

Allogeneic haematopoietic cell transplantation for myelofibrosis: a real-life perspective

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Summary

Myelofibrosis (MF) is a clonal stem cell neoplasm with heterogeneous clinical phenotypes and well-established molecular drivers. Allogeneic haematopoietic stem cell transplantation (HSCT) offers an important curative treatment option for primary MF and post-essential thrombocythaemia/polycythaemia vera MF or secondary MF. With a disease course that varies from indolent to highly progressive, we are now able to stratify risk of mortality through various tools including patient-related clinical characteristics as well as molecular genetic profile. Owing to the high risk of mortality and morbidity associated with HSCT for patients with myelofibrosis, it is important to improve patient selection for transplant. Our primary goal is to comprehensively define our understanding of current practices including the role of Janus Kinase (JAK) inhibitors, to present the data behind transplantation before and after leukaemic transformation, and to introduce novel personalization of MF treatment with a proposed clinical-molecular prognostic model to help elucidate a timepoint optimal for consideration of HSCT.

Keywords: myelofibrosis, allogeneic transplant, ruxolitinib, splenectomy.

Primary myelofibrosis (MF) comprises a group of myeloproliferative neoplasms (MPNs), which is characterized by cytopenia, splenomegaly, mainly in later stages, with a risk of leukaemic transformation to acute myeloid leukaemia (AML) in some patients. Furthermore, the course of the disease may

range from indolent to severely symptomatic and rapidly progressive, with a median survival ranging from months to many years with median survival estimated at six years.¹ Additionally, patients with polycythaemia vera (PV) or essential thrombocythaemia (ET) might develop marrow fibrosis (secondary MF) during the course of their disease, often many years after the initial diagnosis. Interestingly, patients with secondary MF have similar presentation as patients with primary MF.² Furthermore, treatment of patients with primary myelofibrosis has evolved over the years. The introduction of Janus Kinase (JAK1/2) inhibitors revolutionized the outcome of patients with myelofibrosis.³ Nevertheless, some patients do experience disease progression or relapse and in them transplant is inevitable.⁴ The aim of this review is to discuss the role allogeneic haematopoietic stem cell transplantation (HSCT) in patients with MF and to discuss the optimal timing of HSCT, novel distinctions in treatment considerations including role of JAK2 inhibitors both pre and post HSCT, the role of pre-HSCT splenectomy as well as new developments in optimal donor selection and advances in conditioning regimen.

Risk stratification

There are several classification systems for categorizing risk of primary MF (PMF) that predict survival:

- DIPSS (Dynamic International Prognostic Scoring System):⁵ this comprises age > 65 years, anaemia (haemoglobin <100 g/l), leukocytosis (WBC > 25 × 10⁹/l), circulating myeloblasts (≥1%), and constitutional symptoms, applied at any time of the disease course. Categorized into four groups according to the number of factors present: Low (0), Intermediate-1 (1–2), Intermediate-2 (3–4) and High (>4).
- DIPSS-plus:⁶ in addition to the above features, this includes low platelet count (<100 × 10⁹/l), the need for red blood cell transfusions and the presence of adverse

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or unfavourable cytogenetics features including trisomy 8 (+8), monosomy 7 or 7q deletion (-7/7q), monosomy 5 or 5q deletion (-5/5q), 12p deletion, inversion 3 (inv3) and 11q23 rearrangement.

- MIPSS70-plus (Mutation Enhanced International Prognostic Score System for transplant age <70 years):⁷ this incorporates all of the above features plus additional cytogenetic information, which allows a more refined designation of unfavourable karyotype, the absence of calreticulin (*CALR*) gene mutations, high-risk mutations, and bone marrow fibrosis.
- GIPSS (Genetically-Inspired Prognosis Scoring System):⁸ this is based on karyotype (unfavourable or high-risk), absence of type 1-like *CALR* gene mutation and presence of high-risk mutations (*ASXL1*, *SRSF2*, *U2AF1Q157*). It offers a low-complexity prognostic tool for PMF that is only dependent on genetic risk factors and, thus, forward-looking in its essence.

Current data show that the life expectancy of PMF patients without *JAK2* inhibitors and HSCT is favourable, often up to 20 years, if their DIPSS score is 'Low' (no factors).⁵ If the score is 'High' (>4 factors), the median life expectancy decreases to 2–3 years. Overall survival (OS) is also affected by the presence or absence of certain mutations. In 2013, Vannucchi *et al.* showed a worse probability of OS and leukaemic transformation in patients harbouring certain mutations including *EZH2*, *SRSF2* and *ASXL1* mutations, compared to those who lacked these mutations. In the multi-variable analysis, when these mutations were analyzed independently, only *ASXL1* mutation predicted worse OS [hazard ratio (HR) 1.4; $P = 0.04$] in the context of the DIPSS-plus model, which incorporates cytogenetic risk. *ASXL1* shows worse OS in the low/intermediate-1 risk ($P = 0.07$) and intermediate-2 groups ($P = 0.03$) but not in the high-risk group ($P = 0.78$).⁹ In another study of 797 patients with PMF, the prognostic impact of two or more mutated genes was found to be IPSS/DIPSS-independent while the number of high-risk mutations was significant. OS directly correlated with the number of high-risk mutations with ≥ 2 mutations conferring an OS of 2.6 years while 1 and 0 have shown an OS of 7 and 12.3 years, respectively. The presence of ≥ 2 mutations also leads to a shorter leukaemia-free survival (LFS) compared to that in patients who carried mutations with no prognostic implication.¹⁰ In addition, patients harbouring ≥ 3 mutations are less likely to respond to ruxolitinib.¹¹

Impact of genetic mutations

HSCT outcomes are affected by the presence or absence of some mutations; certain driver mutations including *JAK2*, myeloproliferative leukaemia virus (*MPL*) oncogene and *CALR* (variants type 1 and type 2) confer a selective proliferative advantage to the cancer cell and manifest variable clinical outcome. Patients with type 2-like *CALR* mutations

exhibit an essential thrombocythaemia (ET) phenotype with a low risk of thrombosis and indolent disease course, while patients with type 1-like mutations develop an MF phenotype and have a high risk of progression from ET to secondary MF.¹² Patients with *JAK2*- and *MPL*-negative myelofibrosis and ET have a high incidence of *CALR* mutation.¹³ This cohort of patients with mutated *CALR* demonstrate the best long-term survival with a two-year OS of 82% compared to 56% in wild-type *CALR* disease. While patients with *JAK2* or *MPL* mutations possess an intermediate survival, triple-negative patients (negative for all three mutations: *JAK2*- *MPL*-, *CALR*-) have the worst survival ($P = 0.015$). The reason for this survival difference is not entirely clear but possibly related to the lower non-relapse mortality (NRM) for patients with *CALR* mutation compared to more than 50% NRM for triple-negative patients ($P = 0.011$). The cumulative incidence of relapse did not significantly differ between the mutation groups. The data suggest a better OS for *CALR*-mutated patients after HSCT, a lower rate of NRM and a higher incidence of grade I acute graft-versus-host disease (GVHD) and limited chronic GVHD.¹³ Thus, *CALR* seems a valuable diagnostic as well as prognostic marker in PMF and secondary MF patients who will undergo HSCT.

The *ASXL1* mutation is also recognized as conferring a high risk of PMF progression and leukaemic transformation and it may play a fundamental role in the pathogenesis of PMF. It is also known to cause molecular progression of the chronic phase of PV and ET.¹⁴ *ASXL1* was an independent risk factor for lower progression-free survival (PPS; HR, 1.53, $P = 0.008$) than in patients who did not have this mutation.¹⁵ Tamari *et al.* showed that mutations including *ASXL1*, *SRSF2*, *IDH1/2*, *EZH2*, and *TP53* that are known to increase risk of disease progression, did not affect OS or relapse-free survival (RFS) in patients with PMF undergoing HSCT.¹⁶ Their findings contrast with data published by Kröger *et al.* that demonstrated a greater risk of relapse in the presence of *ASXL1* mutations.¹⁵

With the development of novel next-generation sequencing (NGS) technologies and the revolutionary single-cell RNA sequencing (scRNA-seq), we are better able to characterize malignant and stromal cell populations in a tumour. This process affords a powerful means to assess gene expression in a single cell and thus to determine if the presence of these mutations in various cell populations in an individual's blood or bone marrow can impact long-term outcomes. With scRNA-seq, very small subsets of cells that may be relevant to the behaviour of the disease can be studied down to the level of clusters of combined genes. A study of data from patients with AML used scRNA-seq technology developed by 10× Genomics, Inc., (Pleasanton, CA, USA) to yield a comprehensive single-cell data set. It was co-authored with researchers from the Fred Hutchinson Cancer Research Center (FHCRC) who collected transcriptome data from approximately 250 000 single cells across 29 samples. Results showed that this technique for single-cell RNA sequencing

has better scalability and was more robust and had similar sensitivity to existing techniques. The system's rapid cell encapsulation and high cell capture efficiency enabled analysis of precious clinical samples from patients with AML. The system's sensitivity and capability to identify rare populations and to distinguish large immune population was validated through cell lines and synthetic RNAs and profiled 68 000 peripheral blood mononuclear cells. Finally, sequence variation in the transcriptome data was used to determine host and donor chimaerism at single-cell resolution from bone marrow mononuclear cells isolated from transplant patients.¹⁷

Transplant considerations

Patients who are transplant-eligible do benefit from undergoing HSCT; however, this is not without any risk. A large multicentre retrospective study conducted by Gowin *et al.* that analyzed OS in 1 377 patients with MF treated with HSCT and without HSCT showed long-term OS benefit of HSCT in patients with intermediate-1 or higher-risk MF but at the cost of early transplant-related mortality.¹⁸ The higher the DIPSS risk score, the greater the extent of long-term OS benefit with HSCT was. Extramedullary disease (tumour formation outside the bone marrow, e.g. spleen, liver, lungs, lymph nodes), portal hypertension (liver, digestive organs), and pulmonary hypertension increase the risk of post-transplant complications.¹⁹ In fact, a liver biopsy from a patient with post-transplant extramedullary haematopoiesis clearly showed an associated sinusoidal fibrosis. In areas with extensive fibrosis, collagen fibres filled the sinusoidal space and resulted in the loss of hepatocytes.

Pulmonary hypertension (PH) is an impressive complication prevalent in about 50% of patients with MF as a result of extramedullary haematopoiesis yet is not routinely evaluated and diagnosed prior to HSCT despite evidence that it can negatively impact survival post HSCT.²⁰ A recent large retrospective at FHCRC suggested the use of concurrent transthoracic echocardiogram (TTE) as well as chest computed tomography (CT) for the evaluation of pulmonary artery pressures and the pulmonary artery to aorta ratio, respectively as pre-HSCT evaluation to assess presence and degree of PH. The retrospective review found that in 270 patients with PMF who underwent HSCT, only 115 had either a TTE, chest CT or both obtained for any reason prior to HSCT. In this cohort, they noted a general trend toward shorter survival in patients determined to have PH by their combined TTE and CT assessment.²⁰

In the process of long-standing fibrosis, remodelling of the stem cell niche significantly reduces OS even in patients undergoing HSCT with the implication that the effects of microenvironment and stem cell niche are not entirely reversible after HSCT.²¹ A recent study from City of Hope examined the peak pulmonary artery systolic pressure (PASP) pre and post HSCT of 65 patients and found a significant reduction in PASP after HSCT.²² While they concluded an inferior

survival in MF patients with PH prior to HSCT due to increased NRM, they found that PH appeared to be partially reversible after successful HSCT, suggesting that pulmonary vascular changes are not permanent. Overall, there appears to be derived benefit in routine and early monitoring for PH possibly with TTE and chest CT in patients with MF prior to consideration for HSCT.

Role of pre-transplant splenectomy

Splenomegaly noted in the setting of extramedullary haematopoiesis is a pervasive phenomenon in MF. This becomes an important consideration in evaluating such patients for HSCT as splenomegaly has been linked to more frequent graft failures, not to mention the risk of thrombosis, infections and haemorrhage that splenectomy itself poses, complications which could delay or even preclude a patient from undergoing HSCT.^{23,24} On the other hand, pre-transplant splenectomy in patients with MF has been linked with improved haematopoietic recovery as well as improved OS in some cases.^{25,26} Recently, Bossard *et al.* conducted a comprehensive retrospective review of 530 patients from 57 centres to evaluate the association between splenectomy and incidence of HSCT; they discovered 81 splenectomies, 99 deaths prior to HSCT (90 without splenectomies and nine after), and 333 HSCTs of which 65 underwent splenectomies after HSCT.²⁷ A significantly higher rate of transplant was noted among the splenectomized cohort compared to the non-splenectomized cohort particularly in the first four months. Moreover, there was no significant difference in the rate of death between splenectomized and non-splenectomized patients. However, with the advent of JAK2 inhibitors, the utility of routine splenectomy prior to HSCT becomes questionable as JAK2 inhibitors can generate a substantial reduction in splenomegaly. Given the relative safety profile of splenectomy and increase in rate of transplant post splenectomy, splenectomy can be considered in patients with massive splenomegaly who are refractory to ruxolitinib without a detrimental impact on their post-transplant outcome.

Ruxolitinib prior to transplant

Treatment with ruxolitinib prior to transplantation has become the standard of care for most patients with PMF or secondary MF. This drug can provide a rapid and sustained reduction in splenomegaly, improvement in symptoms and quality of life, possibly resulting in longer survival. These effects on metabolic consequences may translate into survival benefits. However, data to show that ruxolitinib improves survival outcomes by transforming the disease course are limited. Furthermore, the rate of drug discontinuation due to side effects or lack of efficacy is 38% and 45% after one and two years, respectively.^{28,29}

A phase I/II clinical study published in 2017 found that survival was poor after ruxolitinib discontinuation, with a

median OS of 14 months.³⁰ Moreover, clonal evolution, defined as the acquisition of ≥ 1 mutation, occurred in 35% of patients while receiving ruxolitinib and also after discontinuation. This clonal evolution may predict a worse prognosis after ruxolitinib discontinuation, with an OS of six *versus* 16 months without clonal evolution. Furthermore, transfusion dependence at baseline is a known marker of disease severity, which has also been associated with the development of clonal evolution. Interestingly, this clonal evolution tends to occur in patients more frequently and earlier in those on ruxolitinib than in those who do not receive this treatment. This may be the result of selective pressure by ruxolitinib exerted on the bone marrow stem cells and if this occurs it is probably best to discontinue treatment and consider either HSCT or other investigational targeted (non-transplant) agents. In an editorial following this publication, the decision on initial treatment using ruxolitinib is depicted by a traffic light signal system where molecular information represents green and yellow lights for starting treatment.³¹ Acquisition of new mutations during therapy with ruxolitinib would turn the traffic light to red, indicating the need to switch direction.

A recently published phase II prospective trial assessed the effects of pre-transplant ruxolitinib in patients with PMF and secondary MF who underwent HSCT.³² The primary endpoint of the study was two-year OS. Twenty-eight patients were transplanted with a median age at transplant of 56 years. Patients who had leukaemic transformation prior to HSCT were excluded. All patients received ruxolitinib prior to HSCT with a median time of treatment of seven months and a minimum duration of eight weeks. Ruxolitinib was tapered prior to HSCT by 5 mg every three days with discontinuation on day -4 of conditioning with no cytokine release syndrome (CRS) observed. All patients attained continued engraftment with a median time to neutrophils engraftment ($>500 \times 10^6/l$) of 19 days and a median time to platelets engraftment ($>20 \times 10^9/l$) of 20 days. Two patients died from NRM and two patients relapsed. After a median follow-up of 13 months, the OS was 86% at two years post transplant and grade III-IV GVHD was observed in 22% of the patients. This study showed that ruxolitinib was well tolerated and pre treatment may improve outcomes post HSCT. Of course, a larger patient group and longer follow-up is needed to confirm these results. The use of ruxolitinib as a bridge prior to HSCT may increase the proportion of patients eligible to undergo HSCT and improve outcomes.³³

The phenomenon of 'ruxolitinib withdrawal syndrome' characterized by rapid progression of the disease, worsening cytopenias, rapidly increasing splenomegaly sometimes leading to haemodynamic instability, shock and possible CRS has been noted with tapering of ruxolitinib.³⁴ In the phase III COMFORT-I trial comparing ruxolitinib to placebo in the treatment of patients with intermediate-2 or high-risk myelofibrosis, no specific withdrawal effect of ruxolitinib was noted.³⁵ However, the tapering schedule is not standardized in the context of HSCT. In the phase II trial mentioned

above, ruxolitinib was tapered progressively every three days by 5 mg until four days prior to stem cell infusion.^{34,35} In another trial, ruxolitinib was tapered by one dose level (5 mg) every 1-2 days over a six-day period.³⁶ In 'real life', tapering schedules vary according to centre protocols. Some centres withdraw ruxolitinib abruptly at the start of the conditioning regimen without reporting adverse events.

A major cause of early morbidity and mortality associated with HSCT in MF patients is early NRM. However, recent studies have shown promising results in improving engraftment, severity of GVHD and lower NRM in patients with MF who received ruxolitinib prior to HSCT. In a recent phase II prospective trial, out of 28 patients with primary and secondary MF who underwent HSCT, the median time that patients received ruxolitinib was seven months; donor source included human leukocyte antigen (HLA)-matched siblings, 11 matched unrelated, one allele-mismatched unrelated and three umbilical cord blood.³² In this cohort, OS was 93% at one year and 86% at two years post HSCT. Similarly, in a two-stage Simon phase II trial evaluating ruxolitinib therapy followed by a reduced-intensity conditioning (RIC) regimen for patients with MF undergoing HSCT, out of 19 transplant recipients, ruxolitinib was tapered successfully without any withdrawal effects and they achieved a graft failure, NRM, acute GVHD, and chronic GVHD at 24 months of 16%, 28%, 64% and 76%, respectively.³⁷ The reduced symptom burden that ruxolitinib affords allows safe commencement of conditioning therapy prior to HSCT. Hence, use of ruxolitinib prior to HSCT might serve as means to improve overall post-transplant outcomes.

In patients requiring discontinuation of ruxolitinib, salvage therapy can be considered prior to HSCT. In a retrospective, single-centre study, Kuykendall *et al.* evaluated salvage treatment options and outcomes in MF after ruxolitinib discontinuation.³⁸ In their cohort, the median OS was 13 months and 16% of the patients progressed to AML with 80% of them progressing while on ruxolitinib. Excluding those who underwent HSCT, those receiving salvage treatment had superior OS compared to those who did not, with a median OS of 15.0 months compared to 4.9 months ($P = 0.02$). Improved OS in patients who underwent HSCT compared to those who did not remained significant even when patients who progressed to AML were excluded ($P = 0.007$). In multivariate analysis, the effect of salvage therapy remained significant after adjusting for haematologic and clinical covariates at the time of ruxolitinib discontinuation ($P = 0.04$). Nevertheless, the best outcomes were observed in patients who underwent transplantation. After discontinuation of ruxolitinib, the authors found that HSCT and immunomodulatory agents, namely, thalidomide and lenalidomide, were the most often utilized salvage treatment options while hydroxycarbamide (hydroxyurea) and investigational agents were found to be most frequently used in the clinical phase I/II study mentioned above, likely reflecting differences in a clinical trial population as well as regional

practices. These findings show that salvage treatment can lead to clinical responses after ruxolitinib discontinuation; however, these responses are rare and outcomes in this patient population are poor but can be improved by including HSCT in the therapeutic strategy.³⁸

Whom and when to transplant?

Biological age is no longer a limiting criterion for transplant. Careful selection is necessary for patients aged over 65; however, successful transplants have been carried out in elderly patients older than 70 years. Comorbidities are very important as they may limit the intensity of the transplant regimen, subsequently affecting the risk of relapse. Karnofsky index has also a strong impact on survival after HSCT, which implies that transplant should be performed early enough in the disease time course before the disease alters the patient's physical state.³⁹ Other considerations are the clinical symptoms of the patient, quality of life, transfusion dependence and response to ruxolitinib.

Certain patients with a DIPSS score of 1–2, or Intermediate-1, may benefit from transplant such as those with $\geq 2\%$ blasts in peripheral blood and/or transfusion dependence/refractoriness (Fig 1).¹ Some younger patients may also elect to proceed with this route even though non-transplant therapy options may be available for them. With a DIPSS score of >3 , or Intermediate-2/High, HSCT is generally advised. The classification of DIPSS-plus Intermediate-1/Intermediate-2/high risk is also an accepted indication for HSCT. With advanced disease or with lack of response to JAK inhibitors, HSCT is the

only viable option even though results are not as good as when HSCT is performed earlier in the disease course.

Karyotype and mutations should be highly considered in the transplant decision-making process. Patients with adverse karyotype or with triple-negative disease (no mutations of *JAK2*, *CALR* or *MPL1*) should undergo HSCT or at least be monitored closely for disease progression in order to not miss a favourable window for transplantation. Gagelmann *et al.* utilized a comprehensive clinical, molecular and transplant-specific information from 361 patients to develop a prognostic scoring system, the Molecular Myelofibrosis Scoring System (MTSS).⁴⁰ Molecular components of the scoring system including the presence of *AXL1* mutation, and non-*CALR/MPL* driver mutation genotype were shown to be independent predictors of outcome in multivariate analysis. Absence of *CALR* type 1 mutation specifically, which portends a risk profile similar to that of triple-negative patients, can influence outcomes after both diagnosis of MF and an HSCT and should be taken into consideration for an early transplantation. In addition, those with two or more high-risk mutations and perhaps PMF with *any* high-risk mutation including *AXSL1* in addition to other clinical and transplant-specific risk factors should also be considered for a transplant. In a commentary provided by Passamonti, this idea of individualizing HSCT decision in patients with MF through the utilization of MTSS is further exemplified as HSCT can ultimately provide a cure in approximately 50% of patients with MF after five years (Table I).⁵

Transplantation before leukaemic transformation

The risk of leukaemic transformation is relatively high for PMF (approximately 25%) while considerably lower in patients with PV or ET (~5%). A retrospective cohort study of 233 patients diagnosed with PMF or secondary MF between March 1990 and December 2014 undergoing transplantation at the FHCRC was published.⁴¹ The aim was to determine if the DIPSS-plus would better predict post-HSCT outcomes than the original DIPSS. None of the patients had leukaemic transformation. Data were analyzed with respect to OS, RFS and non-relapse mortality (NRM). RFS was significantly worse in the higher DIPSS-plus risk groups (log-rank $P = 0.0001$). In the multivariable analysis, the probability of OS and RFS doubled in high risk compared to intermediate-2 risk groups according to the DIPSS-plus score. Furthermore, patients with 'high' risk disease have a higher probability of NRM (log-rank $P = 0.02$) due to organ toxicity, infections and GVHD with more complications occurring in patients with prior organ involvement with the disease. The additional components in the DIPSS-plus classification (adverse cytogenetics, thrombocytopenia and transfusion dependence) all contributed to post-transplant outcomes. This information should enhance the ability to advise patients regarding optimal timing of transplant.

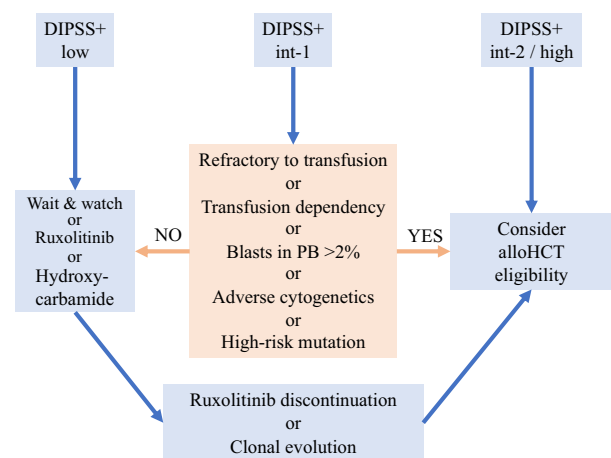


Fig 1. Transplantation algorithm for patients with myelofibrosis. Adverse or unfavourable karyotype is defined by the presence of one of the following abnormalities: trisomy 8 (+8), monosomy 7 or 7q deletion (-7/7q), monosomy 5 or 5q deletion (-5/5q), 12p deletion, inversion 3 (inv3) and 11q23 rearrangement; high-risk mutations are *ASXL1*, *SRSF2*, *EZH2*, *U2AF1Q157* and *IDH1/2*. PB, peripheral blood; alloHCT, allogeneic haematopoietic cell transplantation. [Colour figure can be viewed at wileyonlinelibrary.com]

Table I. Summary of the outcomes in the main studies on allogeneic haematopoietic cell transplantation in myelofibrosis.

Number of patients	Year	Age (years)	Intermediate-2/ high-risk %	NRM % (years)	OS % (years)	Reference
104	2007	49	58%	NA	61% (7)	[69]
100	2008	49	90%	43% (3)	42% (3)	[70]
103	2009	55	83%	16% (1)	67% (5)	[44]
289	2009	46	64%	35–50% (5)	37% (5)	[60]
147	2011	53	83%	29% (4)	39% (4)	[71]
170	2012	51.5	88%	34% (5)	57% (5)	[72]
150	2012	57	98%	NA	60% (5)	[73]
MSD, <i>n</i> = 79 MUD, <i>n</i> = 104 MMUD, <i>n</i> = 50	2014	55	88%	MSD, 23% (5) MUD, 37% (5) MMUD, 56% (5)	MSD, 56% (5) MUD, 48% (5) MMUD, 34% (5)	[45]
MSD, <i>n</i> = 32 MUD, <i>n</i> = 34	2014	55	95%	MSD 22% (2) MUD 59% (2)	MSD 75% (2) MUD 32% (2)	[46]
160	2016	57	NA	FM 44% (7) FB 32% (7)	FM 52% (7) FB 59% (7)	[74]
61	2019	60	95%	33% (2)	59% (2)	[75]
2224	2019	52	NA	MAC 34.6% (5) RIC 34.4% (5)	MAC 53% (5) RIC 51% (5)	[52]
2916	2020	56	61%	37% (5)	50% (5)	[39]

NRM, non-relapse mortality; OS, overall survival; MSD, matched sibling donor; MUD, matched unrelated donor; MMUD, mismatched unrelated donor; NA, not available; FM, fludarabine melphalan; FB, fludarabine busulfan.

While current efforts to improve overall outcomes post transplant are under way, there has been an evident shift in transplant practices over the past decade that has improved the overall course. A recent study evaluating survival, NRM, and relapse-related mortality after allogeneic haematopoietic stem cell transplant conducted in 2003–2007 *versus* 2013–2017 cohorts showed that over the decade, there has been a decrease in the adjusted hazards of day-200 NRM (HR 0.66 [95% confidence interval, CI, 0.48–0.89]) as well as a decrease in relapse of cancer, relapse-related mortality and overall mortality with the latter noted in patients receiving myeloablative and reduced-intensity conditioning as well as for patients whose allograft came from matched sibling *versus* unrelated donor.⁴² This remarkable improvement over the decades can be largely attributed to incremental advances in conditioning therapy, GVHD prophylaxis, prednisone dosing, infection control and supportive measures.

Transplantation after progression/leukaemic transformation

In approximately 10–20% of cases, MF and post-ET/PV MF transforms into AML.⁴³ In the setting of clonal evolution, with or without the progression to leukaemia, a transplant is currently considered the best therapeutic option. For patients who do progress to leukaemia, survival is not as good as it is for those transplanted earlier in the disease course. However, a European study has shown that if patients are treated with chemotherapy and achieve complete remission (CR) prior to transplantation, the probability of survival is significantly better than that seen in those who do not achieve CR.⁴³ This

study included 46 of 1 048 patients from the European Group for Blood and Marrow Transplantation (EBMT) registry who received HSCT for acute leukaemia evolving from MF and showed that HSCT can cure MF that has transformed to leukaemia. Transformed AML in MF results in a median survival of less than five months. This study identified that the only significant factor for survival was CR *versus* no CR before transplantation (69% vs 22%, *P* = 0.008); however, CR was achieved only in eight patients.

Optimal donor selection

As in all haematologic malignancies, in particular myeloid diseases, donor selection plays a significant role in HSCT outcomes in patients with MF. In a phase II study of the Chronic Leukemia Working Party of the EBMT, the use of matched sibling donors (MSD) led to lower NRM (12%) compared to unrelated donors (URD; 38%) at one year.⁴⁴ Another report from the Center of International Blood and Marrow Transplant Research confirmed these results showing higher mortality in patients receiving URD.⁴⁵ Whether the use of MSD *versus* URD both matched or both mismatched impacted mortality and survival was controversial.

In a prospective study by the Myeloproliferative Disorders Research Consortium (MRD-RC) the use of URD led to inferior survival and NRM compared to MSD (32% vs 75% and 59% vs 22% at two years, respectively).⁴⁶ In patients without HLA-matched related donor (MRD), alternative donor source selection asserts a vital role in patient outcomes. In a retrospective study consisting of 224 PMF patients, comparison of outcomes of HSCT was analyzed

based on different haematopoietic stem cell source groups including related donor bone marrow (MSD-BM), related peripheral blood stem cells (MSD-PB), unrelated donor bone marrow (URD-BM), unrelated umbilical cord (URD-CB) and other haematopoietic stem cell grafts.⁴⁷ Higher NRM was noted in patients who received URD-CB transplantation in a multivariate analysis. NRM at one year after MSD-BM, MSD-PB, URD-BM and URD-CB transplantation was 16%, 36%, 30%, and 41%, respectively. OS at one and four years after MSD-BM, MSD-PB, URD-BM and URD-CB were 81% and 71%, 58% and 52%, 61% and 46%, and 48% and 27%, respectively. In selected patients without an HLA-identical related donor, URD-CB and URD-BM sources of donor transplant are viable options with close monitoring due to high risk of NRM with URD-CB transplantation.

Data on HSCT with a haploidentical donor are still limited. In the largest cohort published to date, the Chronic Malignancies Working Party of the EBMT showed that patients with MF undergoing mismatched/haploidentical HSCT can achieve engraftment with acceptable incidence of GVHD and encouraging PFS and OS. In the 56 patients included in the study, the cumulative incidence of acute grade II–IV GVHD and one-year chronic GVHD were 28% and 45%, respectively. The two-year OS, PFS and NRM were 56%, 43% and 38%, respectively. A more recent multi-institutional study retrospectively reviewed 58 patients from 11 centres with MF who underwent haploidentical HSCT with post-transplant cyclophosphamide from 2000 to 2019 with a two-year OS, RFS, relapse and NRM of 69%, 52%, 21% and 27%, respectively.⁴⁸ Interestingly, the spleen size and type of driver mutation had no significant impact on outcomes. Grade 3–4 acute GVHD was seen in five patients. Five patients had graft failure which is similar to rates reported in sibling and unrelated donors. Although further comparisons of URD and haploidentical donor are warranted with longer follow-up, it is now recommended to consider haploidentical HSCT when no MRD or matched URD are available.

Conditioning regimen

Additionally, conditioning regimen may impact outcome of myelofibrosis post transplant in terms of survival, relapse and engraftment. It is important to note that the median age of diagnosis of patient with PMF ranges from 60 to 67 years which impedes the use of myeloablative conditioning (MAC) regimen and inclines transplanters to use reduced toxicity (RTC) or intensity (RIC) conditioning regimens.^{49,50} In the latter MRD-RC trial, RIC regimen was used with fludarabine and melphalan-based conditioning. Neutrophil and platelets engraftment were reached in 97% and 88% of the sibling group and 76% and 59% of the unrelated group, respectively. The median time to neutrophil engraftment was 22 and 18 days in the MSD and URD groups, respectively and the median time to platelet engraftment was 28 days in both groups.⁴⁶

Current guidelines recommend the use of oral busulfan with targeted dosing according to plasma levels and alternatively, considering intravenous busulfan guided by plasma levels.¹ Currently, there is no conclusive evidence to support the use of a particular MA or RIC conditioning regimen, but favourable results have been noted with the implementation of busulfan–cyclophosphamide and fludarabine–busulfan and anti-thymocyte globulin (ATG). In an effort to address the appropriate dose density to improve survival outcomes after transplant, Popat *et al.* conducted a non-randomized, prospective, phase II trial evaluating low-dose escalated to high-dose (myeloablative conditioning) busulfan and fludarabine in MF patients up to age 74.⁵¹ In the study, 15 patients received ‘low-dose’ IV busulfan 130 mg/m²/day on Days –3 and –2 while 31 patients received high-dose conditioning with either 100 mg/m²/day on Days –5 to –2 or pharmacokinetic-guided area under the curve of 4 000 µmol/min (Days –5 to –2); cumulative incidence of NRM was 9.7% at day 100 and at three years in the high-dose group and 0% in the low-dose group at day 100, which increased to 20% at three years. In a multivariate analysis, however, there was no impact in NRM but a trend towards lower relapse in the cohort that received high-dose busulfan. No primary graft failure was observed. The median time to neutrophil and platelet engraftments was 13 and 24 days, respectively.

Many other studies, mostly retrospective, assessed the use of RIC conditioning. In a retrospective registry study of the EBMT, 2 224 patients with myelofibrosis who underwent HSCT were included in which 35% of the patients received MAC and 65% received RIC regimen.⁵² OS was not statistically different between the two regimens at five years, being 53% in the MAC group and 51% in the RIC group ($P = 0.78$). There was a trend toward higher relapse in the RIC group (23%) compared to the MAC group (20.1%) at five years ($P = 0.008$). The median time to neutrophil and platelet engraftment was similar in both groups being 17 and 18 days and 19 and 20 days, respectively. The most frequently used regimens in the RIC group were busulfan/fludarabine and busulfan/melphalan. The most frequently used MAC regimens were busulfan/cyclophosphamide and busulfan/fludarabine. There was no difference in term of OS between the different regimens in the MAC and RIC groups. In the multivariate analysis of OS and NRM, age >50 in the MAC group and age between 60 and 70 in the RIC group had a major impact.

Moreover, the use of thiotepea, busulfan and fludarabine (TBF) has been increasingly explored as a promising regimen in MF patients undergoing HSCT. A retrospective study conducted at two centres in Israel and Poland evaluated 67 patients with MF, of which 45% had prior PV or ET with 36%, 46% and 18% undergoing MAC, RIC and TBF conditioning regimens, respectively. At one year, PFS was noted to be 80%, 54% and 45% with TBF, MAC and RIC, respectively. Median time to neutrophil and platelet engraftment was 15 and 21 days, respectively.⁵³ Another single-centre experience constituting 29 patients with MF who received

TBF conditioning prior to HSCT also showed high engraftment rates and promising PFS of 69% at three years.⁵⁴ All regimens used mainly busulfan-based combinations, which led to comparable and acceptable engraftment rates and median time to engraftment.

One of the prevalent causes of morbidity and mortality in patients undergoing HSCT for MF remains acute and chronic GVHD. Recently, randomized trials have shown a benefit in the use of ATG in reducing the incidence of chronic GVHD in patients with ALL or AML undergoing HSCT.⁵⁵ A similar study was subsequently proposed in patients with MF undergoing an HLA-matched sibling donor transplant as data regarding the use of ATG in this setting is scarce. A retrospective study evaluating 287 patients with MF from the EBMT registry included 135 patients who received *in vivo* T-cell depletion and 152 patients who did not with predominant use of RIC regimens. Patients either received ATG doses of 10 mg/kg or lower (Thymoglobulin®) or doses of 20 mg/kg or higher (Grafalon®). The cumulative incidence of grade II–IV acute GVHD was lower at 26% among patients who received ATG compared to 41% in patients who did not receive ATG. However, ATG did not decrease the risk of chronic GVHD and had no impact on disease-free survival, relapse-free survival and risk of relapse. The elevated pro-inflammatory biomarkers associated with MF particularly as the bone marrow may remain fibrotic up to three months following HSCT likely has an impact in increasing the risk of GVHD in patients with MF in general.⁵⁶ Moreover, the efficiency of ATG may in part be due to suboptimal lymphocyte counts targeted by ATG and usually noted in patients with MF receiving intensive chemotherapy. The study overall showed both a survival benefit as well as a protective effect against acute GVHD. However, further prospective studies need to be conducted in order to truly delineate the benefit of ATG in MF transplanted from HLA-matched related donor.

Poor graft function and failure

However, despite improvements in patient selection, timing of transplant and conditioning strategies, poor graft function (PGF) remains one of the main obstacles for a successful HSCT. PGF is defined as a persistent severe neutropenia, together with an anaemia and/or thrombocytopenia. It may occur in 2–27% of MF patients after HSCT.⁵⁷ In contrast with graft failure, the chimaerism is full donor in patients with PGF. Post-transplant CD34⁺-selected stem cell ‘boost’ can be effective in restoring normal graft function in this context, including in those patients who received haploidentical HSCT.⁵⁸ It allows haematopoietic recovery in the majority of patients with PGF without increasing the risk of GVHD and avoids the use of aggressive toxic strategies, such as a second transplant. In case of mixed chimaerism, new approaches to enable sustained engraftment, lower NRM and reduce relapse need to be developed in prospective trials to improve survival and quality of life of the patients.

In an effort to combat treatment-related mortality and NRM with the increasing use of non-myeloablative conditioning (NMA) and RIC regimens, additional consideration needs to be shed on graft failure which carries an incidence of 2–24% of patients undergoing HSCT for MF. In addition to the type of conditioning regimen utilized, other notable factors contributing to engraftment failure include alternative donor selection (mismatched unrelated donor, cord blood donor or haploidentical sources), inadequate number of CD34⁺ cells infused, negative donor/recipient cytomegalovirus serostatus, degree of splenomegaly and degree of fibrosis and thrombocytopenia prior to transplant.^{39,59,60} In an effort to address one of these potential sources contributing to engraftment failure, Slot *et al.* conducted a retrospective review of 53 patients with MF who had undergone HSCT at three different centres in the Netherlands; patients were evaluated and found to have higher frequency (28%) of graft failure within 60 days primarily associated with conditioning regimen.⁶¹ Neutrophil engraftment at 60 days was lower (56%) in patients who received NMA than in patients who received RIC (84%). Moreover, in the six patients who underwent a second HSCT after graft failure, use of NMA again resulted in graft failure whereas the use of RIC in the second HSCT resulted in successful engraftment. They concluded the use of a more intensive conditioning regimen that incorporates busulfan or melphalan to be optimal preventative strategies in addressing graft failure.

Role of post-transplant ruxolitinib

While there is a clear established role of ruxolitinib in improving outcomes prior to transplant, data regarding the utility of ruxolitinib as post-HSCT maintenance are sparse. In a pilot study conducted by Pu *et al.*, a retrospective review evaluated the feasibility and toxicity of ruxolitinib both pre-HSCT as well as post-HSCT maintenance regimens for MF.⁶² In this cohort, pre-HSCT ruxolitinib was titrated from 10 to 20 mg twice daily and tapered off from five days before initiating conditioning regimen. Post-HSCT dose was 5 mg twice daily, starting after absolute neutrophil count (ANC) reached $0.5 \times 10^9/l$ for three consecutive days. Median duration of ruxolitinib was eight months prior to HSCT and 20 months maintenance regimen post HSCT. Out of four patients who received ruxolitinib both pre and post HSCT, two experienced herpes zoster and cytomegalovirus (CMV) viraemia and one experienced *Clostridium difficile* colitis. All four patients in this cohort also achieved CR at a median of 11.5 months and showed a significantly superior acute GVHD profile with zero patients succumbing to acute GVHD. Not only could ruxolitinib at 5 mg twice daily post HSCT serve as a potential novel approach for GVHD prophylaxis, it can also potentially achieve a greater degree of spleen size reduction, improved resolution of fibrosis as well as rapid engraftment although further large-scale prospective studies are needed prior to implementation as standard of care.

Role of post-transplant cyclophosphamide

The use of post-transplant cyclophosphamide has revolutionized the realm of haploidentical and unrelated allogeneic HSCT in decreasing the incidence of acute and chronic GVHD. The implementation post transplant of cyclophosphamide (PTCy) is particularly appealing with sparse data in the setting of MF. A recent prospective pilot study in Italy evaluated GVHD prophylaxis with PTCy and ruxolitinib in 20 patients with primary or secondary MF.⁶³ Patients received cyclophosphamide 50 mg/kg/day IV once daily on Day +3 and Day +4 as well as ruxolitinib 15 mg three times a day on Days –8 through –2 and ruxolitinib 7.5 mg twice a day on Days +5 through Day +100. All patients received RIC regimen followed by HSCT from related ($n = 7$) or unrelated ($n = 13$) donors. In this cohort, 17 achieved engraftment whereas two died prior to engraftment and one experienced primary graft failure. GVHD prophylaxis with PTCy and ruxolitinib was associated with low toxicity profile (30% experienced grade 3–4 non-haematological toxicity, 45% viral reactivation, 15% severe sepsis), low relapse incidence and decent acute and chronic GVHD control. Two-year NRM, OS and event-free survival were 15%, 85% and 72%, respectively. A significant number ($n = 11$) experienced severe PGF which could possibly be mitigated in the future with dose reduction of ruxolitinib.

Moreover, another recent study also explored the utilization of both ATG and PTCy in patients with MF who underwent HSCT with peripheral blood as the graft source in an effort to improve post-transplant outcomes.⁶⁴ All patients received RIC regimen with ATG, PTCy and ciclosporin. Prior to June 2018, 29 patients received a total dose of 4.5 mg/kg of rabbit-ATG administered on three consecutive days combined with PTCy (50 mg/kg/day for two days on Days +3 and +4) and ciclosporin since Day +5. After June 2018, eight patients received a reduced dose of ATG, from 4.5 mg/kg to a total dose of 2 mg/kg administered on two consecutive days. Utilization of both ATG and PTCy achieved a high OS (74.4% at one year), RFS (71.3% at one year) and a low incidence of acute and chronic GVHD. A total of six patients experienced graft failure. While the ideal ATG dose has not been established, the study did find a non-significant lower incidence of CMV and Epstein–Barr virus reactivation in patients who had received a lower dose of ATG. The goal of the reduced dose was also to reduce NRM and graft failures while preserving GVHD prophylaxis in combination with PTCy.

Relapse post-allogeneic HSCT

Despite advances in allogeneic HSCT which can offer a cure rate of 30–65% in patients with MF, risk of relapse remains a consistent barrier. In patients who have experienced relapse following HSCT, early withdrawal and reduction of post-transplant immunosuppression, administration of donor lymphocyte infusion (DLI) and a second HSCT have been

suggested. DLIs have been successfully used for mixed chimaerism or relapse, either pre-emptively or as salvage therapy.^{65,66} Use of DLI has been established as a safe and effective immune-adaptive strategy in patients with residual disease guided by use of polymerase chain reaction (PCR)-based monitoring of JAK2 V617F and/or chimaerism studies. In a retrospective study conducted by Klyuchnikov *et al.*, 26 patients received a median of three (range, 1–5) DLIs in a dose-escalating mode starting with median dose of 1.2×10^6 up to a median dose of 40×10^6 T cells/kg and found that 10/26 patients achieved a complete response to DLI.⁶⁶ Non-responders to DLI proceeded to a second allogeneic transplant. While DLI for patients with residual disease can be considered, there are little data on the use of DLI in a haploidentical setting for MF and combining immunomodulation with maintenance therapy after HSCT is still under investigation.

There are sparse data on the use of a second allogeneic HSCT following relapse in MF with current literature limited to case reports and smaller cohort studies.⁶⁷ A more recent study, however, specifically focused on the use of a treosulfan-based conditioning regimen in 33 patients with MF with relapse after HSCT and failure of DLI. Each patient received treosulfan dosed at 36–42 mg/m² in combination with fludarabine and ATG for a second HSCT.⁶⁸ All patients in this cohort achieved successful leukocyte engraftment by a median of day 11 and approximately 56% experienced acute grade II–IV GVHD at day 100. Overall, patients experienced a decent toxicity profile, disease-free and OS at five years.

Conclusion

Over the past decade, with an expansion of our understanding of the biology and pathophysiology of the disease course in MF, we have been able to prognosticate various clinical-, patient-related and molecular components of defining the risks of patient's disease course as well as outcomes following HSCT. Certain mutations, particularly *ASXL1*, or triple-negative disease status, confer a higher risk of relapse and mortality and patients exhibiting such features should be considered for early transplant. Moreover, the presence of clonal evolution and lack of response to JAK inhibitors are also considerations for early transplantation while taking into account patient comorbidities, clinical symptoms and quality of life. In terms of future direction, clinical trials including NCT03427866 (clinicaltrials.gov) are currently under way to evaluate the utility of post-HSCT maintenance therapy with agents including ruxolitinib for primary and secondary myelofibrosis.

Author contributions

MS, RD and MM performed the bibliographic search and wrote the first version of the manuscript. All other authors contributed to the design, helped writing, editing and revising this manuscript.

Conflict of interest

MM reports grants and/or lecture honoraria from Janssen, Sanofi, Maat Pharma, JAZZ pharmaceutical, Celgene, Amgen, BMS, Takeda, Pfizer, Novartis, and Roche (all outside the submitted work). RD reports lecture honoraria from Keocyt, Sanofi and Novartis (all outside the submitted work). FM reports lecture honoraria from Therakos/Mallinckrodt, Biocodex, Janssen, Keocyt, Sanofi, JAZZ pharmaceutical and Astellas (all outside the submitted work). AB reports grants and/or lecture honoraria from Novartis, Roche, Janssen, Takeda, Sanofi, JAZZ pharmaceutical, Celgene, Amgen, and Pfizer (all outside the submitted work).

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