteria for even CI. This will be calculated as cumulative incidence with NRM as competing event (Appendix 4, IWG-MRT response criteria).

Progression-free survival: PFS will be estimated for all patients. Failure will include death, progressive disease or relapse. Death due to any cause, progressive disease or relapse (whichever earlier) will be considered as events for this analysis.

Overall survival: OS will be estimated for all patients with death due to any cause as event.

See Appendix 4 for IWG-MRT criteria for response assessment *(from Tefferi et al., 2006)*

10 STUDY DESIGN

10.1 Statistical Considerations

A two- stage Simon Phase II study will be conducted in each of two groups of patients: related and unrelated donor transplants. In each donor transplant group, the first stage of this design will include 11 patients evaluated for death or graft failure by 100 days post-transplant. In each stratum, we will enroll additional patients (up to 20%) of stratum total to take into account exclusions due to donor failure (such as donor deemed unsuitable for stem cell donation due to medical or other reasons) only. Those patients who have toxicities related to Ruxolitinib and not been able to reach HCT due to these toxicities will be included in the estimation of overall failure rates. Only those patients who are excluded based on donor related issues without any regimen related complications will be excluded from the estimation of failure rates. However, all data on these patients will be reported.

Unrelated transplants: Based on data from the MPD-RC 101 study (31), there are 15 observed failures (as defined above) among 34 patients by day 100 (44%; of patients died or experienced graft failure by day 100) who received matched unrelated donor transplants. Based on these preliminary data, we will not pursue a regimen with a failure rate of 50% or greater in this trial.

A minimax two-stage design will be used to test the null hypothesis that the 100 day failure rate as defined above is ≥50% versus the alternative that this failure rate is ≤25%. If the regimen is actually not safe, there is a 0.04 probability of concluding that it is (the target for this value was 0.050). If the regimen is actually safe, there is a 0.19 probability of concluding that it is not (the target power is 80%). After testing the regimen in 11 patients in the first stage, the trial will be terminated if there are 6 or more failures within 100 days. If the trial goes on to the second stage, a total of 26 patients will be studied. If the total number of failures is 9 or more, the regimen will be rejected.

Related transplants: Based on data from the MPD-RC 101 study (31), there are 4 observed failures (as defined above) among 32 patients (12.5%) who received matched related donor transplants in this group of patients.

A minimax (and optimal) two-stage design to test the null hypothesis that the 100 day failure rate as defined above is ≥25% versus the alternative that this failure rate is ≤10%. If the regimen is actually not safe, there is a 0.04 probability of concluding that it is (If the regimen is actually safe, there is a 0.18 probability of concluding that it is not (the target power is 80%).

After testing the regimen in 11 patients in the first stage, the trial will be terminated if there are 3 or more failures within 100 days. If the trial goes on to the second stage, a total of 50 patients will be studied. If the total number of failures is 8 or more, the regimen will be rejected.

All calculations from PASS 2008 (NCSS, J. Hintze, Kaysville, Ut.).

10.2 Definitions of Endpoints

See section 9 for definitions.

10.3 Analysis Plan

The primary objective of this study is to estimate the proportion of patients who experience graft failure or death by day 100 post transplant in patients who receive (a) related donor transplant and in those who receive (b) an unrelated donor transplant. In each of these two groups, if the study continues to the completion of the second stage of the two stage designs, we will estimate the failure rate with exact 95% Clopper Pearson confidence intervals in each group separately.

Baseline patient characteristics, disease history, and treatment related variables will be summarized separately for each strata defined by donor type using descriptive summary statistics and graphical approaches.

Secondary endpoints, including the proportion of patients who are 'cured' (with resolution of splenomegaly and normalization of blood counts), will be presented along with 95% confidence intervals for each stratum separately. Duration of cure will be summarized in these patients. Rates of transplant-related mortality and 95% confidence intervals will be presented. Progression free survival and overall survival will be presented using Kaplan-Meier cumulative survival curves.

Biomarker Analysis: Biomarkers will be assessed prior to transplant (before pre transplant treatment) and at 6, 12, and 24 months post-transplant. Rates of normalization for each individual marker and for combinations of one or more of these markers will be estimated (separately in each of the two strata). Descriptive statistics and graphical displays of the distributions in relation to survival and other post-transplant events will be provided at each time point. For each patient, a graph of each marker over time will be provided. Methods for longitudinal data analysis will also be used to examine the time course of marker changes in these patients**.**

Quality of Life Analysis: Approaches similar to those described for the analyses of biomarkers will be used to examine quality of life over time in this study.

10.4 Anticipated study duration and time lines

The goal of this trial is to accrue 82–86 patients (76 evaluable patients + extra 10–15% patients for patient attrition due to donor unavailability, donor being declared unfit for donation or patient not been able to reach transplant for any reason) over 18 months. Approximately half of recruitment would be from North America (US and Canada) and half of recruitment would be from Europe. It is anticipated that the study will open at 15–20 centers in North America and Europe. We anticipate that by January 2013, at least 3 centers will start enrolling patients, by April 2013, >75% of the participating centers will start enrolment. The last patients entered will be followed for 2 years with annual follow up thereafter, for a maximum of 4 years of follow up from the start of the study period.

11 ADVERSE EVENTS

An adverse event is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product/biologic (at any dose), or medical device and which does not necessarily have to have a causal relationship with this treatment. This includes the onset of new illness and the exacerbation of pre-existing conditions. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of a medicinal product whether or not considered related to the medicinal product:

- occurring in the course of the use of a drug, biological product, or device;
- associated with, or observed in conjunction with product overdose, whether accidental or intentional;
- associated with, or observed in conjunction with product abuse, and/or
- associated with, or observed in conjunction with product withdrawal.

All adverse events must be recorded in the subject's medical records and on the electronic Case Report Form. The onset and end dates, severity, duration, effect on study drug administration (e.g. discontinuation), relationship to study drug, and administration of any other drug(s) to treat this event will be recorded for each adverse event. The severity of the adverse event and relationship to study drug will be assessed according to specific guidelines.

11.1 Reporting of non-Serious Adverse Events

An adverse event should initially be entered under the "Immediate AE Reporting" (Part A) section in the Adverse Events Form found in the MPD-RC database.

Additional information can be entered under the "Intermediate (Part B) AE Reporting" section of the Adverse Events form. Updates on all AEs must be provided every two weeks by the investigators to the coordinating center using the "Intermediate (Part B) AE Reporting" section.

The clinical course of the AE is "concluded" when either the AE has reached a resolution or it becomes a chronic disorder not prone to further evolution. When the AE is "concluded", the investigator is required to inform the coordinating center by completing the "Final (Part C) AE Reporting" section of the Adverse Events Form.

11.2 Reporting of Serious Adverse Events

A serious adverse event must be reported to the Data Monitor at the MPD-RC Central Office within 24 hours of the event following the appropriate MPD-RC Standard Operating Procedures (SOP):

- SOP 2-1: Serious Adverse Event (SAE) reporting – USA sites
- SOP 2-2: Serious Adverse Event (SAE) reporting – EU sites
- SOP 2-3: Serious Adverse Event (SAE) reporting – Canada site

11.2.1 EC/IRB Notification by Investigator

Reports of all serious adverse events (including follow-up information) must be submitted to the local institutional IRB according to the institution's policy. Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's binder.

11.2.2 Notifications by Sponsor

FDA

The MPD-RC Data Center and Regulatory Coordinator will receive reports of SAEs through the coordinating office. The MPD-RC as study sponsor shall notify the FDA by telephone or by facsimile transmission of any unexpected fatal or life-threatening experience associated with the use of the drug as soon as possible but no later than 7 calendar days from the sponsor's original receipt of the information.(1-800-332-0178) of any unexpected fatal or life-threatening experience associated with the use of the drug as soon as possible but no later than 7 calendar days from the sponsor's original receipt of the information. All other unexpected, serious adverse events that are considered related to study treatment will be reported on a MedWatch form by the Study Sponsor to the FDA within 15 calendar days. If a previous adverse event that was not initially deemed reportable is later found to fit the criteria for reporting, the study sponsor will submit the adverse event in a written report to the FDA as soon as possible, but no later than 15 calendar days from the time the determination is made.

Europe

The sponsor is responsible for the prompt notification to all concerned investigator(s), the Research Ethics Committees and competent authorities (e.g. MHRA) of each concerned Member State / Country of findings that could adversely affect the health of subjects, impact on the conduct of the trial or alter the competent authority's authorization to continue the trial in accordance with Directive 2001/20/EC and other regulations as appropriate. In each country a PI designate will be utilized to facilitate this process.

Novartis/Incyte

The MPD-RC or designee (i.e Consorzio Mario Negri Sud) will report all serious adverse events (SUSAR) to the safety office at Novartis Healthcare Pvt. Ltd within 15 days of first notification of the SAE irrespective of causality and in parallel with submission to the FDA or equivalent European regulatory bodies.

11.2.3 MPD-RC Reporting of Suspected Unexpected Serious Adverse Reactions

(SUSARs) Regulatory Bodies and Ethics Committees

All suspected adverse reactions related to an investigational medicinal product (the tested IMP) which occurs in the concerned trial, and that are both unexpected and serious (SUSARs) are subject to expedited reporting. These events should be reported by the individual investigators to the MPD-RC.

11.2.4 MPD-RC Reporting of SUSARs

MPD-RC, or designee, should report all the relevant safety information previously described to the concerned competent authorities and to the Ethics Committee concerned. MPD-RC or

designee shall inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of subjects. All SUSAR reports will also be reported to the FDA, European Research Ethics Committees and competent authorities (e.g. MHRA) of each concerned Member State / Country and Incyte/Novartis.

11.2.5 When to report

Fatal or life-threatening SUSARs

The MPD-RC should notify the Competent Authorities and the Research Ethics Committees/ IRBs as soon as possible but no later than 7 calendar days after the sponsor has first knowledge of the minimum criteria for expedited reporting. In each case relevant follow-up information should be sought and a report completed as soon as possible. It should be communicated to the Competent Authorities and the Ethics Committee within an additional eight calendar days.

Non-fatal and non-life-threatening SUSARs

All other SUSARs and safety issues must be reported to the competent authority and the Ethics Committee in the concerned countries as soon as possible but no later than 15 calendar days after the sponsor has first knowledge of the minimum criteria for expedited reporting. Further relevant follow-up information should be given as soon as possible.

11.2.6 How to report

To ensure subject safety, every SAE, regardless of suspected causality must be reported per protocol to the MPD-RC Central Office within 24 working hours of learning of its occurrence. The SAE should be reported in the Web-based data entry system (WBDES) on the "Immediate AE Reporting" (Part A) e-form and also on a Medwatch form 3500A. A signed and dated Medwatch 3500A should be emailed to the MPD-RC Data Monitor, Study Chair, Safety Officer, and PI of Project 6. At the time of the initial report the following information at minimum should be provided:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment
- Investigational agent dose

Significant new information on ongoing serious adverse events should be provided promptly to the study sponsor by entering the information into the database on the "Intermediate (Part B) AE Reporting" e-form. Update on all SAEs must be provided every two weeks (15 days) by the investigators to the coordinating center using the "Intermediate (Part B) AE Reporting" e-form.

The clinical course of the SAE is "concluded" when either the AE has reached a resolution or it becomes a chronic disorder not prone to further evolution. When the AE is "concluded", the

investigator is required to inform the coordinating center using the "Final (Part C) AE Reporting" e-form.

Minimum criteria for initial expedited reporting of SAEs

Information on the final description and evaluation of an adverse reaction report may not be available within the required time frames for reporting. For regulatory purposes, initial expedited reports should be submitted by the Sponsor within the time limits as soon as the minimum following criteria are met:

a) A suspected investigational medicinal product,
b) An identifiable subject (e.g. study subject code number),
c) An adverse event assessed as serious and unexpected, and for which there is a reasonable suspected causal relationship,
d) An identifiable reporting source, and, when available and applicable:
   - a unique clinical trial identification (the sponsor's trial protocol code number)
   - a unique case identification (i.e. sponsor's case identification number).

Follow-up reports of SAEs

In case of incomplete information at the time of initial reporting, all the appropriate information for an adequate analysis of causality should be actively sought from the reporter or other available sources. The sponsor should report further relevant information after receipt of follow-up reports. In certain cases, it may be appropriate to conduct follow-up of the long-term outcome of a particular reaction.

Format of the SUSARs reports

Electronic reporting should be the expected method of the Sponsor for expedited reporting of SUSARs to the competent authority. In that case, the format and content as defined by the Guidance of the Competent Authorities should be adhered to. The CIOMS-I form is a widely accepted standard for expedited adverse reactions reporting. However, no matter what the form or format used, it is important that the basic information/data elements described in annex 3 of the EU directive or as per country requirement, when available, be included in any expedited report (some items may not be relevant, depending on the circumstances).

11.3 Grading and Relationship Assessment of Adverse Events

Any adverse event will be graded for severity according to the definitions in Table 14Table 14.

All the toxicities related in the phase prior to stem cell infusion (during the phase of Ruxoltinib administration and taper off) will be defined according to CTCAE v4

(http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf)

In the post-transplant period in first 6 weeks, transplant related regimen-related toxicities will be defined according to Bearman's criteria (Appendix 9); which are widely used in BMT literature. Grading of acute and chronic graft-versus-host disease will be done according to NIH criteria (Appendix 10).

All other toxicities in the post-transplant period will be graded according to CTCAE v 4.0 (http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf)

Table 14 Definitions of Adverse Event Severity Categories

Mild (1)	**Awareness of sign, symptom or event, but easily tolerated.**
Moderate (2)	**Discomfort enough to cause interference with usual activity and may warrant intervention**
Severe (3)	**Incapacitating with inability to do usual activities or significantly affects clinical status, and warrants intervention.**
Life Threatening (4)	**Immediate risk of death.**
Fatal (5)	**Fatal**

The Investigator must assess the relationship of any adverse effect to the use of study drug, based on available information, using the following guidelines noted in Table 15.

Table 15 Adverse Event Relationship to Treatment Guidelines

Not related (1)	**No temporal association, or the cause of event has been identified or the drug cannot be implicated**
Unlikely (2)	**A doubtful association exists**
Possibly Related (3)	**Temporal association, but other etiologies are likely the cause. However, involvement of the drug cannot be excluded.**
Probably Related (4)	**Temporal association, other etiologies are possible but unlikely.**
Definitely (5)	**Definite causal relationship identified**

11.4 Monitoring of Adverse Events

Patients having adverse events will be monitored with relevant clinical assessments and laboratory tests as determined by the Investigator. **ALL adverse events must be followed to satisfactory resolution or stabilization of the event(s).**

11.5 Immediately Reportable Adverse Events

Any serious adverse event, whether or not deemed drug-related or expected, must be reported by the physician within 24 hours to adverse event monitoring committee.

Time frame for monitoring Serious Adverse Event

Allogeneic transplant is a complex modality and where a patient can continue to have serious adverse events from the events related to the transplant procedure for life long. For this study, reportable period for the adverse and serious adverse events would be from the start of Ruxolitinib to 100 days after the transplant. The new intervention is this study is use of Ruxolitinib which will

be used for prior to the transplant, and therefore 100 days post-transplant will be a reasonable period to monitor any SAE related to Ruxolitinib.

A patient undergoing transplant may continue to have various serious adverse events such as chronic GVHD, infectious complications, disease relapse or progression related to the transplant procedure itself for an indefinite duration. Information about all these events will be captured in the Electronic case report form (MPD-RC Database) and will be reported with the final study report. The Reporting of post-100 AE and SAEs is to the Drug manufacturer Phamacovigilance group is optional. Events of this category still need to be circulated to the participating sites as stated in the contract. In these cases the sites have the responsibility to report to their IRB's as per their institution's guidelines.

However, if the event is deemed a SUSAR even after 100 days of treatment, the MPD-RC is expected to report this event to the Drug manufacturer Pharmacovigilance group, the IRB and the participating sites, even if at the this point the event is not treatment related, and is most likely GVHD related.

All events happening to the patients should be recorded in the E-CRF in the MPD-RC database and will be part of the study final report.

Reports of all serious adverse events, including deaths must be communicated to the appropriate Institutional Review Board or ethical review committee and/or reported in accordance with local law and regulations.

Definition of serious adverse event

A serious adverse event (SAE) is any adverse drug or biologic or device experience occurring at any dose that results in any of the following outcomes:

a. death;
b. life-threatening AE (that places the subject, in the view of the initial reporter, at immediate risk of death from the AE as it occurs, ie, suicidal ideation/suicide attempt);
c. persistent or significant disability/incapacity;
d. required in-patient hospitalization, or prolonged hospitalization;
e. congenital anomaly or birth defect;
f. other serious (important medical events)

Procedures for Reporting Drug Exposure during Pregnancy and Birth Events

If a woman becomes pregnant or suspects she is pregnant while participating in this study, she must inform her treating physician immediately and permanently discontinue study drug. The pregnancy must be followed through delivery for SAEs.

11.6 Exceptions for Definitions of Serious Adverse Events

The following will **not** be considered serious adverse events:

Any event that results in hospitalization or prolongs an existing hospitalization will not be considered a serious adverse event if the only reason for that hospitalization or prolongation was for:

a. transfusion of blood products
b. administration of study procedures

c. placement of a permanent intravenous catheter
d. hospice placement for terminal care
e. out-patient hospitalization for procedures such as elective day surgery or hospitalization due to convenience purposes, e.g. transportation difficulties.

11.7 Safety Monitoring

This trial will be monitored by the MPD-RC Data Safety Monitoring Board according to the established Charter.

Data Safety Monitoring Board

An External Data and Safety Monitoring Board has been established. All members have experience and expertise in clinical trials. DSMB members are not directly involved in any phase of MPD-RC clinical trials and they have no major financial or intellectual conflict of interest that would prevent them from objectively reviewing the interim data and providing advice to the Trials Steering Committees and the Clinical Advisory Group. They function independently of all other individuals, processes, and progress to ensure study integrity, monitor patient safety (providing quarterly safety reports), evaluate the results of interim analysis to assess efficacy, and make recommendations about protocol amendments and early termination to the Trials Steering Committees. The External Data Safety and Monitoring Board must meet at least two times a year.

The DSMB statistician will possess a copy of the treatment codes for un-blinding purposes if required by the members of the board.

11.7.1 Medical Monitoring

It is the responsibility of the local institutional Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan. Medical monitoring will include a regular assessment of the number and type of serious adverse events.

11.7.2 Monitoring Plan and Period of Observation

The monitoring plan for this study is maintained by the MPD-RC and contains the following elements:

- This study will be conducted according to the policies and procedures of the MPD-RC;
- All MPD-RC provided drug supplies will be returned to the MPD-RC or are to be disposed in accordance with procedure agreed to by investigator and the MPD-RC.

12 REGULATORY REQUIREMENTS

See Section 5.1.1 regarding required regulatory documents. All protocol amendments will be generated through and distributed by the MPD-RC Central Coordinating Office, which will also maintain records of IRB approval, amendments, SAEs, and annual reviews.

12.1 Good Clinical Practice

The study will be conducted in accordance with the International Conference on Harmonisation (ICH) for Good Clinical Practice (GCP) and the appropriate regulatory requirement(s). The investigator should be thoroughly familiar with the appropriate use of the study drug as described in the protocol and Investigator's Brochure. Essential clinical documents should be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

12.2 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments should be submitted to a properly constituted independent EC or IRB, in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study should be made in writing to the investigator and a copy of this decision should be provided to the MPD-RC Central Office before commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the MPD-RC Central Office.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. See section 13 for a copy of the Subject Informed Consent Form. This consent form should be submitted with the protocol for review and approval by the EC/IRB for the study. The consent of a subject, using the EC/IRB-approved consent form, must be obtained before a subject is allowed to participate. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator obtaining the consent.

12.3 Patient Information and Informed Consent

After the study has been fully explained, written informed consent should be obtained from either the patient or his/her guardian or legal representative prior to study participation. The method of obtaining and documenting the informed consent and the contents of the consent should comply with ICH-GCP and all applicable regulatory requirement(s).

12.4 Patient Confidentiality

Information about study subjects should be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information

The patient has the right to revoke their authorization for use of their PHI. In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

12.5 Protocol Compliance

The investigator should conduct the study in compliance with the protocol given approval/favorable opinion by the IRB and the appropriate regulatory authority (ies). Changes to the protocol will require approval of the MPD-RC and written IRB approval/favorable opinion prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to patients. The MPD-RC will submit all protocol modifications to the regulatory authority (ies) in accordance with the governing regulations. Any departures from the protocol must be fully documented in the source documents.

12.6 Management of Information

The MPD-RC at Mount Sinai will maintain sponsorship authority at the Icahn School of Medicine at Mount Sinai and all other participating sites.

Eligibility criteria will be confirmed using the electronic CRF. All inclusion and exclusion criteria are listed; the investigator is obliged to fill in all items. Subjects are enrolled in the study only if he/she fulfills all inclusion and no exclusion criteria.

IRB approval for each site will be submitted to the MPD-RC Central Coordinating Office via e-mail (PDF file preferred) or fax:

MPD-RC Central Coordinating Office
Regulatory Coordinator
Alicia Orellana
Office hours: Monday through Friday 9AM to 5PM EST/EDT

Upon receipt of these documents and confirmation of certification of the study investigators, the site will be granted access to the online registration system and case report forms (section 12.9).

In addition, all protocol amendments will be generated through and distributed by the MPD-RC Central Coordinating Office, which will also maintain records of IRB approval, amendments, SAEs, and annual reviews.

Serious adverse event reporting is detailed in section 11.2.

Protocol deviations will be reported to the MPD-RC Regulatory Coordinator:

Office hours: Monday through Friday 9AM to 5PM

The MPD-RC principal investigators will be notified as soon as possible (and within 7 days) of reporting, and will be responsible for granting approval.

The MPD-RC will monitor study progress on an on-going basis; this will include electronic and telephone correspondence between the central coordinating office and with individual investigators at other sites.

12.7 Drug Accountability

Accountability for the study drug at all study sites is the responsibility of the MPD-RC. The responsible investigator at each participating center will ensure that the study drug is used only in accordance with this protocol, drug accountability records indicating the drug's delivery date to the site, inventory at the site, use by each patient, and return. Accountability records will include dates, quantities, lot numbers, expiration dates (if applicable), and patient numbers.

12.8 Source Documents

Source data includes all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

12.9 Case Report Forms (CRF)

The study CRF is the primary data collection instrument for the study. All data requested on the CRF must be recorded. The CRFs may be found online at the MPD-RC website at . www.marionegri.it The CRF Form Submission schedule is listed below:

Table 16 CRF Form Submission Schedule

MPD-RC #114 Form#	MPD-RC #114 FORM NAME	CRF SUBMISSION SCHEDULE
00	Parameter List	@ Registration
01	ENROLLMENT	@ Registration
02	PB & BM Report	Baseline, day -65 from the start of study drug, day +100 after BMT, month +6, +12, +24, +36, +48 after BMT, @ time of relapse/progression and study termination for any reason
03	Cytogenetic	Baseline, day -65 (start of study drug), day +100 after BMT, month +6, +12, +24, +36, +48 after BMT, @ time of relapse/progression and study termination for any reason
04	BASELINE	Within 1 week after registration
05	Pre-Transplant Patient Status	After Registration
06	HLA Typing Form	Before transplant
07	Pre-Conditioning DOSE DRUG ADMINISTRATION	Weekly until BMT
08	Pre transplant follow-up day -65,	Day -65
09	Pre transplant follow-up day -58, -51, -44, -30, -23, & -16	Day -58, -51, -44, -30, -23, & -16
10	Pre transplant follow-up day -9	Day -9
11	Conditioning Regimen	Pre-BMT
12	Characteristics of Graft	@ BMT
13	DONOR'S LYMPHOCYTES INFUSION (DLI)	After BMT
14	GVHD Prophylaxis	After BMT
15	Post-transplant Follow-up +30 days	After BMT @ +30 days
16	Post-transplant Follow-up +100 days	After BMT @ +100 days
17	Post-transplant Follow-up +6 months	After BMT @ +6 months

MPD-RC #114 Form#	MPD-RC #114 FORM NAME	CRF SUBMISSION SCHEDULE
18	Post-transplant Follow-up +12 months	After BMT @ +12 months
19	Post-transplant Follow-up +18 months	After BMT @ +18 months
20	Post-transplant Follow-up +24 months	After BMT @ +24 months
21	Post-transplant Follow-up +36 months	After BMT @ +36 months
22	Post-transplant Follow-up +48 months	After BMT @ +48 months
23	Additional Follow-up visit not planned in study protocol	After BMT as needed
24	Follow-up visit after drug interruption	Within 2 weeks of drug interruption
25	Adverse Event Form	Immediately report all SAE's and complete all non-reportable A/E's Monthly
26	Outcome event Malignancy	Monthly and when/if it occurs
27	Outcome event Pregnancy-Abortion	Monthly and when/if it occurs
28	Off treatment notice	Within 2 weeks going off treatment
29	Death notification form	Immediately
30	End of study	Within 2 weeks of completing study
31	EORTC QLQ-C30	Pre-registration, days -5, +30, and +100; months +6, +12, +24, +36, +48, @ time of relapse/progression and study termination for any reason
32	FACT BMT	Pre-registration, days -5, +30, and +100; months +6, +12, +36, +48, @ time of relapse/progression and study termination for any reason
33	MPN-SAF	Pre-registration, days -5, +30, and +100; months +6, +12, +36, +48, @ time of relapse/progression and study termination for any reason

MPD-RC #114 Form#	MPD-RC #114 FORM NAME	CRF SUBMISSION SCHEDULE
34	Exploratory questions and patient feedback	Pre-registration, days -5, +30, and +100; months +6, +12, +36, +48, @ time of relapse/progression and study termination for any reason

12.10 Premature Closure of the Study

This study may be prematurely terminated, if in the opinion of the MPD-RC Trial Steering Committee there is sufficient reasonable cause. Written notification documenting the reason for study termination will be provided to the investigator by the terminating party.

Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to patients
- Failure to enter patients at an acceptable rate
- Insufficient adherence to protocol requirements
- Insufficient complete and/or evaluable data
- Plans to modify, suspend or discontinue the development of the study drug

12.11 Record Retention

It is the investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents must be retained at least 2 years following completion of the last follow-up on patients on active study. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

12.12 Endpoint Verification

The endpoint validation committee will centrally review and validate all end points following pre-defined criteria (Section 9 and Appendix 4). All the clinical events included in the primary and secondary endpoints will be validated by an *ad hoc* committee of expert clinicians blinded to the treatment assigned. Copies of physicians' and hospital records and death certificates will be reviewed by a Primary Endpoint Committee for event validation. Each event will be independently evaluated by two evaluators. Disagreement between the two evaluators will be addressed by the chairman of the committee.

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14 INFORMED CONSENT

TITLE OF RESEARCH STUDY:

Title: Exploring the Potential of Dual Kinase JAK 1/2 Inhibitor Ruxolitinib (INC424) with Reduced Intensity Allogeneic Hematopoietic Cell Transplantation in Patients with Myelofibrosis (IND#116275)

PRINCIPAL INVESTIGATOR (HEAD RESEARCHER) NAME AND CONTACT INFORMATION:

Name: ***(Name of Principal Investigator)***
Physical Address: ***(Participating Site Address)***
Mailing Address: ***(Participating Site Address)***
Phone: ***(Participating site phone)***

WHAT IS A RESEARCH STUDY?

A research study is when scientists try to answer a question about something that we don't know enough about. Participating may not help you or others.

People volunteer to be in a research study. The decision about whether or not to take part is totally up to you. You can also agree to take part now and later change your mind. Whatever you decide is okay. It will not affect your ability to get medical care at ***(Participating site)***.

Someone will explain this research study to you. Please take your time and read this Informed Consent Form carefully before you decide whether or not to take part in this research study. Ask the study doctor or a member of the study staff any questions you may have about this study.

You may also like to take a copy of this form home to review it with your family and friends.

Feel free to ask all the questions you want before you decide. Any new information that develops during this research study, which might make you change your mind about participating, will be given to you promptly.

Basic information about this study will appear on the website http://www.ClinicalTrials.gov. There are a few reasons for this: the National Institutes of Health (NIH) encourages all researchers to post their research; some medical journals only accept articles if the research was posted on the website; and, for research studies the U.S. Food and Drug Administration (FDA) calls "applicable clinical trials" a description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

PURPOSE OF THIS RESEARCH STUDY:

The purpose of this study is to find out if adding the study drug Ruxolitinib (INC424) prior to a combination of other chemotherapeutic drugs (Fludarabine and Busulfan) and then giving you a donor's blood cells (bone marrow transplantation) will be successful in people who have

advanced primary myelofibrosis (PMF), post-polycythemia vera myelofibrosis (PPV-MF) or post-essential thrombocythemia myelofibrosis (PET-MF), collectively known as myelofibrosis (MF). MF is a disorder in which bone marrow tissue develops in abnormal sites because the bone marrow itself undergoes fibrosis or scarring. This study plans to add the drug Ruxolitinib to further aid in reducing pre-transplant spleen size, improve physical performance levels and reduce adverse events (side effects) related to the transplant. Ruxolitinib is a drug that is approved by the Federal Drug Administration (FDA) for the treatment of patients with advanced forms of myelofibrosis. Using Ruxolitinib prior to stem cell transplantation is experimental.

You qualify to take part in this research because you have a blood disorder called myelofibrosis (MF), or myelofibrosis following polycythemia vera (PV), or following essential thrombocythemia (ET), and need a stem cell transplant.

Funds for conducting this research are provided by the Myeloproliferative Disorders Research Consortium (MPD-RC) through a grant provided by the National Cancer Institute (NCI). Additional funds are being provided by Incyte Pharmaceuticals. Incyte will provide study drug in the United States and Novartis will provide study drug outside the United States.

LENGTH OF TIME AND NUMBER OF PEOPLE EXPECTED TO PARTICIPATE

Your participation in this research study is expected to last for an unspecified length of time. After the transplantation you will be seen regularly in the clinic, the visits becoming less frequent as time progresses. Your medical condition will be followed yearly or until a time that you might experience a relapse of your disease.

The number of people expected to take part in this research study at ***(Participating site)*** is ***(sites' number)***. The total number of people expected to take part in this multi-center international research study is between 82 and 86, the number dependent upon the number of patients with appropriate donors. Approximately half of the recruitment would be from North America (US and Canada) and half of recruitment would be from Europe.

DESCRIPTION OF WHAT'S INVOLVED:

If you agree to participate in this research the following information describes what may be involved. You will first give consent to be enrolled in the MPD-RC mandatory Biomarker Study (MPD-RC #107 – *"Correlative Biomarker Study for MPD-RC Treatment Studies in the Philadelphia Chromosome Negative MPD").* This study allows us to collect your biological research specimens (blood, bone marrow, nails) at certain time points during the study.

Screening Phase

If you agree to participate in this study, you will be asked to sign this informed consent before any tests are performed. After you sign the informed consent form, you will have what are called "screening tests". These tests will help the study doctor decide if you are eligible to continue to participate in the study.

You will be asked questions about your medical history, including your transfusion history and about any medications you are currently taking or have taken in the past. In addition, you will

have the following tests performed. They may be done on different days depending on when they can be scheduled. You will have the following tests and procedures performed:

- A review of your medical history and a complete physical exam: your study doctor will ask you a series of questions. He or she will examine your entire body. He or she will use a stethoscope (a device placed on your skin to allow the doctor to hear better) to listen to your heart, lungs, and belly. He or she will feel your entire body, including your neck, trunk, arms, and legs.
- Measurements of your vital signs: heart rate, breathing rate, blood pressure, temperature, weight, and height.
- Body surface area: the surface area of your body will be measured to be used in calculating the amount of medication you are to receive.
- ECOG performance status: rating scale to rate your ability to perform daily functions.
- Electrocardiograms (ECGs): measure the electrical activity of your heart. For this test, you will be asked to lie down while 12 sticky pads are applied to each of your arms and legs and to your chest. The ECG will last about five minutes.
- Spleen measurement: through palpation (feeling with the hands) or through an ultrasound if necessary. Ultrasound technology uses sound waves to reflect off of soft tissues to create an image based upon the computer's interpretation of the waves' reflections. The sonographer will use a gel to help transmit the sound waves through the wand. It is cool and rather gooey – but not uncomfortable and the procedure does not last long.
- ECHO / MUGA scan: The function of your heart will be monitored using either a procedure called an echocardiogram or a MUGA (Multigated Acquisition Scan). An echocardiogram (ECHO) is a standard ultrasound examination with no known harmful effects. Images of your heart are taken using a small device about the size of a bar of soap ("transducer") that is attached to a computer. A slippery gel is spread over the area being examined, and the transducer is pressed firmly against the skin and moved across the area being examined.

  A MUGA scan shows the motion of the heart and how well it pumps the blood. It is performed by injecting a small amount of imaging material into your blood stream (arm) and a camera (computer) will collect the data. Blood flow in your heart and heart rate will show on the screen and is observed and measured by a doctor. This will tell you how efficiently your heart is pumping the blood.

- Pulmonary Function Test (PFT): Pulmonary function tests measure how well the lungs take in and exhale air and how efficiently they transfer oxygen into the blood.
- Urinalysis: you will be asked to provide a sample of your urine to determine how well your kidneys are working.
- Blood Tests: (Approximately a total of 23 tablespoons of blood will be collected from a vein in your arm with a needle):
    - Complete blood count (approx. 1 ½ tsp. blood): complete blood count monitors the blood levels; red blood cells, white blood cells, platelets and other components of the blood.
    - Blood chemistries (approx. 1 ½ tsp. blood): tests creatinine, glucose, bilirubin, and other chemicals in the blood that indicate how well the liver and kidneys are

functioning and to check for side effects and sedimentation rate (checks for inflammation).
  - Thyroid Function (approx. 1 ½ tsp. blood): the thyroid is one of the largest endocrine glands in the body. This gland is found in the neck and controls how quickly the body uses energy, makes proteins, and controls how sensitive the body should be to other hormones. This blood test checks how well the thyroid gland is functioning.
  - JAK2 V617F (1½ tsp. blood): blood test to check for the JAK2 V617F mutation in your blood.
  - Pregnancy test (approx. 1 tsp. blood) - Serum HCG: for women of childbearing potential. Women will not be allowed to participate if they are pregnant or nursing. Both men and women who are able to have children must agree to use an effective form of birth control (hormonal or barrier method of birth control, or abstinence) from the time of screening and throughout their participation in the study.
  - Extra Blood and nail clippings: Up to 4 2/3 Tbsp. of blood will be collected for research purposes for the biomarker study. Nail clippings: 2 clippings will only be gathered once (at pre-study visit). Your nail clippings will provide normal material, like DNA, for comparison with the abnormal material from your blood and/or bone marrow.
  - CD34+ test (2 tsp. blood) – to test for markers in the blood.
  - PT/PTT (0.5 tsp. blood) – a test to determine how quickly your blood clots.
  - Class I and II antigens - molecular typing (approx. 3 tsp. blood) – a test to type the molecules that control the immune response, and that are targets in transplantation rejection.
  - ABO and Rh typing (approx. 1-2 tsp. blood) - Blood type tests done before a person gets a blood transfusion. Human blood is typed by certain markers (called antigens) on the surface of red blood cells.
  - Chimerism studies (2 teaspoons) – To have a baseline of engraftment before the transplant
  - Serum CMV, HSV, EBV (approx. 1 tsp. blood) – to test for viruses of the herpes family.
  - Hepatitis A, B, and C tests (approx. 2 tsp. blood) – to detect current or previous hepatitis infection; an inflammation of the liver, most commonly caused by a viral infection (Hepatitis A, B, C).
- Bone marrow aspiration/biopsy/cytogenetics/clonality: Bone marrow aspiration, also called bone marrow sampling, is the removal by suction (through a needle placed into the bone) of fluid from the soft, spongy material that lines the inside of most bones (marrow). (Approx. 1 ½ tsp.) During a bone marrow aspiration/biopsy an area of the hip is numbed and a needle is placed through the skin and into the hip bone. A small sample of bone marrow and bone is withdrawn. The bone marrow will be examined to see if it is abnormal.

It is important that you tell your study doctor about any medications you are taking including over-the-counter medications, vitamins, herbal medications, and alternative medicines. You may need to stop some of your medicines in order to be able to take part in this study.

Ruxolitinib Pre- Hematopoietic cell transplantation (HCT) Phase of Study (Experimental)

After the screening visit if it is determined that you qualify to take part in this study, the following will take place during the Pre-allogeneic Hematopoeitic Cell Transplantation (HCT) phase of the study. Allogeneic hematopoeitic cell transplantation is a procedure in which some of the blood forming cells (the stem cells) from a donor are collected from the blood or from the bone marrow and are transplanted into the patient (the recipient). Prior to the transplant, you will receive treatment with chemotherapy. You will receive the study drug Ruxolitinib (INC424) 60 days (day -65) before receiving your conditioning regimen with the drugs Fludarabine and Busulfan. This chemotherapy that is given immediately prior to your transplant is called the conditioning or preparative regimen, the purpose of which is to help eliminate your disease prior to the infusion of donor stem cells and to suppress immune reactions. Ruxolitinib will be given at the maximum tolerated dose for 56 days (day -65 to day -10), followed by 4 days (day -9 to day -6) of tapering (reducing the amount) of the drug until it is stopped completely at the start of the conditioning therapy (day -5) prior to stem cell infusion. The term 'reduced intensity' transplant does not necessarily mean a safer transplant or one with less risks and side effects. Reduced intensity applies to strength of the drugs being used to prepare your body for the transplant. You will receive medication (cyclosporine or tacrolimus with methotrexate) to help prevent Graft Versus Host Disease (GVHD), which will be discussed more fully later in this section. If you are receiving an unrelated donor or mismatched related donor transplantation you will additionally receive the drug Thymoglobulin (ATG) to aid in preventing GVHD. You will then receive a stem cell transplant from a donor.

Ruxolitinib (INC424) 5mg tablets (taken by mouth) will be started 60 (day -65) days prior to start of conditioning chemotherapy. The dose of ruxolitinib will be determined according to baseline platelet count and will be modified according to platelet count at your follow-up visit.

If there is a delay in the availability of the donor or a delay in the start of the transplant conditioning regimen for any reason, your dose of Ruxolitinib will not be tapered in that instance until the date of the start of the conditioning regimen is known with certainty.

Your platelet counts will be monitored once a week, and if your platelet count falls below 50 x 10^9/L, then your platelet count will be monitored twice weekly. At follow-up, the dose of Ruxolitinib will be modified (increased or decreased).

After 8 weeks (56 days) of Ruxolitinib treatment, the Ruxolitinib taper will be started (day -9) with the goal of stopping the Ruxolitinib at the start of your conditioning therapy.

Study requirements prior to the start of conditioning therapy:

During the time you are receiving the study medication (Weeks 1-8 and during the Ruxolitinib taper period) you will have the following tests and procedures performed during the below stated time points. During certain visits, you will be asked to return all study medication in the bottles provided no matter whether you take all the capsules or not to measure compliance; empty bottles should also be brought back to the clinic.

Do not forget to tell your study doctor if you start taking any new medicines, feel unwell or want to take part in a new study run by another doctor.

Day -65

You will be seen by the study doctor on day -65 and the following procedures will be performed:

- A physical examination including a review of current medical conditions, transfusion history, spleen palpation, medications you are taking, measurement of your weight, blood pressure, heart rate
- You will have a brief assessment about your ability to perform activities of daily living (ECOG)
- You will be asked to complete brief surveys (Symptom assessment package) about your symptoms
- Routine blood will be drawn for hematology, chemistry – 2 teaspoons
- Blood will be drawn for cytokine profile – 2 teaspoons
- You will be assessed for any signs of harmful effects the drug or any additional problems caused by the medical treatment

Days -58, -51, -44, -30, -23 & -16

At each study visit you will have the following tests and procedures performed:

- Routine blood will be drawn for hematology, chemistry – 2 teaspoons
- You will be assessed for any signs of harmful effects the drug or any additional problems caused by the medical treatment
- Your condition will be assessed to determine if your dose of Ruxolitinib needs to be adjusted

Day -37

During this study visit, you will meet with the study doctor and the following tests and procedures will be performed:

- Routine blood will be drawn for hematology, chemistry – 2 teaspoons
- Blood will be drawn for cytokine profile – 2 teaspoons
- You will be assessed for any signs of harmful effects the drug or any additional problems caused by the medical treatment
- Your condition will be assessed to determine if your dose of Ruxolitinib needs to be adjusted, and if you are having a positive response to Ruxolitinib
- A physical examination including a review of current medical conditions, transfusion history, spleen palpation, medications you are taking, measurement of your weight, blood pressure, heart rate

- You will have a brief assessment about your ability to perform activities of daily living (ECOG)
- Review of the Ruxolitinib medication you have been taking to ensure you have been compliant with taking the medication
- Evaluation of your condition to determine if you are responding well to the study drug treatment

Day -9 (Ruxolitinib tapering)

Day -9 will begin the start of the 4 days of tapering (reducing the amount) of the Ruxolitinib drug until it is stopped at the start of the conditioning therapy prior to the stem cell transplant.

During the Day -9 study visit, the following tests and procedures will be performed:

- Routine blood will be drawn for hematology, chemistry – 2 teaspoons
- A physical examination including a review of current medical conditions, transfusion history, spleen palpation, medications you are taking, measurement of your weight, blood pressure, heart rate
- You will have a brief assessment about your ability to perform activities of daily living (ECOG)
- You will be asked to complete brief surveys (Symptom assessment package) about your symptoms
- Pregnancy test (approx. 1 tsp. blood) - Serum HCG: for women of childbearing potential. Women will not be allowed to participate if they are pregnant or nursing. Both men and women who are able to have children must agree to use an effective form of birth control (hormonal or barrier method of birth control, or abstinence) from the time of screening and throughout their participation in the study.
- Review and instruct you on the tapering schedule of the Ruxolitinib study drug.

Should you complete the Ruxolitinib portion of your therapy, you will be required to be hospitalized for approximately a month, and this may mean time away from work.

If you do not already have a device called an “intravenous (IV) central line” implanted in your chest, you will have one surgically inserted as part of the routine standard of care for the administration of the chemotherapy you will receive for the treatment of your disease. You will be seen by a surgeon for this procedure who will explain it to you in detail and answer any questions that you may have. You will be asked sign a separate consent for this standard procedure.

This central line will remain in place for several months so that we will not have to continually stick you for administration of medication or blood draws.

Information on Donor Stem Cells

For the transplant procedure in this research study, peripheral blood stem cells (blood from a vein) or bone marrow stem cells from either related donors (family members, if available) or unrelated donors will be used. Stem cells are immature or undeveloped cells which will eventually develop into white blood cells, red blood cells and platelets. Although peripheral blood stem cells are preferred for Reduced Intensity Transplants, like the one you will have, there may be times when only stem cells from bone marrow can be obtained. When a family donor is not available, unrelated donor stem cells (either from bone marrow or peripheral blood) may be obtained from outside organizations. These donor stem cells are provided to the study team after thorough quality testing.

Your donor will be asked to sign a separate consent form, explaining the risks of stem cell collection. This consent will be provided to the donor by the hospital as part of their standard procedures.

In cases where an unrelated donor or a slightly mismatched donor is used, there is a higher chance for rejection of the donor stem cells. Rejection is when your body's immune system (defense system) attacks the "foreign" donor stem cells. A needle is placed in the donor's veins of the arm, and blood is collected at a steady rate. Blood (which includes stem cells and other blood cells) will be collected from the body into a machine which separates stem cells from the other blood cells. This is so that the stem cells can be collected into a bag to be given to you. Each collection procedure takes three to four hours.

Reduced Intensity Conditioning Regimen (Standard of Care)
After the stem cells have been collected, you will be admitted to the hospital for approximately a month. In the first five days you will receive the reduced intensity conditioning regimen, the purpose of which is to improve your myeloproliferative disorder and to suppress your immunity so that the stem cell transplant is not rejected by your own immune system. Depending on whether you will receive stem cells from a related or unrelated donor, you will be assigned to one of the two following study treatment conditioning regimens: Fludarabine with busulfan OR fludarabine with busulfan and ATG. ATG is only given to subjects undergoing unrelated donor transplantation.

Fludarabine is given by IV infusion (through a needle placed in the vein) daily for 4 days. Busulfan is then given by IV infusion into a vein, daily for 4 days. To allow convenient administration of these two study drugs, you will receive these drugs through a central line which is located in your chest (this is described above).

If you receive stem cells from an unrelated or mismatched donor, you will be given an additional study drug named "anti-thymocyte globulin" or ATG, on 3 consecutive days before the stem cells are given. This drug further weakens your immune system to reduce the chances of you rejecting the transplant (graft), as well as reducing the chance that the graft cells attack your body's cells, a condition known as graft-versus-host disease (as explained in the risks section). ATG is only given to subjects undergoing unrelated donor transplantation.

This conditioning regimen is summarized in the following table, where "Day 0" is the day of stem cell transplantation:

	Day -5	Day -4	Day -3	Day–2	Day–1	Day 0
Fludarabine	X	X	X	X		
Busulfan	X	X	X	X		
ATG (only if unrelated donor)			X	X	X	

Day -5 study requirements

During Day -5, when you will begin the additional chemotherapy regimen, you will have the following additional tests and procedures performed:

- A physical examination including a review of current medical conditions, transfusion history, spleen palpation, medications you are taking, measurement of your weight, blood pressure, heart rate
- You will have a brief assessment about your ability to perform activities of daily living (ECOG)
- You will be asked to complete brief surveys (Symptom assessment package) about your symptoms
- Routine blood will be drawn for hematology, chemistry – 2 teaspoons
- Blood will be drawn for cytokine profile – 2 teaspoons
- You will be assessed for any signs of harmful effects the drug or any additional problems caused by the medical treatment
- Evaluation of your condition to determine if you are responding well to the study drug treatment

Reduced Intensity Transplant (Standard of Care)

Two days after your last dose of fludarabine and busulfan, on the day of transplant (Day 0 on the table above), the donor stem cells will be infused (through the central IV line). These stem cells will eventually develop into white blood cells, red blood cells, and platelets in your body.

Day 0 study requirements

During Day 0, when you will have the stem cell transplant, you will have the following additional tests and procedures performed:

- A physical examination
- Routine blood will be drawn for hematology, chemistry – 2 teaspoons

- You will be assessed for any signs of harmful effects the drug or any additional problems caused by the medical treatment

Post-Transplant (Standard of Care)

Immediately after the Reduced Intensity transplant, you will receive two FDA approved standard drugs to reduce the risks of graft versus host disease (explained in risk section). These drugs are called tacrolimus and methotrexate. Beginning the 2 days before the transplant (day -2) and continuing for at least 180 days after the transplant, you will take the drug known as tacrolimus which is used to reduce the activity of the your immune system and so lower the risk of transplant rejection. Tacrolimus will be given to you by an intravenous infusion only for the first 3 days. Then, for the remaining180 days, you will take tacrolimus orally (by mouth), as a tablet. You will take tacrolimus tablets twice a day, and the number of tablets you will take depends on your weight. This drug will help prevent your body from rejecting the cells you received from your donor. Methotrexate will be given intravenously on days 1, 3 and 6 after transplant. This drug will help prevent your body from rejecting the cells you received from your donor.

All the standard medical care used after standard transplants, including antibiotics, intravenous nutrition, graft versus host disease prevention and transfusions of red cell and platelets, will be used after your Reduced Intensity Transplant as needed. After the reduced intensity transplant, the testing used to evaluate the success of the study treatment will be similar to that used for regular transplants. If your disease were to return or persist after reduced intensity transplant, or if your body does not successfully "take" (accept) the transplanted donor cells, you may receive additional infusions of stem cells from your donor at two-month intervals without any study treatment conditioning regimen.

If the transplant is an effective treatment of the underlying myeloproliferative neoplasm, it will result in production of normal blood. It usually takes approximately two to three weeks for the donor stem cells to produce blood. During this time, you will be dependent on transfusion of blood and platelets and will need antibiotics to prevent and/or treat infections. You will remain in the hospital until your blood counts are fully recovered and you are in good clinical condition. Usually this means a hospital stay of at least three or four weeks after the transplantation.

After the transplantation, you will be seen regularly by your study doctor to have frequent blood tests, similar to the ones explained in the Ruxolitinib phase.

Post-transplant study requirements (Day +30 to Month +48)

After your stem cell transplant, and after your treatment in the hospital, you will be seen by the study doctor and study team at specific intervals: Day +30, +60, +100, 6 months, 1 year, 18 months, 24 months, 36 months, 48 months. At all of these study visits, the following tests and procedures will be performed:

- A physical examination including a review of current medical conditions, transfusion history, spleen palpation, medications you are taking, measurement of your weight, blood pressure, heart rate
- You will have a brief assessment about your ability to perform activities of daily living (ECOG)
- Routine blood will be drawn for hematology, chemistry – 2 teaspoons
- You will be assessed for any signs of harmful effects the drug or any additional problems caused by the medical treatment
- You will be assessed to see whether or not your body is experiencing Graft Versus Host Disease (GVHD), a side effect of bone marrow or stem cell transplantation. In cases of GVHD, the new donor cells treat the body as "foreign" and launch an attack against it.

Additionally, on these specific study visits, the following additional tests and procedures will be done:

Day +30

- Blood will be drawn for cytokine profile – 2 teaspoons
- You will be asked to complete brief surveys (Symptom assessment package) about your symptoms
- ABO and Rh typing (approx. 1-2 tsp. blood) - Blood type tests done before a person gets a blood transfusion. Human blood is typed by certain markers (called antigens) on the surface of red blood cells
- Chimerism studies (to analyze the transplant) – 2 teaspoons

Day +60

- ABO and Rh typing (approx. 1-2 tsp. blood) - Blood type tests done before a person gets a blood transfusion. Human blood is typed by certain markers (called antigens) on the surface of red blood cells
- Chimerism studies (to analyze the transplant) – 2 teaspoons

Day +100

- You will be asked to complete brief surveys (Symptom assessment package) about your symptoms
- Blood will be drawn for cytokine profile – 2 teaspoons
- Research samples for the MPD-RC tissue bank - small sample of your blood (about 4 tablespoons) and an additional 3 teaspoons of bone marrow will be taken to look for these certain molecules in the blood called "biomarkers"
- Bone marrow aspiration/biopsy/cytogenetics/clonality: Bone marrow aspiration, also called bone marrow sampling, is the removal by suction (through a needle placed into the bone) of fluid from the soft, spongy material that lines the inside of most bones (marrow). (Approx. 1 ½ tsp.) During a bone marrow aspiration/biopsy an area of the hip is numbed and a needle is placed through the skin and into the hip bone. A small sample of bone

marrow and bone is withdrawn. The bone marrow will be examined to see if it is abnormal
- Chimerism studies (to analyze the transplant) – 2 teaspoons
- Evaluation of your condition to determine if you are responding well to the study drug treatment

Day +180 (6 months)
- You will be asked to complete brief surveys (Symptom assessment package) about your symptoms
- JAK2 V617F (1½ tsp. blood): blood test to check for the JAK2 V617F mutation in your blood
- Research samples for the MPD-RC tissue bank - small sample of your blood (about 4 tablespoons) and an additional 3 teaspoons of bone marrow will be taken to look for these certain molecules in the blood called "biomarkers"
- Bone marrow aspiration/biopsy/cytogenetics/clonality: Bone marrow aspiration, also called bone marrow sampling, is the removal by suction (through a needle placed into the bone) of fluid from the soft, spongy material that lines the inside of most bones (marrow). (Approx. 1 ½ tsp.) During a bone marrow aspiration/biopsy an area of the hip is numbed and a needle is placed through the skin and into the hip bone. A small sample of bone marrow and bone is withdrawn. The bone marrow will be examined to see if it is abnormal
- Chimerism studies (to analyze the transplant) – 2 teaspoons
- Evaluation of your condition to determine if you are responding well to the study drug treatment

+12 months
- You will be asked to complete brief surveys (Symptom assessment package) about your symptoms
- JAK2 V617F (1½ tsp. blood): blood test to check for the JAK2 V617F mutation in your blood
- Research samples for the MPD-RC tissue bank - small sample of your blood (about 4 tablespoons) and an additional 3 teaspoons of bone marrow will be taken to look for these certain molecules in the blood called "biomarkers"
- Bone marrow aspiration/biopsy/cytogenetics/clonality: Bone marrow aspiration, also called bone marrow sampling, is the removal by suction (through a needle placed into the bone) of fluid from the soft, spongy material that lines the inside of most bones (marrow). (Approx. 1 ½ tsp.) During a bone marrow aspiration/biopsy an area of the hip is numbed and a needle is placed through the skin and into the hip bone. A small sample of bone marrow and bone is withdrawn. The bone marrow will be examined to see if it is abnormal
- Chimerism studies (to analyze the transplant) – 2 teaspoons
- Evaluation of your condition to determine if you are responding well to the study drug treatment

+18 months

- The study doctor will evaluate your condition to determine if you are responding well to the study drug treatment

+24 months

- You will be asked to complete brief surveys (Symptom assessment package) about your symptoms
- JAK2 V617F (1½ tsp. blood): blood test to check for the JAK2 V617F mutation in your blood
- Research samples for the MPD-RC tissue bank - small sample of your blood (about 4 tablespoons) and an additional 3 teaspoons of bone marrow will be taken to look for these certain molecules in the blood called "biomarkers"
- Bone marrow aspiration/biopsy/cytogenetics/clonality: Bone marrow aspiration, also called bone marrow sampling, is the removal by suction (through a needle placed into the bone) of fluid from the soft, spongy material that lines the inside of most bones (marrow). (Approx. 1 ½ tsp.) During a bone marrow aspiration/biopsy an area of the hip is numbed and a needle is placed through the skin and into the hip bone. A small sample of bone marrow and bone is withdrawn. The bone marrow will be examined to see if it is abnormal
- Chimerism studies (to analyze the transplant) – 2 teaspoons
- Evaluation of your condition to determine if you are responding well to the study drug treatment

+36 months and +48 months

- You will be asked to complete brief surveys (Symptom assessment package) about your symptoms
- JAK2 V617F (1½ tsp. blood): blood test to check for the JAK2 V617F mutation in your blood
- Chimerism studies (to analyze the transplant) – 2 teaspoons
- Evaluation of your condition to determine if you are responding well to the study drug treatment

Off-Treatment

In the case you begin taking the study drug, Ruxolitinib, and then need to discontinue taking the medication due to harmful effects it is causing, you have disease progression, or you have disease relapse after the bone marrow transplant, you will have an off-treatment visit, and a follow up visit, 30 days after you have taken the last dose of study medication. During the off-treatment visit, the following tests and procedures will be performed:

- A physical examination including a review of current medical conditions, transfusion history, spleen palpation, medications you are taking, measurement of your weight, blood pressure, heart rate
- You will have a brief assessment about your ability to perform activities of daily living (ECOG)

- Bone marrow aspiration/biopsy/cytogenetics/clonality: Bone marrow aspiration, also called bone marrow sampling, is the removal by suction (through a needle placed into the bone) of fluid from the soft, spongy material that lines the inside of most bones (marrow). (Approx. 1 ½ tsp.) During a bone marrow aspiration/biopsy an area of the hip is numbed and a needle is placed through the skin and into the hip bone. A small sample of bone marrow and bone is withdrawn. The bone marrow will be examined to see if it is abnormal
- Research samples for the MPD-RC tissue bank - small sample of your blood (about 4 tablespoons) and an additional 3 teaspoons of bone marrow will be taken to look for these certain molecules in the blood called "biomarkers"
- Routine blood will be drawn for hematology, chemistry – 2 teaspoons
- The harmful effects of the drug that you are experiencing caused by the medical treatment will be assessed.

During the 30-day follow-up visit, the following tests will be performed:

- A physical examination including a review of current medical conditions, transfusion history, spleen palpation, medications you are taking, measurement of your weight, blood pressure, heart rate
- You will have a brief assessment about your ability to perform activities of daily living (ECOG)
- Routine blood will be drawn for hematology, chemistry – 2 teaspoons
- The harmful effects of the drug that you are experiencing caused by the medical treatment will be assessed.

After you come off treatment, the research team will follow you every 6 months via telephone or your medical records to collect your disease status.

Note on additional Mandatory Sub-Study / Genetic testing

Your participation in genetic testing is a mandatory part of this study and may help to better understand which MPD patients may benefit from the study treatment in the future.

Segments of the DNA called genes are responsible for passing particular traits such as eye color from parents to children. The genes which pass along your traits direct cells in your body to make proteins some of which may play a role in the development of disease such as MPD. The study of gene mutations and variations in genes may help scientist to predict which patients with MPD are most likely to respond to specific treatments. In this study, genetic testing will be performed in order to learn more about factors which may predict response to the study drug treatment pre-transplant.

The doctors at ***(Participating site)***, working with other cancer researchers, are attempting to better understand the causes of myeloproliferative neoplasms and to develop improved methods for the diagnosis and treatment of these diseases. You will be asked to read and sign a consent form for that mandatory study called "Correlative Biomarker Study for MPD-RC Treatment Studies in the Philadelphia Chromosome Negative MPD".

As part of this research study, the study team is asking you to participate in an additional research study where a small sample of your blood (an additional 3 teaspoons of bone marrow and about 4 tablespoons of blood) will be taken to look for these certain molecules in the blood called "biomarkers". Because these extra blood and bone marrow samples will be collected at the same time as regularly scheduled blood and bone marrow tests, you will not undergo any additional procedures. These blood and bone marrow samples will be collected for research purposes only. You are also being asked to provide two nail clippings. These samples will be used to test and analyze the behavior and activity of molecules (the smallest unit of a substance that can exist alone and retain the character of that substance) and genes (the basic biological unit of hereditary) that may change as by- products of the disease itself. Your nail clippings will provide normal material like DNA for comparison with the abnormal material from your blood and/or bone marrow. This information may provide us with a better understanding of what causes the disease and how to develop better treatments. You will not receive the results from research on your sample.

That study will only collect blood, bone marrow, and nail samples to determine genetic characteristics.

These samples will be sent to special laboratories at the New York Blood Center for careful study and analysis. Your samples will be sent to these laboratories using a unique code that does not identify you by name, address or social security number. Researchers with access to your samples will not be able to identify you. The code linking the sample to your name would be stored at ***(Participating site)*** and known only to your doctor and a limited number of research personnel at ***(Participating site)***. The research will not have any effect on your care; therefore, the results of the genetic testing will not be placed into your medical records. If you discontinue from the trial, regardless of the reason (patient, investigator or sponsor decision), no further genetic samples will be collected. However, we will continue to use data previously generated from the genetic samples collected prior to the date of discontinuation.

These samples will be obtained at the following times:

- At diagnosis or before starting study treatment
- Day +100 following Reduced intensity transplant
- Day +180 following Reduced intensity transplant
- Month +12 following Reduced intensity transplant
- Month +24 following Reduced intensity transplant
- If/when your disease comes back (recurrence of disease)

The blood and bone marrow samples we collect would normally be obtained at these times during your treatment and/or routine follow-up. Blood and bone marrow samples for this study will be drawn only when these samples are being obtained for clinical purposes. Therefore, you will not have to undergo any additional procedures to participate in this study.

Nail clippings will be obtained only once at diagnosis or before starting treatment.

Any information derived directly or indirectly from the future research, as well as any patents, diagnostic tests, drugs, or biological products developed directly or indirectly as a result of this

research, are the sole property of the Sponsor (and its successors, licensees, and assigns) and may be used for commercial purposes. You have no right to this property or to any share of the profits that may be earned directly or indirectly as a result of this research. However, in signing this form and donating a blood sample or tumor tissue specimen for future research, you do not give up any rights that you would otherwise have.

You may wish to obtain professional genetic counseling before signing this informed consent so that you are fully aware of the implications.

YOUR RESPONSIBILITIES IF YOU TAKE PART IN THIS RESEARCH:

If you decide to take part in this research study you will be responsible for the following things: complying with requirements of the study, taking prescribed medications, use of effective birth control, and regular attendance at study visits.

COSTS OR PAYMENTS THAT MAY RESULT FROM PARTICIPATION:

There is no additional cost to you for participating in this research study other than those costs associated with the treatment of your disease. You or your insurance company will be responsible for the costs of your study doctor visits and all of the standard study treatments and procedures, including the hospital visits, x-rays, and blood tests. This is because this standard care would be the same even if you were not participating in this study.

The sponsor of this study will pay for the mandatory sub-study blood tests. The sponsors of this study will pay for the biomarker sub-study blood tests and for the study medication, Ruxolitinib (INC424).

Your insurance company must be contacted to pre-authorize the stem cell procedure and all the study treatments in this study. If your insurance company does not authorize these study treatments, you may choose not to participate in this research study. The study doctor will discuss these options with you. If your insurance company does not authorize the stem cell transplant, and no alternative payment arrangement is made, and you choose to go ahead with the procedure, you would be responsible for the bill, which would be approximately $120,000. Please speak to the study doctor and with the financial/billing representative before proceeding with this research study.

POSSIBLE BENEFITS:

It is important to know that you may not get any benefit from taking part in this research. Others may not benefit either. If you respond to the study treatment ruxolitinib plus the conditioning regimen of fludarabine and busulfan, you may have improvement of your disease. The knowledge learned from this research study may be helpful to other people with myeloproliferative disorders. No direct benefit can be promised as a result of your participation in this research study.

REASONABLY FORESEEABLE RISKS AND DISCOMFORTS:

While on this research study, you are at risk for certain known side effects associated with the study medication you will be taking. There may also be other side effects that we can't predict or that are unknown at this time which could be serious, permanent or, in some cases, result in death. Therefore, it is very important that you communicate any and all symptoms that you experience to your study doctor. Medications can be given to you to make side effects less serious and less uncomfortable. If you do experience side effects, we can withhold or stop your study drug. Many side effects go away shortly after the study drug is stopped, but in some cases side effects can be serious, long lasting or permanent. You should also tell your study doctor about any other medications that you are taking. While on this research study, you may experience the following side effects:

Infections:
Because the immune system (body's defense system) will be weakened for long periods of time after the transplant you will be at risk for infection. To prevent this, antibiotics, antifungal, and antiviral medicines will be given for several months after transplantation.

Graft Versus Host Disease (GVHD):
GVHD is a side effect of bone marrow or stem cell transplantation. In cases of GVHD, the new donor cells treat the body as "foreign" and launch an attack against it. The most common sites of attack by cells causing GVHD are the skin, liver, and gastrointestinal tract. If it occurs within 100 days after transplant it is called acute GVHD. If it occurs later it is called chronic GVHD. Symptoms of GVHD can range from mild to severe, and when severe, GVHD can be fatal. All these organs can be minimally, moderately, or severely affected by graft versus host disease, together or separately.

Symptoms of GVHD that may occur include:

- Skin rash
- Liver disease (including jaundice)
- Nausea, vomiting, diarrhea
- Temporary darkening of the skin and hardening and thickening patches of skin and tissue under the skin (occurs with chronic GVHD)
- Weight loss
- Lung disease (chronic GVHD)

Graft versus host disease is observed after regular transplants and also after Reduced Intensity Transplants. The risks of acute GVHD after non-myeloablative and reduced-intensity transplants appear lower than after fully ablative (standard) transplant. This is felt to be due to a lower injury of healthy tissues. Some patients develop serious GVHD and may need treatment with drugs called immunosuppressants and a few patients may experience fatal GVHD. The overall risk of suffering any significant grade of GVHD has been estimated to be 15 to 75%. Fatal acute GVHD after Reduced Intensity Transplant is relatively rare occurring in less than 10% of patients. The use of more intensive graft versus host disease treatment (a drug given to prevent disease or infection) can be used to address severe graft versus host disease in reduced intensity transplants.

Disease relapse/persistence (return of MPN) and failure to engraft:

Preliminary studies show that most patients experience engraftment which is successful "take" (acceptance) of donor cells, and many achieve 100% donor cells in their bone marrow together

with remission of their myeloproliferative disease. However, because the study treatment conditioning regimen used in this study is much less intense, two problems may result from the use of Reduced Intensity Transplants. The first is the persistence or return of the myeloproliferative neoplasm (IM) after transplantation, and the other is lack of or partial "take" of the donor stem cells. Both problems can be addressed by infusing more cells from the donor into the recipient. Immune cells can cause a reaction against tumors called graft-versus-tumor effect. The purpose of infusing more donor cells will be to take maximum advantage of this effect.

Transplant risks:

Likely:

- Lowered white blood cell count that may lead to infection. This can be treated with blood products (transfusions) and/or antibiotics.
- Lowered platelets, which may lead to an increase in bruising or bleeding. This can be treated with blood products (transfusions) and/or antibiotics.
- Lowered red blood cells, which may cause anemia, tiredness, or shortness of breath. This can be treated with blood products (transfusions) and/or antibiotics.
- Tiredness
- shortness of breath
- fever
- chills
- increased risk of infections
- loss of appetite and/or weight loss
- diarrhea
- nausea
- vomiting
- weakness
- dizziness
- muscle aches
- rash
- itching
- elevated liver function tests
- high blood pressure
- difficulty sleeping
- nervousness
- trembling or muscle twitching
- hair thinning or partial hair loss

Less likely, but serious:

- Kidney damage
- lung damage (scarring of the lung)
- liver damage
- mouth and throat sores

- hives, including severe rash leading to sloughing of skin and mucous membranes (redness and flaking of skin)
- gastrointestinal bleeding (stomach and intestines) which could cause blood when vomiting or black tarry stools
- serious disturbances of the immune system which can result in life threatening infections, red blood cell destruction by the immune system, failure to resume production of red blood cells which could be fatal
- too much sugar in the blood
- bladder irritation where blood may be present in the urine
- temporary unsteadiness when walking
- hypertension (high blood pressure)
- heart damage
- chest pain
- hearing loss
- feelings of extreme stress
- depression or confusion
- seizures (Most seizures last from 30 seconds to 2 minutes and do not cause lasting harm. A seizure is uncontrolled electrical activity in the brain, which may produce physical convulsions like shaking and trembling, mental changes like confusion, or a combination of symptoms.)
- Veno-occlusive disease: One of the problems seen after standard allogeneic transplants is the formation of blood clots in the small blood vessels of the liver. This complication is called veno-occlusive disease or VOD and can result in serious damage to the liver. The type of transplant used in this study is called non-myeloablative, which is less toxic than a standard transplant. Although VOD may still happen after non-myeloablative transplants it is less common.

Less likely, but not serious:

- Tingling of the fingers and/or toes
- weight gain and/or swelling
- insomnia
- Headache
- metallic taste in mouth
- rash and hives
- facial swelling
- muscle/joint aches
- pains in abdomen, chest or bones
- enlargement of spleen,
- increased or decreased potassium in the blood (may cause irregular heart beat, muscle cramping, weakness, difficulty breathing)
- decreased amounts of the blood chemical phosphate (there are no symptoms of low phosphate, unless the values are extremely low. Then you may notice trouble breathing or other respiratory problems, confusion, irritability)
- inflamed (redness and swelling) throat or tongue which may cause a burning sensation
- sensitivity to light

- blurred vision
- ringing ears
- painful or difficult urination.

Drug Side Effects

Risks of Ruxolitinib:

The risks of ruxolitinib may not be fully known, and may vary depending upon the disease you are being treated for. Therefore, you will be informed of the important symptoms or medical events (called "adverse events") that have occurred frequently in patients who had serious blood conditions called myelofibrosis (MF) and polycythemia vera (PV). You will also be informed of any adverse events that were rare but serious and might have been related to the study drug. During your participation, you will be given any new information that may affect your willingness to start or continue in the study.

You should discuss the risks listed here with your Study Doctor. Many side effects go away shortly after the study drug is stopped, but in rare cases they may be serious, long lasting, and/or permanent, and may even cause death. If you experience any of the described symptoms or have any other problems, you must immediately tell the appropriate study staff member or the Study Doctor. If you feel that these symptoms or side effects are life threatening seek medical assistance immediately.

The following adverse events were reported as common side effects (occurring in at least 1%) or very common (occurring in at least 10%) of patients who were treated with ruxolitinib for MF or for PV.

Very Common (at least 10%)

- Anemia (low red blood cells
- Thrombocytopenia (low platelets)
- Bruising
- Neutropenia (low white blood cells)
- Raised ALT and AST (blood proteins that may indicate mild liver damage)
- Hypercholesterolemia (increase in cholesterol)
- Hypertriglyceridemia (increase in triglycerides)
- Dizziness
- Headache
- Urinary tract infections
- Weight gain

Common (more than 1% but less than 10%)

- Flatulence (gas)
- Constipation
- Herpes Zoster (shingles)
- Hypertension (high blood pressure)

Ruxolitinib may cause low blood cell counts (white blood cells, red blood cells and platelets). If your white blood cell count becomes low while you take the drug, this means you may have an increased chance of getting an infection, including urinary tract infections and viral infections. You will be checked for any signs of infection before starting ruxolitinib and any serious infections should be treated before you start ruxolitinib; your physician will check you carefully for signs of infection while you are being treated. You also may become anemic (low red blood cell count) while you take the drug, and that may cause you to feel fatigued or short of breath. If your platelet count becomes low while you take the drug, it may lead to bleeding and/or bruising. In some people taking ruxolitinib, the decrease in blood cell counts have been severe. In most cases, low blood cell counts can be reversed by stopping the study drug temporarily or reducing the dose; you will be checked often for this side effect while on study. If your blood cell counts do not recover quickly, the study drug may be stopped for a longer duration to allow the blood cell counts to recover.

Uncommon: (occurring in fewer than 1% of patients)

These events are events that were uncommon, but occurred during ruxolitinib treatment and are potentially serious.

- Non-melanoma skin cancers (NMSCs) are skin cancers such as basal cell cancer or squamous cell cancer that usually develop on the sun-exposed areas of skin, and commonly require surgery to remove. NMSCs have been reported in patients with MF or PV who were treated with ruxolitinib. Most of these patients had histories of extended treatment with medications known to increase the risk of NMSC, and had NMSC or pre-cancerous skin lesions before being treated with ruxolitinib. It is not known whether or not ruxolitinib contributed to these cases of NMSC. Periodic skin examination is recommended for patients who are at increased risk for skin cancer.

(The following conditions have occurred in patients with MF who were treated with ruxolitinib)

- Tuberculosis (TB) has occurred in a small number of patients (less than 1%) with MF who were treated with ruxolitinib, but it is not known whether this was due to MF, ruxolitinib, or other factors that are known to increase the risk of tuberculosis (such as diabetes, bronchitis, asthma, smoking, emphysema, or steroid use). Tell your study doctor if you have been treated for TB in the past, or have ever had a positive skin test for TB. An increase in systolic blood pressure was noted on at least one occasion in more MF patients treated with ruxolitinib than patients treated with comparison drugs (31% vs. 20%), but the average changes in blood pressure very small, generally occurred only once over the treatment period, and their meaning was not clear.
- About one week following interruption or discontinuation of ruxolitinib, some patients with MF experienced a return of symptoms (such as fatigue, bone pain, fever, itching, night sweats, weight loss, or an enlarged spleen). There have been cases of MF patients stopping ruxolitinib during another ongoing illness who became more severely ill, but it was not clear whether stopping ruxolitinib therapy contributed to the patients' conditions worsening. When stopping ruxolitinib therapy, your doctor may choose to gradually decrease the dose of ruxolitinib.
- A rare disease called progressive multifocal leukoencephalopathy (PML) has been reported during ruxolitinib treatment for MF. PML comes from a viral infection that

causes brain damage and can be fatal. It is unknown whether this was due to ruxolitinib treatment since PML has occurred in patients with blood cancers, including MF, who were not treated with ruxolitinib. Tell your study doctor immediately if you have any of the following symptoms or if anyone close to you notices that you have any of these symptoms: confusion or problems thinking, loss of balance or problems walking, clumsiness, difficulty speaking, decreased strength or weakness on one side of your body, blurred and/or loss of vision.

Risks of Fludarabine:

Likely:

- Lowered white blood cell count that may lead to infection. This can be treated with blood products (transfusions) and/or antibiotics.
- Lowered platelets, which may lead to an increase in bruising or bleeding. This can be treated with blood products (transfusions) and/or antibiotics.
- Lowered red blood cells, which may cause anemia (less hemoglobin (protein in red blood cells that carries oxygen) to carry oxygen to your tissues), tiredness, or shortness of breath. This can be treated with blood products (transfusions) and/or antibiotics
- Fever
- Nausea
- Vomiting
- Mouth or throat sores
- Diarrhea
- Ulcers
- Loss of appetite
- Swelling or water retention
- Skin rashes
- Muscle aches
- Nervousness
- Hearing loss
- Sleepiness
- Fatigue

Risks of Busulfan:

Common Side Effects:

- Anxiety
- Back pain
- Constipation
- Diarrhea
- Dizziness
- Dry mouth
- Flushing or hot flashes

- Headache
- Hiccup
- Indigestion
- Loss of appetite
- Mild fever or chills
- Mild redness or swelling at the injection site
- Minor cough or sore throat
- Muscle or joint pain
- Nausea
- Runny nose
- Tiredness or weakness
- Trouble sleeping
- Vomiting.

Severe Side Effects:

- Severe allergic reactions (rash; hives; itching; difficulty breathing; tightness in the chest; swelling of the mouth, face, lips, or tongue)
- Black, tarry stools
- Blurred vision or other vision changes
- Chest pain
- Confusion
- Decreased amount of urine, painful urination, or blood in the urine
- Depression
- Fainting
- Fast or irregular heartbeat
- Hallucinations
- Missed menstrual period
- Red, swollen, or blistered skin
- Seizures
- Severe or persistent pain, redness, or swelling at the injection site
- Severe or persistent cough
- Severe or persistent dizziness or headache
- Severe or persistent nausea, vomiting, or diarrhea
- Shortness of breath or trouble breathing
- Signs of infection (eg, persistent fever, chills, or sore throat)
- Sores in the mouth or trouble swallowing
- Unusual bruising or bleeding (eg, nosebleed)
- Unusual pain or swelling of the legs or calves
- Unusual stomach pain, swelling, or weight gain
- Vomit that looks like coffee grounds
- Yellowing of the skin or eyes.

Risks of Tacrolimus (Prograf):

Likely:

- High blood pressure
- Lowered white blood cell count that may lead to infection. This can be treated with blood products (transfusions) and/or antibiotics.
- Lowered platelets, which may lead to an increase in bruising or bleeding. This can be treated with blood products (transfusions) and/or antibiotics.
- Lowered red blood cells, which may cause anemia, tiredness, or shortness of breath. This can be treated with blood products (transfusions) and/or antibiotics
- Chest pain
- Headache
- Tremors
- Insomnia
- Tingling of the fingers and toes which can cause clumsiness or stumbling
- Nervousness, or anxious feelings
- Abnormal blood chemistries: changes in your blood calcium, sodium, albumin (protein), potassium, and/or magnesium
- Excess body hair
- Nausea
- Vomiting
- Constipation
- Diarrhea
- Appetite loss
- Kidney damage
- Decreased urination
- Abnormal liver function
- Pain
- Fever
- Loss of strength and energy
- Weakness
- Back pain
- Swollen ankles or fingers
- Gum disease
- High blood sugar.

Less likely:

- High potassium in the blood. Diuretics that spare potassium such as triamterene (found in Dyazide and Maxzide), amiloride (found in Moduretic), and spironolactone (Aldactone) are not recommended.
- Confusion
- Seizures (Most seizures last from 30 seconds to 2 minutes and do not cause lasting harm. A seizure is uncontrolled electrical activity in the brain, which may produce physical convulsions like shaking and trembling, mental changes like confusion, or a combination of symptoms.)
- Sadness
- Hallucinations
- Shock like muscle contractions

- Numbness
- Irrational thoughts
- Temporary unsteadiness when walking
- Strange dreams

Aluminum hydroxide, which is found in many antacids, binds tacrolimus in the stomach. Aluminum-containing antacids should not be taken with tacrolimus.

The destruction of tacrolimus by the body may be prevented by a large number of drugs, resulting in higher blood levels of tacrolimus, and possibly increasing its side effects. Such drugs include bromocriptine (Parlodel), cimetidine (Tagamet), cisapride (Propulsid), clarithromycin (Biaxin), cyclosporine (Sandimmune; Neoral), danazol (Danacrine), diltiazem (Cardizem; Tiazac), erythromycin, fluconazole (Diflucan), itraconazole (Sporanox), ketoconazole (Nizoral), metoclopramide (Reglan), methylprednisolone (Medrol), nicardipine (Cardene), troleandomycin (Tao), and verapamil (Calan; Isoptin; Verelan; Covera-HS). Grapefruit juice also may have a similar effect on tacrolimus and should be avoided completely receiving this medication.

Other drugs can stimulate the break-down of tacrolimus, decreasing its blood concentration and possibly reducing its effectiveness. Such drugs include carbamazepine (Tegretol), nifedipine (Procardia; Adalat); phenobarbital, phenytoin (Dilantin), rifabutin, and rifampin.

The study doctor will monitor the subject very closely for side effects. Doses will be adjusted if serious side effects occur.

Risks of methotrexate:

Likely:

- Lowered white blood cell count that may lead to infection. This can be treated with blood products (transfusions) and/or antibiotics.
- Lowered platelets, which may lead to an increase in bruising or bleeding. This can be treated with blood products (transfusions) and/or antibiotics.
- Lowered red blood cells, which may cause anemia, tiredness, or shortness of breath. This can be treated with blood products (transfusions) and/or antibiotics
- Inflammation (redness, swelling, burning) of the mouth, (gums, tongue, throat)
- Inflammation (swelling, burning) of the intestine, especially the small intestine
- Nausea
- Vomiting, sometimes with blood
- Diarrhea
- Loss of appetite
- Mouth sores
- Itching
- Rash
- Sun sensitivity
- Drowsiness
- Blurred vision
- Ringing in ears
- Low energy
- Seizures.

Less likely:

- Pale stools
- Headache
- Back pain and rigidity.

Less Likely, But Serious:

- Liver disease
- Lung disease (which may not be fully reversible)
- Kidney problems
- Bladder problems
- Low sperm counts
- Diabetes

Folic acid may decrease response to methotrexate. NSAIDs (nonsteroidal anti-inflammatory drugs), which are anti-inflammatory pain medications like ibuprofen, Motrin, or Advil may increase methotrexate blood levels. Other drugs can increase or decrease effectiveness of this drug. Patients should not take any folic acid or NSAIDs while on this research study and should tell their study doctor before taking any other drugs or vitamins.

Risks of anti-thymocyte globulin (ATG):

Likely:

- Fever
- Chills

Rarely:

- Hypotension (low blood pressure)
- Allergic reaction (shortness of breath)
- Cause the occurrence of lymphoma (cancer of your immune system) after transplantation

Risk of secondary malignancy

A number of established chemotherapeutic agents have a known risk of causing secondary cancers and/or leukemias. Certain agents in use today, not currently known to be associated with this risk may be shown at a later time to result in the development of these secondary cancers and/or leukemias.

Pregnancy Risks

The effects of the drugs used in this research study on the fetus are unknown. For this reason, to take part in this study, women who are capable of bearing children and men who are capable of fathering a child and who are heterosexually active must use an effective means of birth control throughout the study. You must notify the study doctor if you suspect that you or your partner may be pregnant.

Risks of clipping nails

There are no risks to clipping nails**.**

Potential Medication Interaction

Because some medications (either sold with or without prescriptions) can potentially reduce the effectiveness and/or enhance the side effects of ruxolitinib, you should tell your study doctor about any new treatments or medications you may be taking. This includes all prescription or sold without prescription medications, health food supplements, vitamins or herbal remedies, acupuncture or other alternative therapies.

OTHER POSSIBLE OPTIONS TO CONSIDER:

You may choose not to join this research study without any penalty. The choice is totally up to you. There may be other treatment options that are available to you instead of participating in this research study. These include other non-study treatments for IM, or taking medications to help your symptoms only. You may also decide to use a standard conditioning regimen instead of a reduced intensity regimen. There may also be other research studies available at ***(participating site)*** or another hospital. Your study doctor should explain these other options, including the possible risks and benefits of these other options before you make the decision to participate in this research study. You may also choose not to receive treatment for myeloproliferative disorders.

If you choose not to participate in this research study, you may continue treatments that have already been prescribed or you may choose to receive no treatment. Your doctor will discuss your options with you. If you choose not to participate in this research study, other choices are available to you.

IN CASE OF INJURY DURING THIS RESEARCH STUDY:

If you are injured or made sick from taking part in this research study, medical care will be provided. Generally, this medical care will be billed to you and/or your health care insurance in the ordinary manner and you will be responsible for all treatment costs not covered by your insurance, including deductibles, co-payments, and coinsurance. This does not prevent you from seeking payment for injury related to malpractice or negligence. Contact the investigator for more information.

ENDING PARTICIPATION IN THE RESEARCH STUDY:

You may stop taking part in this research study at any time without any penalty. This will not affect your ability to receive medical care at ***(Name of site)*** or to receive any benefits to which you are otherwise entitled.

If you decide to stop being in the research study, please contact the Principal Investigator or the research staff.

You may also withdraw your permission for the use and disclosure of any of your protected information for research, but <u>you must do so in writing</u> to the Principal Investigator at the address on the first page. Even if you withdraw your permission, the Principal Investigator for the research study may still use the information that was already collected if that information is necessary to complete the research study. Your health information may still be used or shared

after you withdraw your authorization if you should have an adverse event (a bad effect) from participating in the research study.

Samples will be banked from this research study. If you wish to withdraw your specimen from use in research, you must do so in writing to the Principal Investigator of the study.

Withdrawal without your consent: The study doctor, the sponsor or the institution may stop your involvement in this research study at any time without your consent. This may be because the research study is being stopped, the instructions of the study team have not been followed, the investigator believes it is in your best interest, or for any other reason. If specimens or data have been stored as part of the research study, they too can be destroyed without your consent

This research involves the research use of genetic testing to diagnose the presence of a genetic variation linked to a predisposition to a genetic disease or disability in the subject or the subject's offspring. You have the right to withdraw consent to use of the tissue for future use at any time, and the Principal Investigator should be contacted to withdraw consent. If you have consented to storage of the tissue sample and you would like to withdraw your consent, you may do so at any time. A member of the study team who is storing the sample will promptly destroy the sample or portions that have not already been used for research.

CONTACT PERSON(S):

If you have any questions, concerns, or complaints at any time about this research, or you think the research has hurt you, please contact the office of the research team and/or the Principal Investigator at phone number ***(sites' phone number)*****.**

This research has been reviewed and approved by an Institutional Review Board. You may reach a representative of the Program for the Protection of Human Subjects at ***(Participating site)*** at telephone number ***(sites' phone number)*** during standard work hours for any of the following reasons:

- Your questions, concerns, or complaints are not being answered by the research team.
- You cannot reach the research team.
- You are not comfortable talking to the research team.
- You have questions about your rights as a research subject.
- You want to get information or provide input about this research.

DISCLOSURE OF FINANCIAL INTERESTS:

None.

MAINTAINING CONFIDENTIALITY – HIPAA AUTHORIZATION:

As you take part in this research project it will be necessary for the research team and others to use and share some of your private protected health information. Consistent with the federal

Health Insurance Portability and Accountability Act (HIPAA), we are asking your permission to receive, use and share that information.

What protected health information is collected and used in this study, and might also be disclosed (shared) with others?
As part of this research project the researchers will collect your name, address including zip code, telephone number, date of birth, sex, race, and ethnicity.

The researchers will also get information from your medical record **(**includes hospital record from ***(Participating site)*** and referring physician's records)

During the study the researchers will gather information by:

- taking a medical history (includes current and past medications or therapies, illnesses, conditions or symptoms, family medical history, allergies, etc.)
- doing a physical examination that generally also includes blood pressure reading, heart rate, breathing rate and temperature
- completing the tests and procedures explained in the description section of this consent such as CT scans, pulmonary function tests, X-rays, ECGs, pregnancy test results, urinalysis, bone marrow aspirate/biopsy, nail clippings, flow cytometry, Chimerism test results, Hepatitis A, B, C, results, CMV, HSV, and EBV virus results, blood typing results, class I and II antigen results, and pathology results
- reviewing genetic tests

Why is your protected health information being used?
Your personal contact information is important to be able to contact you during the study. Your health information and the results of any tests and procedures being collected as part of this research study will be used for the purpose of this study as explained earlier in this consent form. The results of this study could be published or presented at scientific meetings, lectures, or other events, but would not include any information that would let others know who you are, unless you give separate permission to do so.

The Principal Investigator may also use and share the results of these tests and procedures to treat you.

The research team and other authorized members of ***(Participating site)*** workforce may use and share your information to ensure that the research meets legal, institutional or accreditation requirements. For example, the ***(Participating site)*** Program for the Protection of Human Subjects is responsible for overseeing research on human subjects, and may need to see your information. If you receive any payments for taking part in this study, the ***(Participating site)*** Center Finance Department may need your name, address, social security number, payment amount, and related information for tax reporting purposes. If the research team uncovers abuse, neglect, or reportable diseases, this information may be disclosed to appropriate authorities.

Who, outside ***(Participating site)***, might receive your protected health information?

As part of the study, the Principal Investigator, study team and others in the ***(Participating site)*** workforce may disclose your protected health information, including the results of the research study tests and procedures, to the following people or organizations: (It is possible that there may be changes to the list during this research study; you may request an up-to-date list at any time by contacting the Principal Investigator.)

- Research data coordinating office and/or their representative(s) who will be responsible for collecting results and findings from all the centers: Myeloproliferative Disorders Research Consortium Data Management Center
- Outside laboratory who will be performing laboratory analysis for all the research centers involved in this project: Myeloproliferative Disorders Central Laboratory located at the New York Blood Center in North America and at MPD-RC Tissue Bank, Laboratorio Paolo Belli in Europe.
- The manufacturer of the drug Ruxolitinib, Incyte and Novartis Pharmaceuticals.
- The sponsoring government agency and/or their representative who need to confirm the accuracy of the results submitted to the government or the use of government funds: In the USA this is the National Cancer Institute (NCI), in Europe this will be the individual regulatory authorities for examples the MHRA.
- A Data Safety Monitoring Board or other committee that will monitor the study on an ongoing basis for safety. This will include the ***(Participating site)*** Institutional Review Board (IRB) and the Myeloproliferative Disorders Research Consortium Data and Safety Monitoring Board.
- The United States Food and Drug Administration.
- United States Department of Health and Human Services and the Office of Human Research Protection.

In all disclosures outside of ***(Participating site)***, you will not be identified by name, social security number, address, telephone number, or any other direct personal identifier unless disclosure of the direct identifier is required by law. Some records and information disclosed may be identified with a unique code number. The Principal Investigator will ensure that the key to the code will be kept in a locked file, or will be securely stored electronically. The code will not be used to link the information back to you without your permission, unless the law requires it, or rarely if the Institutional Review Board allows it after determining that there would be minimal risk to your privacy. It is possible that a sponsor or their representatives, a data coordinating office, a contract research organization, will come to inspect your records. Even if those records are identifiable when inspected, the information leaving the institution will be stripped of direct identifiers. Additionally, the monitors, auditors, the IRB, the Food and Drug Administration will be granted direct access to your medical records for verification of the research procedures and data. By signing this document you are authorizing this access. We may publish the results of this research. However, we will keep your name and other identifying information confidential.

For how long will ***(Participating site)*** be able to use or disclose your protected health information?

Your authorization for use of your protected health information for this specific study does not expire.

Will you be able to access your records?

During your participation in this study, you will have access to your medical record and any study information that is part of that record. The investigator is not required to release to you research information that is not part of your medical record.

Do you need to give us permission to obtain, use or share your health information?

NO! If you decide not to let us obtain, use or share your health information you should not sign this form, and you will not be allowed to volunteer in the research study. If you do not sign, it will not affect your treatment, payment or enrollment in any health plans or affect your eligibility for benefits.

Can you change your mind?

You may withdraw your permission for the use and disclosure of any of your protected information for research, but you must do so in writing to the Principal Investigator at the address on the first page. Even if you withdraw your permission, the Principal Investigator for the research study may still use your protected information that was already collected if that information is necessary to complete the study. Your health information may still be used or shared after you withdraw your authorization if you should have an adverse event (a bad effect) from being in the study. If you withdraw your permission to use your protected health information for research that means you will also be withdrawn from the research study, but standard medical care and any other benefits to which you are entitled will not be affected. You can also tell us you want to withdraw from the research study at any time without canceling the Authorization to use your data.

If you have not already received it, you will also be given the ***(Participating site)*** Notice of Privacy Practices that contains more information about how ***(Participating site)*** uses and discloses your protected health information.

It is important for you to understand that once information is disclosed to others outside ***(Participating site)***, the information may be re-disclosed and will no longer be covered by the federal privacy protection regulations. However, even if your information will no longer be protected by federal regulations, where possible, ***(Participating site)*** has entered into agreements with those who will receive your information to continue to protect your confidentiality.

If as part of this research project your medical records are being reviewed, or a medical history is being taken, it is possible that HIV-related information may be revealed to the researchers. If that is the case, the information in the following box concerns you. If this research does not involve any review of medical records or questions about your medical history or conditions, then the following section may be ignored.

Signature Block for Capable Adult

Your signature below documents your permission to take part in this research and to the use and disclosure of your protected health information. A signed and dated copy will be given to you.

DO NOT SIGN THIS FORM AFTER THIS DATE →

Signature of subject	Date and Time
Printed name of subject	

Person Explaining Study and Obtaining Consent

Signature of person obtaining consent	Date and Time
Printed name of person obtaining consent	

If the individual cannot read, a witness is required to observe the consent process and document below:

My signature below documents that the information in the consent document and any other written information was accurately explained to, and apparently understood by, the subject, and that consent was freely given by the subject.

Signature of witness to consent process	*Date and Time*
Printed name of person witnessing consent process	

APPENDICES

Appendix 1 Dynamic International Prognostic Scoring System for Myelofibrosis (DIPSS)

Risk Factors at any time of disease management – Selected for Significant Association with Shorter Survival

No.	Prognostic Factor	Score
1	Age > 65 years	**1**
2	Presence of Constitutional symptoms *(weight loss >10% of the baseline value in the year preceding MF diagnosis and/or unexplained fever or excessive sweats persisting for more than 1 month)*	**1**
3	Hemoglobin (Hb) < 10g/dL	**2**
4	WBC count > 25 x 10^9/L	**1**
5	Blood blasts ≥1	**1**

Definition of the Risk Groups of the Prognostic Scoring System of Myelofibrosis

Risk Group	Score	Median Survival Months (95% CI)
Low	**0**	**Not reached**
Intermediate - 1	**1-2**	**9.8 yrs**
Intermediate - 2	**3-4**	**4.8 yrs**
High	**≥5**	**2.3 yrs**

Appendix 2 Transfusion Dependency

RBC-transfusion dependence is defined as recently described by a panel with expertise on Myeloproliferative disorders ***(Gale et al, Leukemia Research, 2011).*** This is defined as an average RBC-transfusion-frequency of ≥2 units of RBC transfusion every 28 days (in the absence of active bleeding or chemotherapy) over at least the 84 days immediately prior to study entry.

Appendix 3 Definition of Unfavorable Karyotype

Unfavorable karyotypes in myelofibrosis associated with poor prognosis

Unfavorable karyotype includes:

- Complex karyotype
- Single or two abnormalities including +8, −7/7q-, i(17q), −5/5q-, 12p-, inv(3) or 11q23 rearrangement

Appendix 4 IWG-MRT criteria for response assessment

IWG-MRT criteria for response assessment *(from Tefferi et al., 2006)*

Response Criterion Parameter

Complete remission (CR)

i. Complete resolution of disease-related symptoms and signs including palpable hepatosplenomegaly.

ii. Peripheral blood count remission defined as hemoglobin level at least 110 g/L, platelet count at least 100 x 10^9/L, and absolute neutrophil count at least 1.0 x 10^9/L. In addition, all 3 blood counts should be no higher than the upper normal limit.

iii. Normal leukocyte differential including disappearance of nucleated red blood cells, blasts, and immature myeloid cells in the peripheral smear, in the absence of splenectomy.

iv. Bone marrow histologic remission defined as the presence of age-adjusted normocellularity, no more than 5% myeloblasts, and an osteomyelofibrosis grade no higher than 1.

Partial remission (PR)

Requires all of the above criteria for CR except the requirement for bone marrow histologic remission. However, a repeat bone marrow biopsy is required in the assessment of PR and may or may not show favorable changes that do not however fulfill criteria for CR.

Clinical improvement (CI)

Requires one of the following in the absence of both disease progression (as outlined below) and CR/PR assignment (CI response is validated only if it lasts for no fewer than 8 weeks):

i. A minimum 20 g/L increase in hemoglobin level or becoming transfusion independent (applicable only for subjects with baseline hemoglobin level of less than 100 g/L).

ii. Either a minimum 50% reduction in palpable splenomegaly of a spleen that is at least 10 cm at baseline or a spleen that is palpable at more than 5 cm at baseline becomes not palpable.

iii. A minimum 100% increase in platelet count and an absolute platelet count of at least 50 000 x 10^9/L (applicable only for subjects with baseline platelet count below 50 x 10^9/L).

iv. A minimum 100% increase in ANC and an ANC of at least 0.5 x 10^9/L (applicableonly for subjects with baseline absolute neutrophil count below 1 x 10^9/L).

Progressive disease (PD)

Requires one of the following:

i. Progressive splenomegaly that is defined by the appearance of a previously absent splenomegaly that is palpable at greater than 5 cm below the left costal margin or a minimum 100% increase in palpable distance for baseline splenomegaly of 5-10 cm or a minimum 50% increase in palpable distance for baseline splenomegaly of greater than 10 cm.

ii. Leukemic transformation confirmed by a bone marrow blast count of at least 20%.

iii. An increase in peripheral blood blast percentage of at least 20% that lasts for at least 8 weeks.

Stable disease (SD)

None of the above.

Relapse

Loss of CR, PR, or CI.

In other words, a subject with CR or PR is considered to have undergone relapse when he or she no longer fulfills the criteria for even CI. However, changes from either CR to PR or CR/PR to CI should be documented and reported.

Appendix 5 Myeloproliferative Neoplasm symptom assessment form (MPN-SAF) and Brief Fatigue Inventory (BFI)

Instructions: Please fill out all questions, as best able, reflecting how these symptoms affected you over the **LAST WEEK** unless directed otherwise. Complete forms until the STOP instruction toward the end of the packet.

Symptom	1 to 10 (0 if absent) ranking* 1 is most favorable and 10 least favorable
Please rate your fatigue (weariness, tiredness) by circling the one number that best describes your fatigue right **NOW**	(No Fatigue) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Please rate your fatigue (weariness, tiredness) by circling the one number that best describes your USUAL level of fatigue during past 24 hours	(No Fatigue) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Please rate your fatigue (weariness, tiredness) by circling the one number that best describes your WORST level of fatigue during past 24 hours	(No Fatigue) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Circle the one number that describes how, during the past 24 hours, fatigue has interfered with your	
General activity	(Does not Interfere) 0 1 2 3 4 5 6 7 8 9 10 (Completely Interferes)
Mood	(Does not Interfere) 0 1 2 3 4 5 6 7 8 9 10 (Completely Interferes)
Walking ability	(Does not Interfere) 0 1 2 3 4 5 6 7 8 9 10 (Completely Interferes)
Normal work (includes work both outside the home and daily chores)	(Does not Interfere) 0 1 2 3 4 5 6 7 8 9 10 (Completely Interferes)
Relations with other people	(Does not Interfere) 0 1 2 3 4 5 6 7 8 9 10 (Completely Interferes)
Enjoyment of life	(Does not Interfere) 0 1 2 3 4 5 6 7 8 9 10 (Completely Interferes)

Circle the one number that describes how, during the past Week how much difficulty you have had with each of the following symptoms	
Filling up quickly when you eat (Early satiety)	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Abdominal pain	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Abdominal discomfort	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Inactivity	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Problems with headaches	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Problems with concentration Compared to prior to my MPD	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Dizziness/ Vertigo/ Lightheadedness	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Numbness/ Tingling (in my hands and feet)	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Difficulty sleeping	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Depression or sad mood	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Problems with sexual desire or Function	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Cough	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Night sweats	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Itching (pruritus)	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Bone pain (diffuse not joint pain or arthritis)	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Fever (>100° F)	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Daily)
Unintentional weight loss last 6 months	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
What is your overall quality of life?	(As good as it can be) 0 1 2 3 4 5 6 7 8 9 10 (As bad as it can be)

Appendix 6 EORTC QLQ-C30 (VERSION 3)

ENGLISH

EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials: _____

Your birthdate (Day, Month, Year): _____

Today's date (Day, Month, Year): 31 _____

	Not at All	A Little	Quite a Bit	Very Much
1. Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2. Do you have any trouble taking a long walk?	1	2	3	4
3. Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4. Do you need to stay in bed or a chair during the day?	1	2	3	4
5. Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:	Not at All	A Little	Quite a Bit	Very Much
6. Were you limited in doing either your work or other daily activities?	1	2	3	4
7. Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8. Were you short of breath?	1	2	3	4
9. Have you had pain?	1	2	3	4
10. Did you need to rest?	1	2	3	4
11. Have you had trouble sleeping?	1	2	3	4
12. Have you felt weak?	1	2	3	4
13. Have you lacked appetite?	1	2	3	4
14. Have you felt nauseated?	1	2	3	4
15. Have you vomited?	1	2	3	4
16. Have you been constipated?	1	2	3	4

Please go on to the next page

ENGLISH

During the past week:	**Not at All**	**A Little**	**Quite a Bit**	**Very Much**
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

30. How would you rate your overall quality of life during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

Appendix 7 FACT-BMT (VERSION 4)

FACT-BMT (Version 4)

Below is a list of statements that other people with your illness have said are important. **Please circle or mark one number per line to indicate your response as it applies to the past 7 days.**

	PHYSICAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GP1	I have a lack of energy	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	I am forced to spend time in bed	0	1	2	3	4

	SOCIAL/FAMILY WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GS1	I feel close to my friends	0	1	2	3	4
GS2	I get emotional support from my family	0	1	2	3	4
GS3	I get support from my friends	0	1	2	3	4
GS4	My family has accepted my illness	0	1	2	3	4
GS5	I am satisfied with family communication about my illness	0	1	2	3	4
GS6	I feel close to my partner (or the person who is my main support)	0	1	2	3	4
Q1	*Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please mark this box* ☐ *and go to the next section.*					
GS7	I am satisfied with my sex life	0	1	2	3	4

FACT-BMT (Version 4)

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

	EMOTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GE1	I feel sad	0	1	2	3	4
GE2	I am satisfied with how I am coping with my illness	0	1	2	3	4
GE3	I am losing hope in the fight against my illness	0	1	2	3	4
GE4	I feel nervous	0	1	2	3	4
GE5	I worry about dying	0	1	2	3	4
GE6	I worry that my condition will get worse	0	1	2	3	4

	FUNCTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GF1	I am able to work (include work at home)	0	1	2	3	4
GF2	My work (include work at home) is fulfilling	0	1	2	3	4
GF3	I am able to enjoy life	0	1	2	3	4
GF4	I have accepted my illness	0	1	2	3	4
GF5	I am sleeping well	0	1	2	3	4
GF6	I am enjoying the things I usually do for fun	0	1	2	3	4
GF7	I am content with the quality of my life right now	0	1	2	3	4

FACT-BMT (Version 4)

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

	ADDITIONAL CONCERNS	Not at all	A little bit	Some-what	Quite a bit	Very much
BMT1	I am concerned about keeping my job (include work at home)	0	1	2	3	4
BMT2	I feel distant from other people	0	1	2	3	4
BMT3	I worry that the transplant will not work	0	1	2	3	4
BMT4	The effects of treatment are worse than I had imagined	0	1	2	3	4
C6	I have a good appetite	0	1	2	3	4
C7	I like the appearance of my body	0	1	2	3	4
BMT5	I am able to get around by myself	0	1	2	3	4
BMT6	I get tired easily	0	1	2	3	4
BL4	I am interested in sex	0	1	2	3	4
BMT7	I have concerns about my ability to have children	0	1	2	3	4
BMT8	I have confidence in my nurse(s)	0	1	2	3	4
BMT9	I regret having the bone marrow transplant	0	1	2	3	4
BMT10	I can remember things	0	1	2	3	4
Br1	I am able to concentrate	0	1	2	3	4
BMT11	I have frequent colds/infections	0	1	2	3	4
BMT12	My eyesight is blurry	0	1	2	3	4
BMT13	I am bothered by a change in the way food tastes	0	1	2	3	4
BMT14	I have tremors	0	1	2	3	4
B1	I have been short of breath	0	1	2	3	4
BMT15	I am bothered by skin problems (e.g., rash, itching)	0	1	2	3	4
BMT16	I have trouble with my bowels	0	1	2	3	4
BMT17	My illness is a personal hardship for my close family members	0	1	2	3	4
BMT18	The cost of my treatment is a burden on me or my family	0	1	2	3	4

Appendix 8 Patient Global Impression of change (PGIC)

- Since starting treatment, my *overall quality of life* is: *(please circle one)*

-3	-2	-1	0	1	2	3
Very much worse	Moderately worse	A little worse	About the same	A little better	Moderately better	Very much better

- Since starting treatment, my *physical condition* is: *(please circle one)*

-3	-2	-1	0	1	2	3
Very much worse	Moderately worse	A little worse	About the same	A little better	Moderately better	Very much better

- Since starting treatment, my *emotional state* is: *(please circle one)*

-3	-2	-1	0	1	2	3
Very much worse	Moderately worse	A little worse	About the same	A little better	Moderately better	Very much better

Appendix 9 Regimen Related Toxicity: Bearman Toxicity Criteria

	Grade I	Grade II	Grade III
Cardiac toxicity	*Dysrhythmias - recurrent or persistent, no therapy required. *EF/CHF - asymptomatic decline of resting ejection fraction by more than 20% of baseline value *Ischemia - asymptomatic ST and T wave changes suggesting ischemia *Pericardial - pericarditis (rub, chest pain, ECG changes)	*Dysrhythmias - requires monitoring or treatment *EF/CHF - mild CHF, responsive to therapy *Ischemia - angina without evidence for infarction *Pericardial - symptomatic effusion, drainage required	*Dysrhythmias with hypotension or ventricular tachycardia or fibrillation *EF/CHF - severe or refractory CHF *Ischemia - acute myocardial infarction *Pericardial - tamponade; drainage urgently required.
Pulmonary toxicity	*Dyspnea -no CXR changes -no infection -no congestive heart failure *CXR showing isolated infiltrate or mild interstitial changes -no infection -no congestive heart failure -asymptomatic	*CXR with extensive localized infiltrate or moderate interstitial change combined with dyspnea and not caused by infection or CHF. *Decrease of PO_2 (>10% from baseline) but not requiring mechanical ventilation or >50% O_2 on mask and not caused by infection or CHF.	*Interstitial changes requiring mechanical ventilatory support or >50% oxygen on mask and not caused by infection or CHF.
Stomatitis toxicity	*Pain and/or ulceration not requiring a continuous IV narcotic drug.	*Pain and/or ulceration requiring a continuous IV narcotic drug (morphine drip).	*Severe ulceration and/or mucositis requiring preventive intubation. *severe ulceration resulting in documented aspiration pneumonia with or without intubation.
GI toxicity	*Watery stools >500ml but <2000ml every day not related to infection.	*Watery stools >2000ml every day not related to infection. *Macroscopic hemorrhagic stools with no effect on cardiovascular status not caused by infection *subileus not related to infection.	*Ileus requiring nasogastric suction and/or surgery and not related to infection. *Hemorrhagic enterocolitis affecting cardiovascular status and requiring transfusion.

	Grade I	Grade II	Grade III
Hepatic toxicity	*Mild hepatic dysfunction with bilirubin >34.2 micromoles/litre and bilirubin <102.6 micromoles/litre *Weight gain >2.5% and < 5% from baseline, of noncardiac origin. *SGOT increase more than 2-fold but less than 5-fold from lowest preconditioning.	*Moderate hepatic dysfunction with bilirubin >102.6 micromoles/litre and <342 micromoles/litre. *SGOT increase >5 -fold from preconditioning/ *Clinical ascites or image documented ascites >100ml. *Weight gain >5% from baseline of noncardiac origin.	*Severe hepatic dysfunction with bilirubin >342 micromoles/litre. *Hepatic encephalopathy. *Ascites compromising respiratory function.
CNS toxicity	*Somnolence, patient is easily arousable and oriented after arousal.	*Somnolence with confusion after arousal *Other new objective CNS symptoms with no loss of consciousness not more easily explained by other medication, bleeding, or CNS infection.	*Seizures or coma not explained (documented) by other medication, CNS infection, or bleeding.
Bladder toxicity	*Macroscopic hematuria after 2 days from last chemotherapy dose. -no subjective symptoms of cystitis -no infection	*Macroscopic hematuria after 7 days from last chemotherapy dose -no infection *Hematuria after 2 days with subjective symptoms of cystitis -no infection.	*Hemorrhagic cystitis with frank blood necessitating invasive local intervention with instillation of sclerosing agents, nephrostomy or other surgical procedure.
Renal	*Increase in creatinine up to twice the baseline value (usually the last recorded before start of conditioning).	*Increase in creatinine above twice baseline -no dialysis.	*Requirements of dialysis.

Appendix 10 Assesment of Acute and Chronic GvHD

The staging and grading of acute GvHD should be done according to consensus criteria as defined by Przepiorka et al, BMT, 1994.

Extent of organ involvement			
	Skin	Liver	Gut
Stage			
1	Rash on <25% of skin	Total bilirubin 2-3 mg/dl or 35-50 μmol/L	Diarrhea >500 ml/day or persistent nausea
2	Rash 25-50% of skin	Total bilirubin 3-6 mg/dl or 51-102 μmol/L	Diarrhea >1000 ml/day
3	Rash >50% of skin	Total bilirubin 6-15 mg/dl or 103-225 μmol/L	Diarrhea >1500 ml/day
4	Generalized erythroderma with bullous formation	Total bilirubin >15 mg/dl or >225 μmol/L	Severe abdominal pain with or without ileus
Grading			
I	Stage 1-2	None	None
II	Stage 3 or	Stage 1 or	Stage 1
III	-	Stage 2-3 or	Stage 2-4
IV	Stage 4	Stage 4	-

Grading of chronic GVHD

Chronic GVHD should be graded as mild, moderate or severe according to NIH consensus criteria (Filipovich et al, 2005, BBMT). The scoring for each organ involved is done as

Score 0 = no involvement
Score 1 = Mild involvement (no significant impairment of daily living)
Score 2 = moderate involvement (significant impairment of daily living)
Score 3 = severe impairment (major disability)

Global scoring of severity of chronic GvHD is done as follows:

Global scoring of severity of chronic GvHD

Number of organs	Mild cGvHD	Moderate cGvHD	Severe cGvHD
1	Score 1	Score 2	Score 3
2	Score 1	Score 2	Score 3
3		Score 1	Score 3
Lung involvement		Score 1	Score 2

Mild cGVHD = 1 or 2 organs involved (except for lung) with maximum score of 1
Moderate cGvHD= lung score of 1 or 3 organs with score of 1 or at least 1 organ with score of 2
Severe cGvHD= lung score of 2 or score of 3 in any organ

Appendix 11 Request for Release of Nonconforming HCT/Ps

I certify that patient_____

MRN#_____

Has a medical emergency_____ **that requires that he/she receives nonconforming HCT/Ps**

Products to be released for infusion:

	Product UPN	Nature of Nonconformance
1.	_____	_____
2.	_____	_____
3.	_____	_____
4.	_____	_____

Comments:_____

Signature: _____ **Date:** _____