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JAK Inhibitor Prior to Allogeneic Stem Cell Transplant for Patients with Primary and Secondary Myelofibrosis: A Prospective Study

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TABLE OF CONTENTS

1.0 INTRODUCTION

Myelofibrosis (MF) can present as a de novo disorder (PMF) or evolve from polycythemia vera (PV) or essential thrombocythemia (ET). Regardless of the etiology, MF is characterized as a clonal stem cell disorder associated with elevated levels of pro-inflammatory and pro-angiogenic cytokines such as TNF alpha, IL-6 and IFN gamma resulting in a bone marrow stromal reaction that includes varying degrees of reticulin and collagen fibrosis and osteosclerosis. Clinically, MF is characterized by progressive anemia, leukopenia or leukocytosis, thrombocytopenia or thrombocythemia and multi-organ extramedullary hematopoiesis that frequently involves the spleen resulting in massive splenomegaly, severe constitutional symptoms, a hypermetabolic state and cachexia. The median age at diagnosis is 60 to 65 years. The clinical course is heterogeneous ranging from indolent disease in some patients who may survive for years or decades to aggressive disease in other cases with survival measured in months. The most common causes of death are progressive marrow failure leading to infection or hemorrhage, transformation to acute myelogenous leukemia and complications of portal hypertension.

While in the past drug therapies including hydroxyurea, busulfan, 6-mercaptopurine, anagrelide, thalidomide, lenalidomide, interferon, corticosteroids, androgens, and erythropoiesis stimulating agents or other growth factors were used as the first line treatment of MF, none of these have been shown to improve survival. Ruxolitinib, a selective JAK-1/2 inhibitor, was recently FDA approved for treatment of primary and secondary MF. Impressive symptom control in MF patients on ruxolitinib treatment is predominantly mediated by profound suppression of inflammation, mirrored by a significant reduction in pro-inflammatory and pro-angiogenic cytokines. [1] Reduction of these cytokines is typically accompanied by a significant reduction in spleen size. To date, the only potentially curative therapy for MF is allogeneic hematopoietic stem cell transplantation (HCT). Whether JAK-2 inhibitor therapy prior to HCT by improving patient performance status and the degree of splenomegaly, can lead to more favorable post-transplant outcomes is an important area of investigation. However, no prospective studies have been performed to date. Herein, we aim to prospectively assess the effects of pre-transplant JAK-2 inhibitor therapy as an adjunct to HCT with the goal of improving post-transplant outcomes in patients with myelofibrosis.

2.0 BACKGROUND

2.1 Prognostic factors in myelofibrosis

Prognosis of MF varies with the presence or absence of specific risk factors. In 2009, Cervantes et al published a multi-center analysis of risk factors and their impact on prognosis in patients with myelofibrosis.[2] Age of greater than 65 years, presence of constitutional symptoms including weight loss, fever, or night sweats, anemia (hemoglobin < 10 g/dL), leukocytosis (white blood cells (WBC) > 25 x 109/L), and a circulating blast percentage ≥ 1% were identified as most predictive of outcome. Patients with no risk factors (low-risk group) had a median survival of 135 months, patients with one risk factor (intermediate-1 risk) had a median survival of 95 months, patients with two risk factors (intermediate-2 risk) had a median survival of 48 months, and patients with 3 or more risk factors (high-risk) had a median survival of 27 months. [2]

This risk stratification is referred to as the international prognostic scoring system (IPSS). The *Dynamic* IPSS (DIPSS) uses the same 5 variables but can be used at any time in the disease course; Hb < 10 receives a score of 2 points. The risk groups are scored as follows: Low risk = (score) 0, Intermediate 1 = 1 or 2, Intermediate 2 = 3 or 4, High risk = 5 or 6. The DIPSS *plus* scoring system includes, in addition, transfusion dependence, unfavorable and complex karyotype and platelet count.

Current recommendations suggest that the potential risk of transplant related complications is justified in transplant-eligible patients with < 5 years expected survival if not transplanted. Based on DIPSS data this would include patients in the intermediate 2 and high risk groups. Those are, indeed, the patients included in most clinical transplant trials; however, lower risk patients actually have better outcome following HCT, raising questions as to the selection of patients for HCT. Published data from our center showed that the 6 year post-HCT survival for Low, Intermediate 1, Intermediate 2 and High risk patients was 80%, 67%, 54% and 38%, respectively, validating the DIPSS as an accurate prognosticator for post-HCT outcome in MF.[3]

2.2 Rationale for use of JAK inhibitors

Myelofibrosis is a clonal hematopoietic stem cell disease characterized by molecular (*JAK2*V617F, *MPL*W515L/K) and cytogenetic (13q-, 20q-) markers. [4,5] The *JAK2*V617F mutation has been identified in over 95% of patients with PV and approximately 50% of patients with ET and PMF. In clinical trials, treatment of MF patients with the JAK inhibitor ruxolitinib resulted in prompt resolution of constitutional symptoms concurrent with reduction in the plasma inflammatory cytokine profile. [6-8] In a phase 3 randomized trial of ruxolitinib, 42% of treated patients experienced a reduction in spleen size/volume of at least 35% compared with 0.7% in those receiving placebo. [8] This was true in both JAK-2 mutated and non-mutated patients. Ruxolitinib also improved constitutional symptoms coincident with normalization of cytokine profiles.

In a phase 1-2 study of ruxolitinib therapy at MD Anderson, OS was significantly superior in ruxolitinib treated patients as compared with the historical cohort in an analysis adjusted for IPSS. [7] Spleen volume reduction alone was associated with survival advantage in ruxolitinib treated patients; 63% of patients achieved >50% reduction in spleen volume with a median duration of approximately 2 years. Patients with >50% reduction in splenomegaly had superior survival when compared with patients with < 25% spleen size reduction ($p < 0.0001$). Furthermore, the finding of superimposable survival curves for high-risk and intermediate-2 risk patients treated with ruxolitinib suggests that ruxolitinib therapy may downgrade an individual's prognostic score category and improve predicted survival. In fact, in the Comfort 1 trial, patients randomized to ruxolitinib versus placebo had a better median OS. [8] In the Comfort 2 study, 61% of patients on the best supportive care arm eventually received ruxolitinib. [6] Still, a modest survival advantage was seen among patients originally randomized to ruxolitinib. The reduction of an inflammatory state with improvement of performance status and cachexia may in itself represent a form of disease modification contributing to improved survival.[6]

2.3 Clinical to Date

Myeloablative Conditioning Regimens

While a broad range of conditioning regimens of different intensities has been developed, initial studies of allogeneic HCT for MF used high intensity regimens, which by most investigators would now be considered "myeloablative" (MAC). At that juncture, concern for graft failure/rejection due to fibrotic marrow was a concern, but subsequent experience showed that this was largely unfounded. In the largest retrospective analysis to date of 289 patients reported to the CIBMTR, 229 of whom underwent MAC, day 100 treatment related mortality (TRM) was 22%-42% (dependent on performance status, URD donor, and peripheral blasts) and overall survival (OS) was 30%-40% at 5 years.[9] Graft failure was more frequent in patients receiving cells from unrelated donors (URD). Among patients with favorable risk factors including a Karnofsky Performance Score (KPS) > 90%, no circulating blasts, and matched sibling donors, 1- and 3-year OS probabilities were 69% and 60%, respectively. For MAC regimens, graft failure rates of <5% [5] to 30% [10] have been reported reflecting the heterogeneity in patient population and specific conditioning regimens. Published TRM rates of 20% at 1 year to 34% at 5 years and OS from 39% at 3 years to 50% at 5 years have been reported **(Table 1).** [5,11-13] Data from our institution showed a 7 year survival of 61% in patients conditioned with targeted IV Busulfan and Cytoxan, which was significantly higher than among those not receiving this regimen [14]. A more recent study demonstrated that administering Cytoxan 120mg/kg over 2 days followed by targeted Busulfan for 4 days conferred significantly less liver toxicity than Busulfan *followed* by Cytoxan in patients with MF. [15] This translated into decreased early TRM. Based on the above data we have chosen to use the Cytoxan/targeted Busulfan combination as conditioning in patients with HLA-matched sibling and HLA-matched unrelated donors in the current study.

Reduced Intensity Conditioning Regimens

TRM has generally been high in older patients and those with comorbidities. To improve outcomes in those patient groups, several small studies of older patients transplanted after RIC have reported encouraging results with decreased TRM. Kroger et al 2005 treated 21 patients with MF with fludarabine 180mg/m2 and busulfan 10mg/kg and ATG followed by related donor or URD HCT and observed no graft failures and no 100-day TRM.[16] One year TRM was 16%. At 3 years, the relapse free survival (RFS) was 84%; 78% of patients with JAK-

2 mutations became PCR negative. A follow-up report included 103 patients treated with fludarabine/busulfan/ATG, with a median age of 55 yrs; 33 had matched sibling donors, and among the 60 URD transplants, 30% were mismatched. [17] Relapse incidence was 22% at 3 yrs and 29% at 5 yrs. NRM was 16%. NRM was higher with HLA mismatched donors. 5-yr RFS was 51% and OS was 67%. RFS was inferior with HLA-mismatched transplants and advanced Lille score, and OS was lower with patient age > 55 years and HLA mismatched transplant. Rondelli et al reported on 21 patients most of whom were conditioned with fludarabine and melphalan or TBI or thiotepa-based RIC. [18] RFS was 74% and OS was 85% at 3 years. The CIBMTR series included 60 patients who underwent RIC. Among these, graft failure occurred in 7 patients, none of whom survived. TRM rate at 1 year was 15% for HLA matched sibling transplants, but 49% in those transplanted from URD. RFS at 3 years was 39% for matched sibling HCT but 17% with URD.

On this background we have chosen to use fludarabine and melphalan for patients receiving matched sibling or URD grafts and require a RIC regimen.

Selected transplant studies in patients with myelofibrosis (modified from Alchalby 2010)[19]

Study	Patients, *n*	Conditioning regimen	Median age, *y*	Transplant-related mortality	Overall survival
Myeloablative conditioning					
Ballen et al. 2005	170 sibling	Various	45	22% at day 100	39% at 5 y
	117 MUD		47	42% at day 100	31% at 5 y
	33 alternative		40	27% at day 100	31% at 5 y
Guardiola et al. 1999	55	TBI-based (*n*=35), various (*n*=20)	42	27% at 1 y	47% at 5 y
Deeg et al. 2003	56	Busulfan-based (*n*=44), TBI-based (*n*=12)	43	20% at 1 y	58% at 3 y
Kerbauy et al. 2007	104	TBI-based (*n* = 15), busulfan-based (*n*=80), reduced-intensity (*n*=9)	49	34% at 5 y	61% at 7 y
Reduced-intensity conditioning					
Hessling et al. 2002	3	Busulfan/fludarabine	51	0% at 1 y	100% at 1 y
Devine et al. 2002	4	Melphalan/fludarabine	56	0% at 1 y	100% at 1 y

Study	Patients, *n*	Conditioning regimen	Median age, *y*	Transplant-related mortality	Overall survival
Hertenstein et al. 2002	20	TBI or fludarabine	50	37% at 1 y	54% at 1 y
Rondelli et al. 2005	21	Various	54	10% at 1 y	85% at 2.5 y
Kröger et al. 2005	21	Busulfan/fludarabine	53	16% at 1 y	84% at 3 y
Bacigalupo et al. 2007	39	Thiotepa-based	51		50% at 5 y
Bacigalupo et al. 2009	46	Thiotepa-based	51	24% at 5 y	45% at 5 y
George et al. 2008	6	Busulfan/fludarabine	51	0% at 16 mo	100% at 16 mo
Kröger et al. 2009	103	Busulfan/fludarabine	55	16% at 1 y	67% at 5 y

AHSCT allogeneic hematopoietic stem cell transplantation, *mo* months, *MUD* matched unrelated donor, *NS* not significant, *pt* patients, *TBI* total-body irradiation, *y* year(s)

Umbilical Cord Blood Transplantation

The incidence of delayed engraftment/graft failure associated with UCB transplants (UCBT) is of concern in this patient population due to the presence of a marrow fibrosis-induced "hostile" microenvironment. The Japanese UCBT program recently reported a case series of 14 patients who underwent RIC UCBT.[20] Neutrophil engraftment occurred in more than 92% of patients (median 23 days,) and platelet engraftment in 43% of patients (median 56 days). Complete donor chimerism was achieved in all evaluable patients. Estimated 4-yr survival was 28.6%. The most prevalent regimen used in these patients consisted of fludarabine 125mg/m2, melphalan 80mg/m2 and TBI 4Gy. Based on these limited data, we are adopting this regimen for patients receiving UCB as their source of stem cells on this protocol.

A second Japanese study focused on second transplants with UCB. Seven patients with various hematologic malignancies were treated with busulfan 16mg/kg, fludarabine 100mg/m2 and cyclophosphamide 120mg/kg. All but one patient achieved myeloid reconstitution, and four patients were alive at 4-40 months. Busulfan and Cytoxan alone as a cord blood conditioning regimen has not demonstrated high rates of successful engraftment. [21] However, the addition of fludarabine to the busulfan/Cytoxan regimen was effective in securing successful engraftment of UCB. Based on those data and the encouraging results with Cytoxan *followed* by busulfan presented by Rezvani et al[15] we will use fludarabine + Cytoxan/busulfan as MAC for the respective patients receiving UCB transplants.

2.4 Role of Surgical Splenectomy

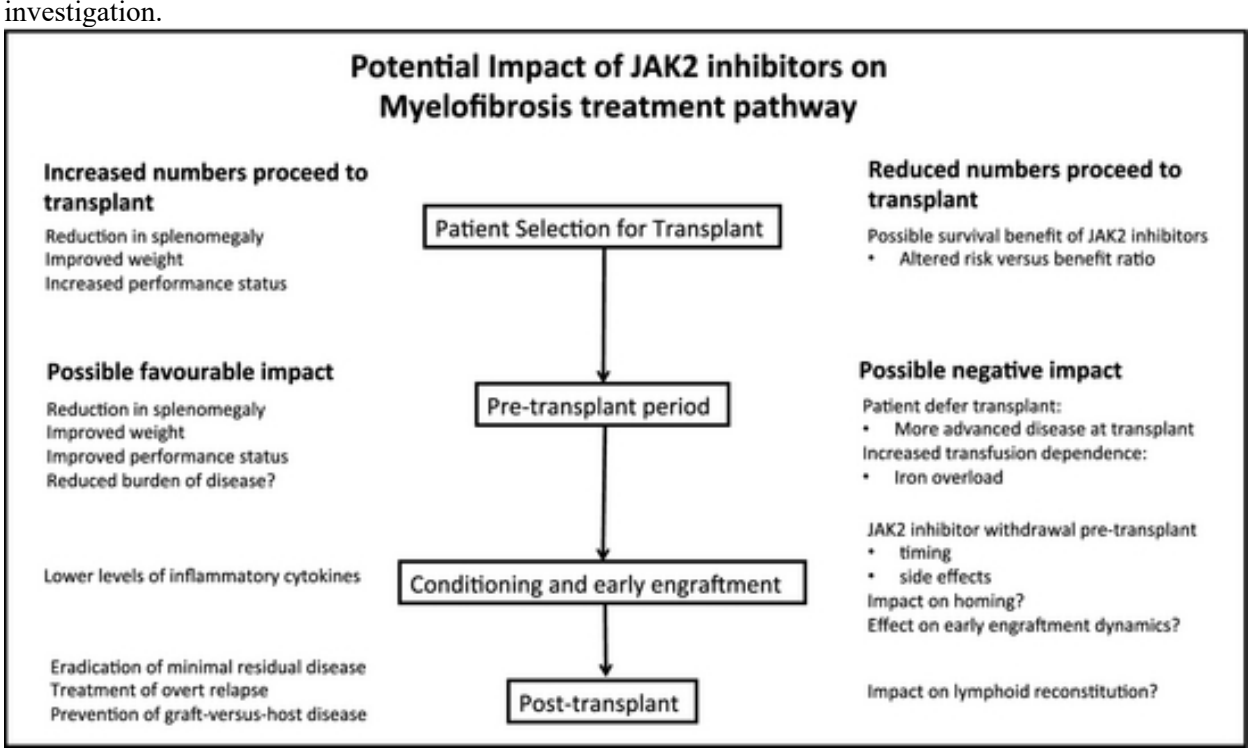
Splenectomy was performed in approximately 10% of the patient cohort reported by Cervantes et al. [2] Data from Mayo Clinic on 314 MF patients undergoing splenectomy showed significant perioperative complications in 28% of patients and a mortality of 6.7%. [22] The report by Ballen et al 2010 did not suggest an effect of prior splenectomy on graft failure or disease free survival. [9] Ciurea 2008 noted that successful engraftment could still

occur despite even massive splenomegaly. [23] Finally, Michallet et al 2009 showed greater severity of GVHD in splenectomized patients. [24]However, there are also proponents for pre-HCT splenectomy in myelofibrosis patients. Li et al 2001 showed that splenectomized patients had faster neutrophil recovery and decreased transfusion requirements.[25] Robin et al 2011 in multivariate analysis of patients receiving peripheral blood stem cells, showed faster engraftment in patients who had had absence of splenomegaly or who had undergone splenectomy pre-transplant. [26] Kroger et al demonstrated a trend toward more rapid neutrophil engraftment in splenectomized vs non splenectomized RIC HCT patients.[17] However, a higher rate of relapse at 3 years was seen in those who had undergone splenectomy. On the other hand, the retrospective analysis by Scott et al in 170 patients transplanted at the FHCC, suggests that splenectomized patients have a superior post-HCT survival. Bacigalupo et al (2010), suggested that spleen size > 22 cm was an independent risk factor for survival.

In the present study we propose, therefore, that patients whose spleen size does not decrease to 22 cm or less (as determined by the clinical provider) despite JAK inhibitor therapy, or are undergoing a UCBT, will be evaluated for surgical splenectomy.

2.5 Rationale for Pretransplant JAK Inhibitors Followed by Allogeneic Transplant in Myelofibrosis

Presently ruxolitinib (Rux) is the only FDA approved therapy for MF, and the only non-HCT therapy to date associated with a survival benefit. Using Rux to reduce spleen size and improve performance status pre-HCT may be useful in improving engraftment, but no structured studies have to been performed to date. Rux also is known to decrease proinflammatory cytokines including IL-6 and has been shown to have benefit in treating acute GVHD. Whether Rux treatment prior to HCT can improve post-transplant outcomes is an important area of investigation.



Diagrammatic representation of the postulated impact of JAK inhibitors on the myelofibrosis AHSCT pathway. (McLornan et al 2012)

3.0 STUDY OBJECTIVES

3.1 Primary Objective

The primary objective of this study is to optimize the role of allogeneic transplantation for primary and secondary MF in the JAK inhibitor era.

4.0 STUDY DESIGN

4.1 Description of Study

This Phase II trial is a single center study of JAK inhibitor therapy followed by myeloablative or reduced intensity HLA-matched related, unrelated or umbilical cord blood transplant for patients with primary and secondary myelofibrosis.

Primary Endpoint

Probability of 2-year survival in 63 patients with MF who receive treatment with a JAK inhibitor followed by an allogeneic transplant.

4.2 Secondary Endpoints

A. To determine the overall incidence of primary and secondary graft failure/rejection
B. Day 100 and 1 year NRM
C. Incidence of relapse at 1 year
D. Incidence and severity of acute and chronic GVHD.

5.0 PATIENT SELECTION

5.1 Inclusion Criteria for Part 1: JAK Inhibitor Administration

A. Age $\geq$ 18 years
B. Disease Criteria
   1. Diagnosis of PMF as defined by the 2008 World Health Organization classification system or diagnosis of secondary MF as defined by the IWG for Myeloproliferative Neoplasms Research and Treatment criteria (Appendix A)
   2. Patients meeting the criteria for intermediate-1, intermediate-2 or high-risk disease by the DIPSS or DIPSS-plus scoring system (Appendix A)
C. Ability to understand and the willingness to sign a written informed consent document
D. Patient must be a potential hematopoietic stem cell transplant candidate

5.2 Exclusion Criteria for Part 1: JAK inhibitor administration

A. Evidence of HIV infection or known HIV positive serology.
B. Uncontrolled viral, bacterial, or fungal infections at the time of study enrollment.
C. History of Prior Allogeneic Transplant
D. Pregnant or breastfeeding (Only if patients have not been started on Rux by their primary oncologist prior to enrollment).

5.3 Inclusion Criteria for Part 2: Allogeneic Stem Cell Transplant

A. Meeting criteria for 1st phase as above, at time of initiation of JAK inhibitor, including ability to understand and willingness to sign a written informed consent. Patients arriving to our instition for transplant and not enrolled in Part 1 may still be enrolled in Part 2 if Part 1 criteria met. These patients will have Part 1 endpoints transcribed from medical records
B. Received Ruxolitinib for at least 8 weeks immediately prior to conditioning and be able to continue until Day -4 pre-transplant per Section 9.2.

C. Performance status score (Appendix B)
Karnofsky ≥ 70

D. Renal Function
Calculated creatinine clearance using the Cockcroft-Gault formula or 24 hr urine creatinine clearance must be > 60 ml/min. .

E. Hepatic Function

Total serum bilirubin must be <3mg/dL unless the elevation is thought to be due to Gilbert's disease or hemolysis.

Transaminases must be < 3x the upper limit of normal

Patients with clinical or laboratory evidence of liver disease will be evaluated for the cause of liver disease, its clinical severity in terms of liver function, and the degree of portal hypertension. Patients with fulminant liver failure, cirrhosis with evidence of portal hypertension or bridging fibrosis, alcoholic hepatitis, hepatic encephalopathy, or correctable hepatic synthetic dysfunction evidenced by prolongation of the prothrombin time, ascites related to portal hypertension, bacterial or fungal abscess, biliary obstruction, chronic viral hepatitis with total serum bilirubin > 3mg/dL, and symptomatic biliary disease will be excluded.

F. Pulmonary function
DLCO corrected >60% normal.
May not be on supplemental oxygen.

G. Cardiac function
Left ventricular ejection fraction >40% OR
Shortening fraction > 26%

H. Comorbidity Index < 5 at the time of pre-transplant evaluation (Appendix B)

5.4 Exclusion Criteria for Part 2: Allogeneic Stem Cell Transplant

A. Uncontrolled viral or bacterial infection at the time of study enrollment
B. Active or recent (prior 6 month) invasive fungal infection without ID consult and approval
C. History of HIV infection
D. Pregnant or breastfeeding
E. Patients without an HLA-identical or 1-allele-mismatched related donor or unrelated donor or umbilical cord blood units that meet transplant criteria.

5.5 Controls

A. 15 de-identified samples will be requested from bioreporsitory protocol FH 1713 as controls for this protocol. The samples will be from patients who did not receive Ruxolitinib prior to transplant and will be used as control samples for the GVHD biomarker analysis.
B. 20 de-identified samples will be requested from protocol IR7801 - FHCC Infectious Disease Sciences/Virology Specimen and Data Repository, to also be used as controls. The samples will be from patients who did not receive Ruxolitinib prior to transplant and will be used as control samples for the GVHD biomarker analysis.

6.0 Donor Selection

Allowable donors include the following:

A. HLA-matched or 1 antigen mismatched sibling donor
B. 10 of 10 HLA-matched or 1 allele mismatched (9 of 10) unrelated donor
C. Peripheral blood is preferred over bone marrow for non-umbilical cord blood recipients.
D. Umbilical cord blood units will be selected according to the following umbilical cord blood graft selection criteria. One or 2 CB units may be used to achieve the required cell dose.

1. The CB graft(s) must be matched at 4-6 HLA-A, B, DRB1 loci with the recipient and therefore may include 0-2 mismatches at the A or B or DRB1 loci. Unit selection will be based on cryopreserved nucleated cell dose and intermediate resolution A, B antigen and DRB1 allele typing for determination of HLA-match. While HLA-C antigen/allele level typing is not considered in the matching criteria, if available, it may be used to optimize unit selection.
2. Selection of two CB units is allowed to provide sufficient cell dose (see below for algorithm to determine single versus double unit transplant). When multiple units are selected, the following rules apply:
    a. The CB unit with the least HLA disparity (with the patient) will be selected first (i.e., selection priority is 6/6 match >5/6 match>4/6 match). Additional CB units then may be selected to achieve the required cell dose, as outlined below. If a second unit is required, this unit will be the unit that most closely HLA matches the patient and meets minimum size criteria outlined below of at least 1.5 x 10^7 TNC/kg (i.e. a smaller, more closely matched unit will be selected over a larger, less well matched unit as long as minimum criteria are met).
    b. If two CB units are used:
        i. The total cell dose of the combined units must be at least 3.0 x 10^7 TNC per kilogram recipient weight.
        ii. Each CB unit MUST contain at least 1.5 x 10^7 TNC per kilogram recipient weight.
    c. Algorithm for determining single versus double unit cord blood transplant:

	Single Unit Allowed for:
Match Grade	**TNC Dose**
6/6	$\geq$ 2.5 x 10^7/kg
5/6, 4/6	$\geq$ 4.0 ($\pm$ 0.5) x 10^7/kg

3. General Comments:
    a. Units will be selected first based on the TNC dose and HLA matching.
    b. CD34+ cell dose will not be used for unit selection unless 2 units of equal HLA-match grade are available. In this case, the unit with the larger $CD34^+$ cell dose (if data available) should be selected.
    c. A CB unit that is 5/6 mismatched but homozygous at the locus of mismatch should be chosen over a 5/6 unit with bidirectional mismatch even if the latter unit is larger (has more cells). This also applies to 4/6 units. This is only applicable to choosing units within a given match grade.
    d. Other factors to be considered:
        i. Within the same HLA match grade, matching at DR takes preference.
        ii. Cord blood banks located in the United States are preferred.
    e. Up to 5% of the cord blood product (s), when ready for infusion, may be withheld for research purposes as long as thresholds for infused TNC dose are met. These products will be used to conduct studies involving the kinetics of engraftment and immunobiology of double cord transplantation.

7.0 INFORMED CONSENT OF SUBJECT AND DONOR

Patients referred to the FHCC for consideration of a transplant may be consented for Part 1 of the study, JAK inhibitor administration, using forms approved by the Institutional Review Board.

Part 2 of the study is the transplant. The patient and related donor will be completely evaluated following the Center's Standard Practice Guidelines. The PI, or his designee, will corroborate that eligibility criteria are met. Patients may be enrolled directly in Part 2 if they meet eligibility criteria for Part 1 and Part 2. In this case, Part 1 data will be extracted from the patient's medical record.

The patient and related donor will meet separately with an attending physician from the SCCA Transplant Team to discuss Part 2 of the study protocol, alternative treatment options and all known risks from participating in the study. Unrelated donors will meet with the evaluating physician at their respective institutions to discuss the

details of the collection procedure and give their informed consent, using forms specific for their institution. Cord blood donor units will be completely evaluated.

Patients who have not started Jakafi within 6 months of signing Part 1 consent will be required to be reevaluted for Part 1 eligibility. If they do not meet Part 1 eligiblity, they will be taken off study.

The protocol will be discussed thoroughly with the patient and family, including requirement for data collection and release of medical records and all known risks to the patient will be described. The procedure and alternative forms of therapy will be presented as objectively as possible and the risks and hazards of the procedure explained to the subject. Consent will be obtained using forms approved by the Institutional Review Board. A summary of the conference will be dictated for the medical record detailing what was covered.

Subjects meeting the criteria set forth in the protocol will be offered the opportunity to participate in the study in a meeting in person or over the telephone.

Patients who enroll on Part 1 of this protocol and meet eligibility criteria for Part 2, may continue on Part 2 of FH Protocol 9033, or enroll on Part 2 of either FH Protocol 10093 (continuing JAK inhibitor therapy through the transplant) or 10441 (using a haploidentical donor) if this is recommended by their treating clinician and agreed to by the PI. Part 1 of Protocol 10093 and Protocol 10441 are identical to the current protocol. Because the data and samples gathered on Part 1 data greatly inform the results of Part 2, patients will be asked to consent for the use of their 9033 data and samples to be carried over to their new study if they switch.

15 de-identified blood samples will be received from FH protocol 1713. The samples will be accompanied by a subject ID # issued for protocol 1713, but our team will not have access to the key to the code, nor have any other way to ascertain the identity of the patients. The patients consented under protocol 1713 for their leftover samples to be used for future research. The activities to be performed under this protocol align with what the subjects have consented to. No additional consent will be sought from these individuals.

8.0 SUBJECT REGISTRATION

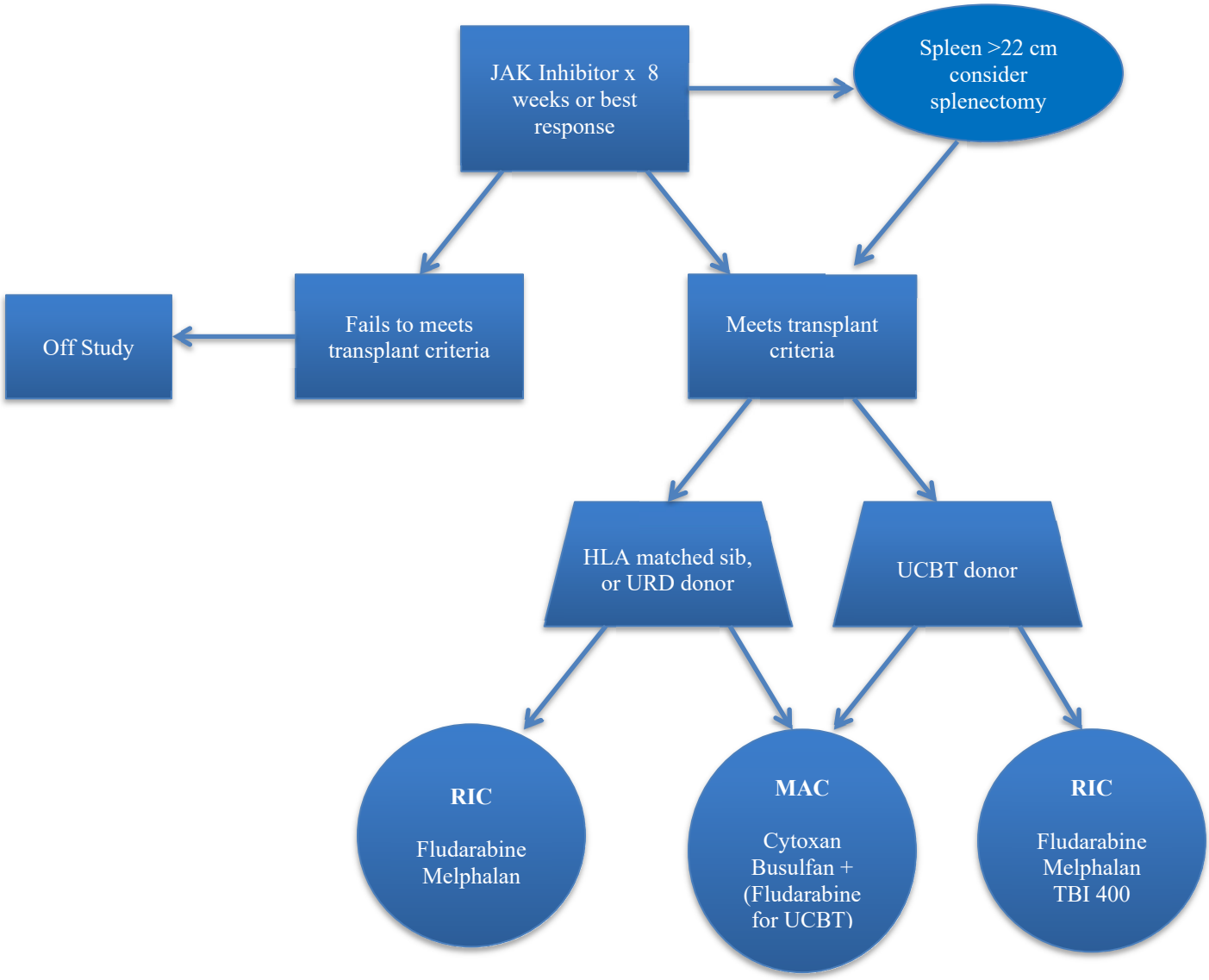
Informed consent must be signed prior to the performance of any study related procedures or assessments.

Subjects will be registered into the system by the Clinical Coordinators Office (CCO) (Intake Office) and assigned a UPN (Unique Patient Number). The CCO will register the subject on to the protocol through the Data Management Office.

9.0 TREATMENT PLAN

9.1 Treatment Schema

This schema is a pictorial overview only; please see narrative Treatment and Evaluation sections of the protocol for specifics and allowable deviations from time frames shown.



9.2 Ruxolitinib Therapy

A. Ruxolitinib will be started in all patients regardless of the presence of splenomegaly at least 8 weeks prior to the start of HCT conditioning and will be continued until best response through day -4 before transplantation. The starting dose will be determined by the patient's treating clinician. Recommended taper schedule for Ruxolitinib: Start tapering by 5 mg BID every two to three days to be completed after their dose on day -4.
B. Ruxolitinib will be dose adjusted or stopped for cytopenias as necessary per treating clinician.
C. Ruxolitinib will be continued through Day - 4 of transplantation with a taper schedule.
D. Patients will have CBC performed as clinically indicated by their treating physician.
E. Patients will be evaluated by physician visits with the physician of their choice at an interval deemed clinically appropriate by this physician.
F. If patients had been receiving Ruxolitinib therapy prior to arrival to the SCCA, we will use their pre-therapy data from their medical records as their baseline. These patients may continue to follow with their home physicians until they arrive to a transplant team at the SCCA..
G. If a patient's spleen remains > 22 cm following at least 8 weeks of treatment with Ruxolitinib (or > 16 cm in patient's undergoing cord blood transplant), they will be referred for consultation for surgical splenectomy to be performed at a local surgical center as deemed appropriate by the study PI.
H. If the patient is on an alternate JAK inhibitor they will be required to change their therapy to Ruxolitinib for 8 weeks prior to the start of conditioning.

9.3 Conditioning Regimens

MAC vs. RIC determination will be at the discretion of the clinical provider together with the CCO.

A. Myeloablative Conditioning Regimen

Day -8	Fludarabine 25 mg/m2 IV *
Day -7	Fludarabine 25 mg/m2 IV * Cyclophosphamide 60 mg/kg IV Mesna 60mg/kg
Day -6	Fludarabine 25 mg/m2 IV * Cyclophosphamide 60 mg/kg IV Mesna 60mg/kg Dilantin starts
Day -5	Busulfan 4 mg/kg IV
Day -4	**Targeted Busulfan Stop JAK inhibitor
Day -3	**Targeted Busulfan
Day -2	**Targeted Busulfan
Day -1	Rest
Day 0	Stem Cell infusion

*Umbilical cord blood recipients only

** Dose to be adjusted as recommended by Busulfan Pharmacokinetics Lab to achieve plasma steady-state BU concentrations of 800 - 900 ng/mL, per SPM.

1. Cyclophosphamide will be given IV at doses of 60 mg/kg/day for 2 days, beginning on day -7 before transplantation. Adjusted body weight should be used for calculating initial doses if patient's actual weight is > 100% of ideal body weight.
2. The initial dose of Busulfan will be administered IV at 4 mg/kg, infused over 3 hours. Adjusted body weight should be used for calculating initial doses if patient's actual weight is > 100% of ideal body weight. The Busulfan Pharmacokinetic Laboratory (206-667-4572) must be notified of patient enrollment. Serial blood samples will be drawn after doses 1, 2, and 3 of intravenous Busulfan in accordance with standardized physicians' orders to determine the steady-state concentration of Busulfan. Subsequent doses of Busulfan will be adjusted to achieve plasma steady-state BU concentrations of 800-900 ng/ml. Prophylactic phenytoin (Dilantin) will be

started as per the Standard Practice Manual on day –6 after the second dose of cyclophosphamide has been administered. On day -1 phenytoin will be discontinued.

3. For patients who have UCB as their donor source, Fludarabine will be administered at doses of 25 mg/m2 IV over one hour once daily on each of 3 consecutive days starting on Day -8 for a total dose of 75 mg/m2. Patients > 120% of ideal weight, BSA will be calculated using adjusted weight.

B. Reduced Intensity Conditioning Regimen for patients with related or unrelated stem cell donors

Day -6	Fludarabine 30 mg/m2 IV
Day -5	Fludarabine 30 mg/m2 IV
Day -4	Fludarabine 30 mg/m2 IV Stop JAK inhibitor
Day -3	Fludarabine 30 mg/m2 IV Melphalan 70 mg/m2 IV
Day -2	Fludarabine 30 mg/m2 IV Melphalan 70 mg/m2 IV
Day 0	Stem Cell infusion

1. Fludarabine will be administered at doses of 30 mg/m2 IV over one hour once daily on each of 5 consecutive days starting on Day -6 for a total dose of 150 mg/m2. Patients > 120% of ideal weight, BSA will be calculated using adjusted weight.
2. Melphalan will be administered at doses of 70mg/m2 IV over 15 to 30 minutes daily on 2 consecutive days starting on D-3 for a total dose of 140mg/m2. Patients >120% of ideal weight, BSA will be calculated using adjusted weight (per Standard Practice)

B. Reduced Intensity Conditioning Regimen for Umbilical cord blood recipients only

Day -6	Fludarabine 25 mg/m2 IV
Day -5	Fludarabine 25 mg/m2 IV
Day -4	Fludarabine 25 mg/m2 IV Stop JAK inhibitor
Day -3	Fludarabine 25 mg/m2 IV Melphalan 40mg/m2 IV
Day -2	Fludarabine 25 mg/m2 IV Melphalan 40mg/m2 IV
Day -1	TBI 4 Gy *
Day 0	Stem Cell infusion

1. Fludarabine will be administered at doses of 25 mg/m2 IV over one hour once daily on each of 5 consecutive days starting on Day -6 for a total dose of 125 mg/m2. Patients > 120% of ideal weight, BSA will be calculated using adjusted weight.
2. Melphalan will be administered at doses of 40mg/m2 IV over 15 to 30 minutes daily on 2 consecutive days starting on D-3 for a total dose of 80mg/m2. Patients >120% of ideal weight, BSA will be calculated using adjusted weight (per Standard Practice)
3. Total Body Irradiation (TBI) will be administered in two 2 Gy fractions to umbilical cord blood recipients only on Day -1.

9.4 Immunosuppressive Therapies

All patients will receive GVHD prophylaxis with 2 drugs as follows:

A. HLA-matched Related, Unrelated and Cord Blood Donor recipients:

1. Tacrolimus.

a. Tacrolimus will be given day –1 for patients receiving related or unrelated stem cells, and on day -3 for patients receiving umbilical cord blood. If a patient is inpatient, they will receive their initial dose of Tacrolimus as a continuous IV infusion at 0.03 mg/kg/day; if they are outpatient the initial dose should be 0.015 mg/kg/dose every 12 hours IV over 1 to 2 hours . Therapeutic monitoring should be done on day +2 and day +5, and the dose adjusted accordingly to achieve a steady-state level of 5-15 ng/ml of serum, with an optimal level of 10 ng/ml. Once the appropriate level is achieved, monitoring should be done at least weekly. Once the patient is therapeutic, administration of tacrolimus should be changed to the oral formulation at a dose conversion of 1:4 (IV:oral) and given in divided doses twice daily (every 12 hours) as long as the patient is tolerating oral medications. If the patient vomits within one hour of administration of oral tacrolimus, repeat the dose. If vomiting persists, the drug should be given intravenously at the appropriate dose (PO:IV ratio 4:1). Dosing should be based on the patient's ideal body weight.
b. In the absence of GVHD, the tacrolimus taper will start on day +56 for related donors, and day 100 for unrelated and umbilical cord blood donors. The dose should be tapered by 20% per month or over 4 months per clinical judgement.. In the presence of GVHD, or if the patient is receiving corticosteroids or other therapy for GVHD, tacrolimus will not be tapered at day +56, and dosing will be maintained to target therapeutic levels. The attending physician and LTFU will determine the duration of therapy. Once tacrolimus taper is initiated, levels should be obtained only if toxicity is suspected.

B. Related and unrelated donor recipients only

1. Methotrexate. Methotrexate will be given at doses of 10 mg/m^2 IV on day +1 (at least 24 hours after PBSC infusion) and on days +3, +6, and +11. Indications for methotrexate dose adjustment include Grade 4 mucositis, severe renal impairment and significant third space fluid accumulation. Dose adjustments and leucovorin rescue, if necessary, will be performed per Standard Practice Guidelines.

C. Umbilical cord blood donor recipients only

1. MMF. All patients will receive MMF at the dose of 15 mg/kg every 8 hours with a maximum of 1 gram/dose. MMF should be started on day zero, 4 to 6 hours after cell infusion for patients receiving UCBT.
    a. MMF will be given every 8 hrs daily until day 40 post transplant and then, in the absence of GVHD, tapered by 12%/week with MMF discontinued after day + 96.
    b. MMF may be given IV or PO with preference that patients receive the IV form if and when they are admitted to the hospital.
    c. Markedly low (<40%) donor T cell chimerism after UCBT may indicate impending graft rejection. MMF should be continued at full dose or, if MMF taper has been initiated, reinstitution of full dose MMF should occur. If MMF has been discontinued, MMF should be reinstituted at full dose.
    d. If patient is diagnosed with GVHD and started on systemic steroids, and patient has stable chimerism, MMF may be tapered following discussion with PI.

9.5 Growth Factor

A. UCBT patients will receive G-CSF 5 mcg/kg/day SC (dose rounded to vial size) based on the actual body weight beginning on Day +1 after UCB infusion. G-CSF will be administered daily until the ANC exceeds 2.5 x 10^9/L for three consecutive days and then discontinued. If the ANC decreases to ≤1.0 x 10^9/L, G-CSF will be reinstituted and titrated to maintain an ANC >1.0 x 10^9/L.

9.6 Supportive Care

A. Patients will receive transfusions, infection prophylaxis (bacterial, fungal, viral), and other therapy (including GVHD) according to Institutional Guidelines.

9.7 Hematopoietic cell collection and Infusion (see also Protocol Specific Infusion Orders)

A. Bone marrow vs PBSC
    1. PBSC mobilization is preferred over bone marrow harvest but either will be accepted per clinicians preference.
B. HLA-matched related donors
    1. Related donors who consent to PBSC donation will receive 5 daily doses of G-CSF, 16 µg/kg/day by subcutaneous injection, commencing on day –5. PBSCs will be collected in the afternoon of day -1, stored at 4ºC overnight, and infused as soon as possible on day 0.
    2. If the collection on day –1 contains less than 5.0 X 10^6 CD34+ cells per kg of recipient weight, a second collection will be performed the following morning and transfused on day 0.
    3. Bone marrow harvest
C. Matched unrelated donors
    1. For unrelated donors, timing of PBSC collection is prearranged through the URD office, via NMDP or other donor centers.
    2. G-CSF will be administered at 10 µg/kg/day for 5 days by subcutaneous injection to unrelated donors per NMDP protocol.
    3. Collection centers will perform leukapheresis for 1 or 2 days and exchange up to a total of 24 liters of blood, with a target to collect a minimum of 5.0 X 10^6 CD34+ cells per kg recipient weight.
    4. The Cellular Therapy Lab will perform quantitation of CD34 and CD3 cells. If possible, for unrelated donor products, cryopreservation of a portion of PBSC should take place for the purpose of possible donor lymphocyte infusion.
    5. Bone marrow harvest
D. Umbilical Cord Blood donors
    1. Procedures for requesting, receiving and characterizing the cord blood unit(s) for infusion will be according to institutional protocol.
    2. The cord blood unit will be thawed and infused per SCCA CTL SOPss. Cord blood products should be infused without delay as soon as the product arrives on the floor.

        a. The thawed product (either one or two units) will be delivered to the patient floor/bedside where the product is double-checked by a nurse with the technologist from the Cellular Therapy Laboratory. Visual inspection of the product is also made at this time. The unit(s) is verified according to 1) the infusion order sheets, 2) the patient's identification number on the cell product, 3) the product (cell) identification number and 4) the patient wristband.
        b. If the cord blood unit(s) fail to pass inspection or if there is insufficient information to verify the cell product for the patient, notify the Director of the Cell Therapy Lab (Shelly Heimfeld, 667-4004) and the PI, Rachel Salit (667-1317, pager 314-2845) immediately.
        c. The goal infusion time of each cord blood unit is 30 minutes, as clinically possible. Pre-medications (if any) prior to cord blood infusion will be at the discretion of the attending. Under no circumstances is the cord blood to be irradiated. No medications or fluids should be given piggyback through the catheter lumen that is being used for cord blood infusion.
        d. The product is infused via IV drip directly into the central line according to standard practice with gravity-filtered tubing.
        e. Vital signs should be monitored before beginning the infusion and periodically during administration. Notify the attending physician, fellow or PA immediately if the patient exhibits signs or symptoms of a reaction.
        f. Benadryl, epinephrine, and hydrocortisone should be available at the bedside for emergency use if necessary. Oxygen with nasal prongs for standby use should be present in the room.
        g. If the patient is a double cord blood recipient, the two units may be given consecutively with no wait between infusion of the units. However, infusion of the second unit will **not** begin until any acute toxicities from the first unit have been controlled. The start and stop time of each unit should be recorded on the infusion record.

9.8 Management of Pre-Engraftment Immune Reactions in UCBT patients

A. A well-recognized clinical entity consisting of skin rash, fever, and, often, loose stools and respiratory distress has been noted to occur prior to engraftment among cord blood patients, generally between Days +7 and +21. This clinical syndrome likely involves cytokine activation, and though clinically similar to acute or hyperacute graft versus host disease, it appears to be a distinct entity, "pre-engraftment syndrome." This syndrome is often controlled with brief steroid bursts, thus avoiding a commitment to extended steroid exposure. Patients should be monitored carefully for this syndrome.

If patients have moderate to severe symptoms as described above and alternative etiologies (i.e., infection) have been excluded or are being appropriately evaluated, recommendations for management are:

1. For patients not on steroid therapy when the syndrome occurs: methylprednisolone should be given at 1 mg/kg IV q day for three days. If symptoms have abated, steroids should be stopped. If symptoms persist, 1 mg/kg methylprednisolone can be continued through six days then stopped if symptoms have abated. If symptoms persist for more than six days, the patient should be considered to have acute/hyperacute GVHD and should be treated with prolonged steroids as deemed appropriate.

2. For patients already on steroids for other reasons when the syndrome occurs: methylprednisolone should be given at a dose of 3-5 mg/kg IV (max dose 500 mg) q 12 hours x 48 hours, followed by a rapid taper to 1 mg/kg IV q 12 hours. Patients should be weaned after response as tolerated.

10.0 EVALUATION

10.1 Pre JAK Inhibitor Evaluation for patients enrolled in Part 1

A. All patients will have a complete history taken, including details of prior therapy for myelofibrosis such as cytotoxic treatment, growth factor administration, transfusion support and splenectomy (or splenic irradiation). Previous thrombotic or hemorrhagic complications will be noted. Constitutional symptoms such as weight loss, fever or night sweats will be recorded.
B. The Dynamic International Prognostic Scoring System (DIPSS) (Appendix A)
C. Karnofsky performance status (Appendix B).
D. Physical examination including assessment by exam for splenomegaly and hepatomegaly.
E. Complete blood and platelet count with leukocyte differential.
F. Basic metabolic and hepatic function panels.
G. Pregnancy test (blood or urine) in females of childbearing potential.
H. Bone marrow: All patients should have diagnostic bone marrow biopsies and, if possible, aspirates taken for morphologic (microscopic) examination, cytogenetic analysis, and flow cytometric analysis within 6 months of enrollment .
I. Peripheral blood: peripheral blood $CD34^{+}$count (send to SCCA Cellular Therapy Lab), cytogenetic analysis (if not possible on marrow)
J. Serum iron and TIBC, and ferritin levels will be obtained.
K. Hotspot lab test (MF mutations).

L. Bone marrow or peripheral blood (not both): Should be sent fresh for JAK mutations (V617F, exon 12) , MPL1 and CALR. Send these only if patient's mutation status is unknown. If patient is known to be positive for any of these mutations these tests do not need to be done.
M. Research: Additional peripheral blood, up to 30 ml, to be used for research on the mechanism of responses to JAK inhibitors and biology of post-transplant disease progression and response. This will include but is not limited to analysis of cytokine levels and activity and genome sequencing. 100mcl of plasma may also be collected from stored peripheral blood samples and sent deidentified to Incyte Corporation for GVHD biomarker analysis. Additional marrow up to 5ml to be used for future research on additional mutations and disease mechanisms will also be collected if possible.

N. Patients who present to the SCCA already on treatment with a JAK inhibitor, and not enrolled in Part 1 will have the pre JAK inhibitor evaluation information extrapolated from their medical records.
O. Results of tests and/or procedures conducted as per standard of care may be used for eligibility determination.

10.2 Pre-Transplant Evaluation

Refer to FHCC Standard Practice Manual for Pre-Transplant Evaluation Guidelines for Allogeneic Transplant.

In addition to Standard Practice Guidelines complete the following protocol specific requirements:

A. The Dynamic International Prognostic Scoring System (DIPSS) (Appendix A)
B. Comorbidity Index Score (Appendix B)
C. Physical examination including assessment by exam for splenomegaly and hepatomegaly.
D. Peripheral blood: peripheral blood $CD34^{+}$count (send to SCCA Cellular Therapy Lab), cytogenetic analysis (if not possible on marrow).
E. Serum iron and TIBC, and ferritin levels will be obtained.
F. Heme Gene Panel if not performed at the time of enrollment on Part 1, or if > 6 months since last evaluation.
G. Bone marrow or peripheral blood: Should be sent fresh for JAK-2 mutations (V617F, exon 12), or MPL1 or CALR if positive pre JAK inhibitor. If mutational status is known, only test for the mutation that was positive pre JAK inhibitor. If mutational status was unknown pre JAK inhibitor therapy test marrow or peripheral blood (not both) for JAK-2 mutations V617F, exon 12), MPL1 and CALR.
H. Research: Additional peripheral blood, up to 30 ml,to be used for research on the biology of post-transplant disease progression and response. This will include but is not limited to analysis of cytokine levels and activity and genome sequencing. 100mcl of plasma may also be collected from stored peripheral blood samples and sent deidentified to Incyte Corporation for GVHD biomarker analysis. Additional marrow up to 5ml to be used for future research on additional mutations and disease mechanisms will also be collected if possible.
    1. Blood samples from patients who did not receive Ruxolitinib prior to transplant will be taken from previously collected pre-transplant samples on Fred Hutch 1713 or 7801 biorepository protocols and sent to Incyte Corporation to be used as control samples for the GVHD biomarker analysis.
I. MRI (bone marrow and STIR) of pelvis, femoral heads and shafts and lumbar spine will be obtained (these films will also provide additional information on spleen size). If patient is unable to have an MRI performed, CT chest, abdomen and pelvis, or ultra sound abdomen/pelvis, may be substituted to assess spleen volume as well as extramedullary sites of disease.
J. Echocardiography with measurement of the left ventricular ejection fraction (LVEF) or left ventricular shortening fraction. MUGA is not preferred but can be used in the event echocardiography is unable to be obtained in a timely manner.
K. Chest CT without contrast to exclude occult infection prior to transplant within 30 days of start of conditioning.
L. Pulmonary function tests.
M. Viral screening including CMV PCR
N. DNA specimen from cord blood unit(s) for chimerism studies (FHCC patients: submitted to CIL)
O. Panel Reactive Antibody (PRA) per Institutional Guidelines
P. Pregnancy test per Institutional Guidelines in females of childbearing potential.
Q. Results of tests and/or procedures conducted as per standard of care for pre transplant work-up may be used for eligibility determination.

10.3 Patient Evaluations from Day 0 through day 28.

Refer to FHCC Standard Practice Manual for Post-Transplant Evaluation Guidelines for Allogeneic Transplant.
In addition to Standard Practice Guidelines complete the following protocol specific requirements:

A. Peripheral blood donor chimerism cell sorted for CD3+, CD33+ cells (and CD56+ if clinically indicated) will be evaluated at Day 28 for patients receiving UCBT and patients receiving reduced intensity conditioning only, unless clinically indicated
B. Bone marrow biopsy and aspirate if possible on Day 28 for disease restaging (morphology, immunophenotyping, cytogenetics and molecular studies as indicated).
C. GVHD evaluation (Appendix C) weekly or as clinically indicated
D. Research: Additional peripheral blood, on Days 0, 7 and 28, not to exceed 30 ml,to be used for research on the mechanism of responses to Ruxolitinib and biology of post-transplant disease progression, and response. 100mcl of plasma may also be collected from stored peripheral blood samples and sent deidentified to Incyte Corporation for GVHD biomarker analysis. This will include but is not limited to analysis of cytokine levels and activity and genome sequencing.

10.4 Patient Evaluations through Day 100, 6 Months, 1 Year and 2-5 Years

Refer to FHCC Standard Practice Manual for Post-Transplant Evaluation Guidelines for Allogeneic Transplant.

In addition to Standard Practice Guidelines complete the following protocol specific requirements:

A. Karnofsky/ECOGperformance status (Appendix B) once between Day 80 and Day 100, and at 1 year.
B. MRI (bone marrow and STIR) of pelvis, femoral heads and shafts and lumbar spine will be obtained (these films will also provide additional information on spleen size), between day 80 and 100, and also at the 1 year Long Term Follow-up. If patient is unable to have an MRI performed, CT chest, abdomen and pelvis, or ultra sound abdomen/pelvis, may be substituted to assess spleen volume as well as extramedullary sites of disease. If the patient had one of these assessments at baseline, it is acceptable to repeat the same modality for comparison.
C. Peripheral blood for the molecular mutation (JAK-2, CALR, MPL) that was previously positive to be done between day 80 – 100 with work-up for discharge from transplant service, at 6 months (only if mutation was positive at day 80-100 evaluation), and yearly until 5 years post transplant.
D. Bone marrow aspirate and biopsy between Day 80 and 100 and 1 year for assessment of donor engraftment, disease restaging, and chimerism analysis. BM should be performed at Day 180 if Day 80-100 marrow demonstrates residual disease, mixed chimerism or molecular mutation is still positive. At the 2-5 year time points BM biopsy will only be done if residual disease in marrow, mixed chimerism or the patient has molecular mutation that is still positive in the marrow at the 1 year evaluation. If chimerism is less than 50% donor, patient should be considered for DLI.
E. Peripheral blood for chimerism studies cell sorted for $CD3^+$ and $CD33^+$, (and if clinically indicated CD56+) between days 80-100, and 1 year. At Day 180 and years 2-5 PB chimerism should be performed as clinically indicated (ie in patients with unresolved or new cytopenia or mixed chimerism). If chimerism is less than 50% donor, patient should be considered for DLI.
F. Peripheral blood for chimerisms at day 56 if CD3 or CD33 is less than 50% donor, at day 28 or if clinically indicated.
G. The Dynamic International Prognostic Scoring System (DIPSS) (Appendix A) at 1 year and yearly until 5 years.
H. GVHD evaluation (Appendix C) weekly and/or as clinically indicated through Day 100 (or longer if clinically indicated), then at 6 months, 1 year and 2-5 years
I. Autopsy report, if available, if death occurs before the 5 year follow-up

J. Research: Additional peripheral blood, up to 30 ml,to be used for research on the biology of post-transplant disease progression and response will be done between days 80 and 100 and at 1 year. This will include but is not limited to analysis of cytokine levels and activity and genome sequencing. Additional marrow up to 5ml at **the one year time point only** to be used for future research on additional mutations and disease mechanisms will also be collected if possible.

10.5 Residual/Recurrent Disease Evaluation

A. Patients will be evaluated routinely for evidence of recurrent malignancy as per Institutional Guidelines on Day 28 and Day 80-100. If at any time the attending physician suspects recurrent disease, additional analyses will be performed as clinically indicated.

B. Research: Additional peripheral blood, up to 30 ml,to be used for research on the biology of disease progression and response. 100mcl of plasma may also be collected from stored peripheral blood samples and sent deidentified to Incyte Corporation for GVHD biomarker analysis This will include but is not limited to analysis of cytokine levels and activity and genome sequencing. Additional marrow up to 5ml to be used for future research on additional mutations and disease mechanisms of recurrence will also be collected if possible.

10.6 Study Evaluation Windows

The target dates for post-transplant study evaluations are outlined in the table below. In certain clinical circumstances (e.g., relapse, clinical status or terminal illness) study tests may be omitted at the physician's or PI's discretion.

Evaluation Timepoint Post Transplant	**Window****
Day 0	**- 2 days**
Day +10	**+/- 3 days**
Day +28	± 7 days
Day +56	± 7 days
Day +80 to +100	± 7 days
Day +180	± 30 days
1 year	± 90 days
2-5 years	*

* Every effort will be made to complete the 1 year and 2-5 year evaluations as close to these dates as possible, taking into consideration patient's circumstances at these time points.

** These timepoints may be delayed beyond window due to a patient's unstable clinical condition as determined by the provider.

11.0 DRUGS, IRRADIATION AND CORD BLOOD INFUSION: TOXICITIES AND COMPLICATIONS

11.1 Ruxolitinib

Ruxolitinib has been studied in over 650 patients with MF and over 40 patients with rheumatoid arthritis and the following risks have been observed:

Likely Side Effects (over 10%)	Less Likely Side Effects (3-9%)	Rare Side Effects (under 2%)
• **Diarrhea**	• **Vomiting** • **Low white blood cell count** • **Increased risk of infection** • **Low to moderate grade fever** • **Pneumonia**	• **Urinary tract infection** • **Inflammation of the bowel** • **Gastrointestinal bleeding** • **Gas** • **High potassium count** • **Low levels of sodium**

• **Nausea** • **Mild to moderately low blood cell counts (red blood cells (anemia) and platelets)** • **Shortness of breath** • **Swelling of the hands or feet** • **Feeling hot** • **Headache** • **Increased risk of bruising and bleeding** • **Weight gain** • **Heart murmur** • **Changes in blood pressure**	• **Bronchitis** • **Fatigue** • **Sleep disturbances** • **Pain in the arms or legs** • **Itchiness** • **Rash (viral skin infections, herpes zoster or singles)**	• **High cholesterol** • **Abdominal pain** • **Fainting** • **Neck ache** • **Infection or pain in joints** • **Severe and life-threatening form of inflammation of the pancreas** • **Bone marrow suppression** • **Significantly low red blood cells, platelets, and ANC (a type of white blood cell)** • **Intracerebral hemorrhage (bleeding)** • **Upper respiratory infection** • **High-grade fever** • **Sore throat** • **Low oxygen level** • **Inflammation of the lungs** • **Weakness** • **Anxiety** • **Acute response to drug withdrawal** • **Depression** • **Cardiomyopathy (weakening and enlargement of the heart muscle)** • **Heart failure** • **Acute myocardial infarction** • **Liver toxicity** • **Necrotizing fasciitis (rare infection of the deeper layers of skin and tissues underneath the skin)** • **Blurred vision/vision loss** • **Necrosis of talus bone (located in the top of the foot joining ankle)**

As the purpose of this study is to find out if JAK inhibitors, when given intentionally as part of a treatment plan prior to transplant, will improve transplant outcomes in patients with MF, patients enrolled in clinical trials for pacritinib and momelotinib will be allowed to also enroll in this study.

11.2 Busulfan

Busulfan can increase your chance of having seizures. Treatment with Dilantin an antiseizure medication both prior to and during busulfan treatment can decrease your chance of having seizures

Likely Side Effects (over 10%)	Less Likely Side Effects (3-9%)	Rare Side Effects (under 2%)
• Lower white blood cell count with increased risk of infection • Diarrhea • Vomiting and nausea • Liver damage • Lower sperm production in men • Hair loss • Loss of appetite • Missing or stopping menstrual cycle in women	• Sores in mouth or on lips • Blood in urine • Fatigue • Lower platelet count (mild) with increased risk of bleeding, especially with an injury like falling • Darkening of nail beds	• Lung fibrosis with cough and shortness of breath • Heart failure with high doses • Decrease in sodium level in the blood with high doses • Secondary cancers

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11.3 Cyclophosphamide (Cytoxan)

Cyclophosphamide can cause bleeding in your bladder. Getting more fluid through a vein or your catheter and drinking extra liquids may prevent this.

Likely Side Effects (over 10%)	Less Likely Side Effects (3-9%)	Rare Side Effects (under 2%)
• Nausea and vomiting • Diarrhea • Loss of appetite • Low white blood cell count with increased risk of infection • Lower sperm production in men • Hair loss • Liver problems • Missing or stopping of menstrual periods in women	• Anemia • Low platelet count with increased risk of bleeding • Sores in mouth or on lips • Blood in urine • Fatigue • Darkening of nail beds • Fetal damage if pregnancy occurs while taking cyclophosphamide	• Lung fibrosis with cough and shortness of breath • Heart failure with high doses • Decrease in sodium level in the blood with high doses • Secondary cancers

11.4 Fludarabine

Likely Side Effects (over 10%)	Less Likely Side Effects (3-9%)	Rare Side Effects (under 2%)
• Low white blood cell count with increased risk of infection • Low platelet count with increased risk of bleeding • Anemia	• Nausea and vomiting • Diarrhea • Fatigue	• Rash • Visual changes • Numbness and tingling in hands or feet • Severe problems with brain (coma at high-dose, confusion) • Pneumonia • Irregular heart beats • Renal failure

11.5 Methotrexate

Likely Side Effects (over 10%)	Less Likely Side Effects (3-9%)	Rare Side Effects (under 2%)
• Nausea and/or vomiting • Loss of appetite • Mouth sores that are painful	• Low white blood cell count and increased risk of infection • Lower platelet count with increased risk of bleeding • Diarrhea or loose stools	• Damage to the liver (may be permanent or cause death) • Allergic inflammation of the lung with fever, cough, and feeling short of breath • Hair loss • Skin reactions (rash, itching)

	• **Kidney damage (may be permanent)** • **Greater risk of sunburn** • **Skin changes in areas where previous radiation was given**	• **Feeling dizzy** • **Blurred vision** • **Hard to think** • **Headaches** • **Redness of eyes and maybe itching but not serious conjunctivitis**

11.6 Tacrolimus

Likely Side Effects (over 10%)	Less Likely Side Effects (3-9%)	Rare Side Effects (under 2%)
• **Hypertension (high blood pressure)** • **Tremor (shaking of the hands)** • **Nausea and/or vomiting** • **Altered levels of magnesium, calcium, potassium, and sugars in the blood**	• **Headache** • **Pain in the hands and/or feet** • **Increases in cholesterol and triglyceride** • **Changes in how clearly one can think** • **Trouble sleeping** • **Increased hair growth** • **Destruction of red blood cells (hemolysis)**	• **Seizures** • **Renal failure from damage to the blood vessel walls and destruction of red blood cells by a condition called hemolytic uremic syndrome (HUS)**

11.7 Mycophenolate Mofetil (MMF)

Likely Side Effects (over 10%)	Less Likely Side Effects (3-9%)	Rare Side Effects (under 2%)
• **Nausea** • **Miscarriage or birth defects if become pregnant while taking and within 6 weeks after stopping MMF**	• **Vomiting** • **Diarrhea (loose stools) and abdominal discomfort** • **Lower red blood cell count that is reversible** • **Lower white blood cell count with increased risk of infection**	• **Stomach and bowel bleeding (blood in stools)** • **Secondary cancers** • **Progressive multifocal leukoencephalopathy (a serious brain infection that can cause weakness, clumsiness and confusion and can lead to death)**

11.8 Melphalan

Likely Side Effects (over 10%)	Less Likely Side Effects (3-9%)	Rare Side Effects (under 2%)
• **Vomiting** • **Nausea** • **Mouth ulcers** • **Diarrhea** • **Skin redness** • **Hair loss** • **Loss of appetite** • **Fatigue** • **Change in taste of food** • **Nail discoloration**	• **Pulmonary scar tissues to form**	• **Confusion and coma** • **Lowers the white blood cell count, which, in turn, increases the risk of infection, which can be life threatening** • **Lowers the platelet count (necessary for blood clotting) which may lead to serious or life-threatening bleeding complications.**

11.9 Granulocyte Colony Stimulating Factor (G-CSF)

Likely Side Effects (over 10%)	Less Likely (1-10%)

• **Muscle aches or pain** • **Bone pain** • **Itching** • **Skin rashes** • **Headache**	• **Blood vessel inflammation** • **Ruptured spleen**

11.10 Total body irradiation (TBI)

Likely Side Effects (over 10%)	Less Likely Side Effects (3-9%)
• **Nausea** • **Fatigue** • **The irradiation dose used may result in sterility, and there is a risk of major genetic damage to any children conceived after transplantation**	• **Temporary hair loss** • **Vomiting** • **Cataracts (an opacity or whitening of the lens) may develop in the eye** • **Inflammation of the salivary gland** • **Diarrhea** • **Painful swelling of the parotid gland (a gland under the chin) for a few days** • **Secondary cancers** • **Mucositis (temporary damage to the lining of the mouth)**

12.0 GUIDELINES FOR ADVERSE EVENT REPORTING

12.1 Adverse Event Reporting/Institutional Policy

The following guidelines are the minimum Cancer Consortium IRB adverse event (AE) reporting guidelines.

In accordance with institutional policy, all adverse events which in the opinion of the principal investigator are unexpected and related or possibly related to the research and serious or suggest that the research places research participants or others at greater risk of physical or psychological harm than was previously known or recognized are to be reported to the IRB within 10 calendar days of learning of the problem.

Definitions:

Adverse Event - Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, medical treatment or procedure and which does not necessarily have to have a causal relationship with this treatment. An adverse event 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, medical treatment or procedure whether or not considered related to the medicinal product.

Life-threatening Adverse Event – Any adverse event that places the patient or subject, in view of the investigator, at immediate risk of death from the reaction.

Unexpected Adverse Event – An adverse event is "unexpected" when its nature (specificity), severity, or frequency are not consistent with (a) the known or foreseeable risk of adverse events associated with the research procedures described in the Protocol-related documents, such as the IRB-approved research protocol, informed consent document and other relevant sources of information such as product labeling and package inserts; and are also not consistent with (b) the characteristics of the subject population being studied including the expected natural progression of any underlying disease, disorder or condition any predisposing risk factor profile for the adverse event.

Serious Adverse Event (SAE) – Any adverse event occurring that results in any of the following outcomes:

- death

- a life-threatening adverse event (real risk of dying)
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly
- requires intervention to prevent permanent impairment of damage

Attribution - The following are definitions for determining whether an adverse event is related to a medical product, treatment or procedure:

- An adverse event is "**related or possibly related to the research procedures**" if in the opinion of the principal investigator, it was more likely than not caused by the research procedures.
- Adverse events that are solely caused by an underlying disease, disorder or condition of the subject or by other circumstances unrelated to either the research or any underlying disease, disorder or condition of the subject are not "related or possibly related."
- If there is any question whether or not an adverse event is related or possibly related, the adverse event should be reported.

12.2 Protocol –Specific Collection, Grading and Reporting of Adverse Events
Patients enrolled in this study are receiving pre-transplant treatments and other transplant-related procedures that are generally associated with high rates of "expected" adverse events. Refer to Appendix D for a list of potential adverse events associated or expected with hematopoietic cell transplantation. Toxicities will be graded using the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. The scale in its entirety can be found at: http://www.acrin.org/Portals/0/Administration/Regulatory/CTCAE_4.02_2009-09-15_QuickReference_5x7.pdf

The following events occurring from the start of the conditioning regimen through day 100 (or through discharge prior to that date) will be captured in protocol-specific case report forms:

- Non-hematologic adverse events of ≥ grade 3
- Grade ≥4 blood and lymphatic system disorder-related adverse events occurring between Day 43 and Day 100
- Serious adverse events as defined above

The following events are not identified as AEs in this study:

- Disease progression or relapse. However, clinical events associated with progression/relapse may be reportable as AEs.
- Hospitalization for the purpose of facilitating stem cell transplant, for protocol-scheduled procedures, blood product transfusions, or for social reasons (i.e., awaiting transport home) will not be considered a serious adverse event. Hospitalization occurring at any time pre- or post-transplant will be considered a SAE only if it is unexpected or the duration of the hospital stay is unexpected.
- Medical or surgical procedures in and of themselves, including those that require hospitalization (e.g., surgery, endoscopy, biopsy procedures) are not considered AEs. However, an event or condition requiring such procedures may be an AE.
- All patients undergoing hematopoietic stem cell transplant are expected to have ≤ Grade 4 pancytopenia as an intended therapeutic effect. These hematologic AEs will therefore be tracked and recorded between day 0 and 43 only as time to recovery of blood counts/engraftment.
- Abnormal laboratory values will be identified and recorded as AEs only if clinical intervention is required as a result.

When a grade 3 adverse event increases in severity to grade 4 or above, the event will be captured at its highest grade. If a patient experiences relapse or graft failure and goes on to further treatment off protocol, adverse events will no longer be collected with the exception of death.

In accordance with FHCC institutional policy, all AEs which in the opinion of the FHCC principal investigator meet all three of the following criteria will be reported to the Coordinating Center IRB within 10 calendar days of learning of the problem:

A. unexpected
B. related or possibly related to the research
C. serious or suggests that the research places research participants or others at greater risk of physical or psychological harm than was previously known or recognized

Graft versus Host Disease assessment, performed by the clinical team as part of routine care, will be reviewed once weekly through Day +100. After Day +100, GVHD data will be captured at Day +180, 1 year, then once yearly through 2 years after Day 0. GVHD data is captured in a GVHD-specific CRF.

Relapse, graft failure and death data will be captured as they occur in CRFs specific to those events. If a patient experiences relapse or graft failure and goes on to further treatment off protocol, adverse events will no longer be collected with the exception of death..

13.0 DATA AND SAFETY MONITORING PLAN

This is single institution Phase 2 clinical trial that is monitored by the principal investigator (PI) and a Data and Safety and Monitoring Board (DSMB). The PI of the study will have primary responsibility for ensuring that the protocol is conducted as approved by the FHCC Scientific Review Committee and Institutional Review Board. The PI will ensure that the monitoring plan is followed, that all data required for oversight of monitoring are accurately reported to the DSMB and FHCC/UW Cancer Consortium Data and Safety Monitoring Committee (DSMC), that all adverse events are reported according to the protocol guidelines, and that any adverse reactions reflecting patient safety concerns are appropriately reported. The PI will personally review with the Research Nurse the clinical course of all the enrolled patients at least twice monthly.

Under the provisions of the FHCC/UW Cancer Consortium Data and Safety Monitoring Plan (DSMP), the Cancer Consortium Clinical Research Support Office provides monitoring for quality process and compliance by qualified monitors unaffiliated with the conduct of the study. Monitoring visits occur at specified intervals determined by the assessed risk level of the study and the findings of the previous visit. The scope of monitoring is specified in the Cancer Consortium DSMP:
http://www.cancerconsortium.org/content/dam/consortium/Resource-Documents/Data-Safety-Monitoring/DSMPlan.pdf

This protocol will be monitored by the MDS/Aplastic Anemia DSMB. This committee meets at six-month intervals and all outcome data is reviewed including all adverse events. The DSMB determines whether the trial has met any stopping rules and reviews any patient safety problems potentially necessitating discontinuation of the trial. A report from the DSMB is submitted to the FHCC IRB and to the study coordinators/PI of this protocol. The DSMB will discontinue the review of outcomes when this protocol is closed to accrual.

An annual review of the progress of the study with respect to the monitoring plan will be performed by the DSMC. The DSMC reviews accrual, serious adverse events, stopping rules and adherence to the protocol-specific data and safety monitoring plan.

14.0 DATA MANAGEMENT/CONFIDENTIALITY

Study data will be recorded in a password-protected research database. Case report forms are generated from the database for review by the principal investigator and study monitors. Clinical Statistics maintains a subject database at FHCC to allow storage and retrieval of subject data collected from a wide variety of sources. The investigator will ensure that data collected conform to all established guidelines for coding, collection, key entry and verification. Each subject is assigned a unique patient number to assure subject confidentiality. Subjects will not be referred to by this number, by name, or by any other individual identifier in any publication or external presentation. The licensed medical records department, affiliated with the institution where the subject receives medical care, maintains all original inpatient and outpatient chart documents. Subject research files are scanned

and stored in a secure database (OWL). OWL records are maintained by the FHCC data abstraction staff. Access is restricted to personnel authorized by the Division of Clinical Research.

Control samples will be received with no associated identifiying information. They would be sent to Incyte Corporation to be used as control samples for the GVHD biomarker analysis.

15.0 STATISTICAL CONSIDERATIONS

ANTICIPATED/PLANNED LOCAL ENROLLMENT: Number of Participants (*must provide exact numbers. i.e., no range)*			
Ethnic Categories	**Sex/Gender**		
	Females	Males	Total
Hispanic or Latino	3	3	6
Not Hispanic or Latino	20	22	42
Ethnic Categories: Total of All Participants*	23	25	48
Racial Categories			
American Indian/Alaska Native	2	2	4
Asian	3	2	4
Native Hawaiian or Other Pacific Islander	2	2	3
Black or African American	2	2	3
White	15	15	19
More Than One Race	0	1	0
Racial Categories: Total of All Participants *	24	24	48

15.1 Statistical Analysis. The primary objective of this single-arm Phase II trial is to assess the potential efficacy of the administration of a JAK inhibitor among patients transplanted for MF. The primary endpoint for these purposes is two-year overall survival. In a previous report, it was shown that DIPSS correlated with mortality following HCT. We hypothesize that the use of a JAK inhibitor will lower the DIPSS risk category, and as a result will lead to improved survival relative to that seen historically for a similar group of patients. We expect to see an absolute improvement in two-year overall survival of 20%. Our previous data show an estimated two-year survival of 50% among 51 patients transplanted for high-risk MF, 64% among 50 patients treated for intermediate-2-risk MF, and 77% among patients treated for intermediate-1-risk MF[3]; patients with various donor sources were included in this study. If the true one-year overall survival is 80%, then 33 patients will provide 90% power to observe a statistically significantly improved probability relative to the fixed value of 60% (at the one-sided significance level of .10). The exact benchmark that we will use for comparison will be determined from the mix of DIPSS categories among those enrolled and treated with JAK on the current trial. From this mix, we will create an expected two-year survival as a weighted average of the data cited above. This weighted average will be used as the benchmark to which the data from the current trial will be compared.

15.2 Stopping Rules. If sufficient evidence exists to suggest that the true probability of treatment-related death by day 100 exceeds 30%, we will reevaluate the prescribed conditioning regimens in combination with JAK inhibitor therapy and consider stopping the protocol.

16.0 TERMINATION OF STUDY

The PI may terminate the study at any time. The IRB also has the authority to terminate the study should it be deemed necessary.

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Appendix A-Disease Criteria and Staging

2008 WHO Classification System

Requires A1 - A3 and any two B criteria	
A1	Megakaryocyte atypia and fibrosis or megakaryocyte atypia, increased granulcytic and decreased erythroid cellularity without fibrosis
A2	Not meeting WHO criteria for PV, CML, MDS or other myeloid neoplasm
A3	Acquired mutation or clonal marker or no reactive cause for fibrosis
B1	Leukoerythroblastic blood film
B2	Increased lactate dehydrogenase[1]
B3	Anemia[2]
B4	Palpable splenomegaly[3]
[1]Degree of abnormality may be mild or marked;	
[2]Hemoglobin < 11.5g/dL for men, < 10 g/dL for women	
[3]Weight loss >10% over 6 months, drenching night sweats or diffuse bone pain	

Tefferi A, Vardiman JW. Classification and diagnosis of myeloproliferative neoplasms: the 2008 World Health Organization criteria and point-of-care diagnostic algorithms. Leukemia. 2008;22:14-22.

IWG for myeloproliferative neoplasms research and treatment criteria

	Major Criteria	Additional Criteria
Diagnosis of post–polycythemia vera MF (PPV-MF) or post–essential thrombocythemia MF (PET-MF) requires meeting both major criteria and 2 additional criteria[2]		
PPV-MF	1 Previous diagnosis of PV as defined by WHO criteria 2 Bone marrow fibrosis grade 2-3 (0-3 scale) or grade 3-4 (0-4 scale)[a]	1 Anemia[b] or sustained loss of requirement of phlebotomy (in the absence of cytoreductive therapy) or cytoreductive treatment for erythrocytosis 2 Leukoerythroblastic peripheral blood picture 3 Increasing splenomegaly: Increase in palpable splenomegaly of ≥5 cm or appearance of newly palpable splenomegaly 4 Development of ≥1 symptoms: >10% weight loss in 6 months, night sweats, unexplained fever (>37.5°C)
PET-MF	1 Previous diagnosis of ET as defined by WHO criteria 2 Bone marrow fibrosis grade 2-3 (0-3 scale) or grade 3-4 (0-4 scale)[a]	1 Anemia[b] and a ≥2 mg/mL decrease from baseline hemoglobin level 2 Leukoerythroblastic peripheral blood picture 3 Increasing splenomegaly: Increase in palpable splenomegaly of ≥5 cm or appearance of newly palpable splenomegaly 4 Increased LDH (above reference level) 5 Development of ≥1 symptoms: >10% weight loss in 6 months, night sweats, unexplained fever (>37.5°C)

Barosi G, Mesa RA, Thiele J, et al; International Working Group for Myelofibrosis Research and Treatment (IWG-MRT). Proposed criteria for the diagnosis of post-polycythemia vera and post-essential thrombocythemia myelofibrosis: a consensus statement from the International Working Group for Myelofibrosis Research and Treatment. Leukemia. 2008;22:437-438.

DIPSS Scoring System

Table 1. Prognostic score systems developed for patients with primary myelofibrosis.

Variable	IPSS [11]	DIPSS [13]	DIPSS-plus [14]
Age >65 years	✓	✓	✓
Constitutional symptoms	✓	✓	✓
Hb <100 g/L	✓	✓	✓
Leukocyte count >25×10^9/L	✓	✓	✓
Circulating blasts ≥1%	✓	✓	✓
Platelet count <100×10^9/L			✓
RBC transfusion need			✓
Unfavorable karyotype*			✓
	1 point each	1 point each but Hb=2	1 point each

*Unfavorable karyotype includes any of the following: +8, -7/7q-, i(17q), inv(3), -5/5q, 12p-, 11q23 rearrangements.
DIPSS: dynamic IPSS; Hb: hemoglobin; IPSS: International Prognostic Scoring System; RBC: red blood cell.

Image courtesy of International Journal of Clinical Reviews
http://www.remedicajournals.com/International-Journal-of-Clinical-Reviews/BrowseIssues/May-2012/Article-Targeted-Therapy-in-PC-NCMN

Appendix B - Performance Status Criteria and Comorbidity Index

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

The Hematopoietic Cell Transplant-Comorbidity Index (HCT-CI)

Instructions: Circle applicable scores and provide actual value or cause of' co-morbidity.

Comorbidities	Definitions	HCT-CI weighted scores	Actual Lab Values/Comments
Arrhythmia	Atrial fibrillation or flutter, sick sinus syndrome, and ventricular arrhythmias	1	
Cardiac	Coronary artery disease‡, congestive heart failure, myocardial infarction, or EF≤50%	1	
Inflammatory bowel disease	Crohn's disease or ulcerative colitis	1	
Diabetes*	Requiring treatment with insulin or oral hypoglycemics, but not diet alone	1	
Cerebro-vascular disease	Transient ischemic attack or cerebro-vascular Accident	1	
Psychiatric Disturbance	Depression anxiety requiring psychiatric consult or treatment	1	
Hepatic -mild*	Chronic hepatitis, Bilirubin >ULN- 1.5 X ULN, or AST/ALT >ULN-2.5XULN	1	
Obesity*	Patients with a body mass index > 35kg/ m^2	1	
Infection*	Requiring continuation of anti-microbial Treatment after day 0	1	
Rheumatologic	SLE, RA, polymyositis, mixed CTD Polymyalgia rheumatic	2	
Peptic ulcer*	Requiring treatment	2	
Moderate/severe renal*	serum creatinine>2mg/dl, on dialysis, or prior renal transplantation	2	
Moderate pulmonary*	DLCO and/or FEV, >65%-80% or Dispend on slight activity	2	
Prior solid tumor	*Treated at any time point in the patient's past history, excluding non-melanoma skin cancer*	3	
Heart valve disease*	Except mitral valve prolapse	3	
Severe pulmonary*	DLCO and/or FEV_1 <65% or dyspnea at rest requiring oxygen	3	
Moderate/severe Hepatic	Liver cirrhosis, Bilirubin>1.5XULN or AST/ALT>2.5XULN	3	
Please provide Karnofksy performance Score=_____% (KPS):		**Total Score =_____**	

Completed by (print):_____ Date completed:_____

Signature:_____

*Comorbidity is currently active or patient requires medical treatment +

‡One or more vessel-coronary artery stenosis, requiring medical treatment, stent, or bypass graft

EF indicates ejection fraction; ULN, upper limit of normal; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; CTD, connective tissue disease;

DLCO, diffusion capacity of carbon monoxide; FEV_1, forced expiratory volume in one second; AST, aspartate aminotransfcrase; ALT, alanine aminotransferase

Appendix C - GVHD Assessment

ACUTE GVHD ASSESSMENT

Staging by Individual Organ Involvement

SKIN: measured by rash first appearing generally between 10 and 70 days after transplant. (excludes rashes of known viral or other origin)

Stage	Description
1	Maculopapular rash <25% BSA
2	Maculopapular rash 25 – 50% BSA
3	Generalized erythroderma
4	Generalized erythroderma with bullous formation and desquamation

LIVER*: measured by total serum bilirubin

Stage	Description
1	2.0 – 2.9 mg/dL
2	3.0 – 5.9 mg/dL
3	6.0 – 14.9 mg/dL
4	$\geq$ 15.0 mg/dL

GUT:** includes only diarrhea occurring after Day +21

Score	Adult	Pediatric***
1	upper GI (anorexia, nausea, vomiting) with diarrhea of <1000 mL/day	upper GI (anorexia, nausea, vomiting) with diarrhea of <555 mL/m^2/day
2	1000 – 1499 mL/day diarrhea	556-833 mL/m^2/day diarrhea
3	$\geq$ 1500 mL/day diarrhea	>833 mL/m^2/day diarrhea
4	severe abdominal cramping, bleeding or ileus caused by GVHD	

* In cases where another cause of hyperbilirubinemia antedated the onset of rash, the liver score should be decreased by one stage.

** In cases where peak GI symptoms are exacerbated by a cause other than GVHD, the gut score should be decreased by one stage.

*** Pediatric patients <17 years of age

ACUTE GVHD ASSESSMENT

Overall Grade

The determination of an overall GVHD grade should be based on the organ stage, response to treatment and whether GVHD was a major cause of death.

Overall Grade	Organ Stage	Qualifying Conditions	Additional Qualifying Conditions
I	Stage 1 -2 skin	No liver or gut	Indicates that the prophylactic immunosuppressive regimen was not sufficient to prevent all manifestations of aGVHD.
II	Stage 3 skin or Stage 1 liver or Stage 1 gut	N/A	Indicates that the prophylactic immunosuppressive regimen was not sufficient to prevent all manifestations of aGVHD, but glucocorticoid treatment after the onset of GVHD was generally sufficient to control the disease.
III	Stage 4 skin or Stage 2-4 liver or Stage 2-4 gut	**without** GVHD as a major contributing cause of death	Indicates that the prophylactic immunosuppressive regimen was not sufficient to prevent all manifestations of aGVHD and that additional treatment after the onset of GVHD did not readily control the disease.
IV	Stage 4 skin or Stage 2-4 liver or Stage 2-4 gut	**with** GVHD as a major contributing cause of death	GVHD was resistant to both the prophylactic immunosuppressive regimen and any additional treatment after the onset of the disease.

References:

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9033

Chronic graft-versus-host disease grading*

In all cases, concomitant processes (i.e. infections or drug reactions) must be ruled out. Karnofsky or Lansky Clinical Performance scores, 60%, > 15% weight loss, and recurrent infections are usually signs of clinical extensive chronic GVHD. Abnormalities that could indicate chronic GVHD are categorized by organ systems as listed below.

Skin	Erythema, dryness, pruritus, pigmentary changes (i.e. hyperpigmentation, vitiligo), mottling, papulosquamous plaques, nodules, exfoliation, macular-papular or urticarial rash, scleroderma, morphea (one or several circumscribed, indurated and shiny lesions)
Nails	Ridging, onychodystrophy, onycholysis
Hair	Premature graying, (scalp hair, eyelashes, eyebrows), thinning scalp hair, alopecia, decreased body hair
Mouth	Dryness, burning, gingivitis, mucositis, striae, atrophy, erythema, lichenoid changes, ulcers, labial atrophy or pigmentary changes, tooth decay, tightness around the mouth
Eyes	Dryness, burning, blurring, gritty eyes, photophobia, pain
Vagina/vulva	Dryness, dyspareunia, stricture or stenosis, erythema, atrophy or lichenoid changes not included
Liver	Elevated liver function tests not due to other causes (alkaline phosphatase ≥ 3x upper limit of normal, AST or ALT ≥4x upper limit of normal or total serum bilirubin ≥ 2.5; in the absence of chronic GVHD involving other organs, liver biopsy is required to confirm diagnosis)
Lung	Bronchiolitis obliterans (see diagnostic indicators), cough, wheezing, dyspnea on exertion, history of recurrent bronchitis or sinusitis
GI	Anorexia, nausea, vomiting, weight loss, dysphasia, odynophagia, malabsorption
Fasciitis	Stiffness and tightness with restriction of movement, occasionally with swelling pain, cramping, erythema and induration, most commonly affecting forearms, wrists and hands, ankles, legs, and feet, inability to extend wrists without flexing the fingers or the elbows, contractures
Serositis	Chest pain or cardiopulmonary comprise due to pericarditis or pleuritis
Muscle	Proximal muscle weakness, cramping
Skeletal	Arthralgia of large proximal girdle joints and sometimes smaller joints

* From Standard Practice Guidelines for "Chronic Graft-versus-Host Disease Classification at the time of presentation" developed by Long Term Follow-Up at the FHCC

Laboratory testing and diagnostic indicators of chronic GVHD*

Eye	Schirmer's test with a mean value ≤ 5mm at 5 minutes, or symptomatic with values of 6-10mm or keratitis detected by slit lamp examination
Liver	Elevated liver function tests not due to other causes (see definition of clinical limited and extensive chronic GVHD)
Lung	New obstructive lung defect defined as FEV1 < 80% of predicted with either an FEF 25-75 <65% of predicted or RV >120% of predicted, or a decrease of FEV1/FVC by > 12% within a period of less than 1 year. A diagnosis of bronchiolitis obliterans requires negative microbiological tests from bronchoalveolar lavage and evidence of air trapping by high resolution end-expiratory and end-inspiratory CAT scans o the chest. A thoracoscopic lung biopsy may be necessary in order to confirm the diagnosis of bronchiolitis obliterans in patients who have obstructive lung disease without air trapping when chronic GVHD involving other organs is absent
Esophagus	Esophageal web formation, stricture or dysmotility demonstrated by barium swallow, endoscopy or manometry
Muscle	Elevated CPK or aldolase, EMG findings consistent with myositis
Blood	Thrombocytopenia (usually 20,000-100,000/μl), eosinophilia, hypogammaglobulinemia, hypergammaglobulinemia, and autoantibodies occur in some cases

* From Standard Practice Guidelines for "Chronic Graft-versus-Host Disease Classification at the time of presentation" developed by Long Term Follow-Up at the FHCC

Appendix D
Potential Adverse Events Associated or Expected with Hematopoietic Cell Transplantation

1. Graft versus host disease: GVHD is a major toxicity associated with the infusion of allogeneic donor stem cells. GVHD may be acute or chronic and may affect multiple organ systems, including the skin, liver, and GI tract.

2. Opportunistic infections, including viral and fungal infections, can result in severe pulmonary, neurologic, hepatic and other organ dysfunction, and possible death.

3. Gastrointestinal toxicity. Nausea and vomiting can be anticipated during the entire course of ablative therapy. Mucositis and diarrhea should be expected. Prednisone can cause GI bleeding.

4. Cardiac toxicity. Cardiotoxicity (congestive heart failure, pericardial effusion, EKG changes) is uncommonly associated with the chemotherapy agents and TBI used in the regimen and these sequelae may prove lethal.

5. Pulmonary toxicity. Diffuse interstitial pneumonitis of unknown etiology and diffuse alveolar hemorrhage occurs with some regularity after BMT and interstitial fibrosis occurs much more rarely. Both are well-described complications of intensive chemotherapy and TBI regimens and may prove lethal.

6. Hepatic toxicity. Veno-occlusive disease of the liver is a common toxicity of high-dose chemoradiotherapy and may result in death. Cyclosporine may cause elevation of ALT/AST.

7. Renal dysfunction. Chemoradiotherapy may uncommonly cause renal dysfunction. More commonly, nephrotoxicity results from cyclosporine and generally responds to dose reduction. Rarely, idiopathic or calcineurin inhibitor-associated hemolytic-uremic syndrome may occur and may be progressive and fatal. A syndrome of moderate renal insufficiency and hemolysis has been seen 5-7 months post HSCT after intensive multi-agent conditioning plus TBI.

8. Hemorrhagic cystitis, manifested either as gross or microscopic hematuria, is a common toxicity after high-dose chemoradiotherapy, but usually associated with regimens that include cyclophosphamide. Hemorrhagic cystitis may predispose to a long-term increased risk of bladder cancer.

9. Central nervous system toxicity. Radiation and chemotherapy can cause CNS toxicity, including seizures, depressed mental status, or leukoencephalopathy. Calcineurin inhibitors can cause seizures or other CNS toxicity.

10. Marrow aplasia. Severe neutropenia, thrombocytopenia, and anemia, is expected to occur for a period of 7 to 42 days following infusion of marrow. Transfusion of platelets and red blood cells is expected as supportive care. Transfusion of blood products may be associated with acquisition of HIV or a hepatitis virus. Neutropenia may increase the risk for acquiring serious infection. Thrombocytopenia may increase the risk of life-threatening hemorrhage. Hemorrhagic or infectious complications during the expected period of aplasia may result in death.

11. Miscellaneous. Alopecia and sterility are expected complications of the program as a whole. Cataract development is possible after TBI and/or steroids. Deficiencies of growth hormone, thyroid hormone, and sex hormones are possible after TBI. Calcineurin inhibitors can cause transient gingival hyperplasia, tremor, seizure, hypertension, headache, dysesthesia and hirsutism. Steroid therapy can also contribute to fluid retention, easy bruising, hypertension, aseptic necrosis of bone and increased susceptibility to infection. MMF can cause spontaneous abortions and birth defects. Hospitalization during conditioning and recovery period is expected to be 5-9 weeks in duration.

Appendix E - FHCC IRB Policies

IRB Policy Web Page: http://extranet.fhcrc.org/EN/sections/iro/irb/policy/index.html

Adverse Events and Noncompliance are addressed in the following policies:

Policy 1.9: "Noncompliance"

Policy 1.11: "Reporting Obligations for Principal Investigators"

Policy 2.6: "Unanticipated Problems Involving Risks to Subjects or Others"

Appendix F Schedule of Study Evaluations see section 10.8 for evaluation windows

Study Evaluations	Prior to JAK inhibitor	Prior to Conditioning			Long Term Follow-up							
			Daily to engraftment	Weekly to Day 100	Day 0	Day +10	Day+28	Day+56	Day+80-100	6 months	1 year	2-5 years
Informed Consent	X[9]	X										
H & P Exam	X	X		X			X			X	X	X
DIPPS status	X	X									X	X
Karnofsky	X[9]	X							X		X	
MRIMUGA/Echo, EKG, Chest CT		X								PRN	PRN	PRN
CBC w/differential	X	X	X	X			X		X	X	X	X
Blood Chemistry	X	X		X			X		X	X	X	X
Hepatic Panel	X	X		X			X		X	X	X	X
Serum Iron, TIBC, ferritin	X[9]	X										
PB CD34	X[9]	X										
Pregnancy Test	X[9]	X										
Viral screening		X										
CMV Surveillance by PCR		X		X[6]								
Urinalysis		X										
Disease Restaging: BM aspirate/biopsy	X[9]	X					X		X	X[1]	X	X[1]
Comorbidity		X										
Spleen assessment[5]		X							X		X	
Chimerism – Bone Marrow									X	X[2]	X	X[2]
Chimerism – Peripheral Blood							X[8]	X[7]	X	X[3]	X	X[3]
Research blood	X[9]	X			X	X	X		X		X	
Research marrow[11]	X[9]	X									X	
Sample Collection for V617F , CAL1R, MPL	X[9]	X							X	X[10]	X	X
GVHD Evaluation				X			X		X	X	X	X
Hot spot lab test	X	X[10]										

Notes: [1] Day 180 will only be done if residual disease in marrow or JAK mutation still positive at day 80 and 2-5 year BM biopsy will only be done if residual disease in marrow or JAK mutation still positive at 1year or prior yearly check. [2] Day 180, and 2-5 year chimerisms will only be done only if previous chimerism did not show greater than 95% engraftment. [3] Chimerisms should be performed as clinically indicated (in patients with unresolved or new cytopenia or mixed chimerism. [5] If patient is unable to have an MRI performed, CT chest, abdomen and pelvis, or ultra sound, may be substituted to assess spleen volume as well as extramedullary sites of disease. [6]Twice weekly for UCBT. [7]If clinically indicated. [8]UCBT and non-myeloablative only. [9] If enrolled in Part 1. [10]If positive at day80-100 evaluation. [10]If not enrolled in part 1 or not done when enrolled in part 1. [11]Collect research bone marrow if possible