

### Mixed myeloid chimerism and relapse of myelofibrosis after allogeneic stem cell transplantation

Allogeneic stem cell transplantation (allo-SCT) remains the only potentially curative treatment for myelofibrosis (MF).<sup>1,2</sup> The 5-year overall survival (OS) rates in MF patients after allo-SCT have improved over the past several years, ranging from 47% to 62%.<sup>3-7</sup> However, relapse after transplantation remains a frequent cause of death, with relapse rates ranging from 10-43% after allo-SCT.<sup>1,8</sup> Strategies for detecting early relapse and improving outcomes in these high-risk patients represent an unmet need.

Assessing response and confirming early relapse after transplant for MF is often challenging based on the clinical criteria,<sup>9</sup> and it usually takes a few months for fibrosis to resolve and the bone marrow morphologic remission to be achieved.<sup>10</sup> Detecting the *JAK2* V617F mutation after SCT in MF is a strong predictor of relapse and a potential marker for guiding adoptive immunotherapy.<sup>7,11-13</sup> Few studies have focused on the potential role of mixed chimerism in predicting early relapse, particularly in those with mixed myeloid chimerism (MMC).<sup>14-18</sup> The influence of MMC on posttransplantation relapse of MF has not been well studied. Therefore, we aimed to determine the role of MMC in predicting relapse in MF patients after allo-SCT. We further explored the correlation between myeloid chimerism and molecular relapse of MF.

Eighty-two consecutive patients with primary or secondary MF who underwent their first allo-SCT at The University of Texas MD Anderson Cancer Center from January 2005 to July 2015 were identified. Patients with double cord or haploidentical donors or with primary graft failure were excluded. MF relapse was defined as any evidence of persistent or recurrent morphologic disease. Molecular relapse was defined as any patient with persistent and/or reappearance of pretransplant molecular genetic abnormalities (*JAK2* V617F, *CALR* and *MPL*). In this cohort, all patients with molecular relapse had evidence of morphologic persistent and/or relapsed bone marrow disease, except for one patient. Patients were determined to have MMC if they had less than 95% of donor myeloid cells at any time after day 30 after transplantation. This study was approved by the institutional review board.

Chimerism testing was performed using eight highly polymorphic microsatellite markers. It included lineage-specific analysis *via* separation of myeloid cell and T-lymphocyte populations as described previously.<sup>19</sup> For the majority of patients, peripheral blood chimerism testing and molecular testing for MF were performed routinely per institutional policies at months 1, 3, 6, 9, 12, 18, 24, and 36 after transplant, and more frequently as indicated at the discretion of the treating physician.

The primary end point was the frequency of MMC among patients with evidence of morphologic and/or molecular relapse. Secondary end points were the progression-free survival (PFS) and OS rates. Treatment responses were defined as described previously.<sup>20</sup> Additionally, molecular remission was defined as *JAK2* V617F or *CALR/MPL* negativity in patients previously positive for them. Survival estimates were calculated for all patients and according to myeloid chimerism status using the Kaplan-Meier method

Forty-four of the 82 patients were male, and the median age at allo-SCT was 57.5 years. Forty-seven patients (57%) were positive for a molecular marker before trans-

**Table 1.** Patient, disease, and transplant characteristics of study cohort.

Number of patients	82
Sex	
Male	44
Female	38
Median age in years at transplant (range)	57.5 (27-74)
Diagnosis	
Primary MF	53
Secondary MF	25
Other MPN	4
Molecular mutation status	
<i>Jak2</i> V617F positive	41
<i>CALR</i> positive	5
<i>MPL</i> positive	1
Not available	5
Donor type	
Sibling	38
Unrelated	44
Source of stem cells	
Marrow	10
Peripheral blood	72
Conditioning regimen	
Myeloablative	66
Reduced intensity	16
Mixed myeloid chimerism	
Yes	35
No	47

MF: myelofibrosis; MPN: myeloproliferative neoplasm.

plantation. Thirty-five patients (43%) developed MMC, of which 24 patients received a myeloablative conditioning regimen. Twenty-nine of these 35 patients had initial full donor myeloid chimerism after transplant before they developed MMC. Table 1 summarizes the patient, disease, and transplant characteristics of study population.

During the study period, a total of 34 patients (41%) had persistent or relapsed disease after SCT. The flow charts in Figure 1 and the *Online Supplementary Figure S1* show the study patients and their disease outcomes according to the MMC status and by molecular status. Only one patient with full donor myeloid chimerism (n=47) experienced disease progression during the study period (Figure 1). In contrast, all but two patients with MMC had morphologic and/or molecular relapse either at the time when MMC was detected or soon afterwards. When we analyzed the study patients with relapsed disease (n=34), all but one patient had MMC.

Among the 47 patients with pretransplant positive molecular marker, 21 patients developed MMC of whom 95% (n=20) had concomitant molecular relapses. The exception was a patient with complete conversion to full chimerism after immunosuppression reduction. Similarly, for the 30 patients with negative molecular testing before transplantation, 13 of 14 patients (93%) with MMC eventually experienced morphologic relapse. The exception was a patient with the successful conversion to full donor chimerism after immunosuppression reduction.

The *Online Supplementary Table S1* and *Online Supplementary Figure S2* summarize the patient characteristics, disease outcomes, and interventions done of the 35 patients with MMC. The most common cause of death for these patients was persistent/recurrent disease. Thirteen of the 18 deaths were attributed to progressive disease, of which seven had transformed acute myeloid

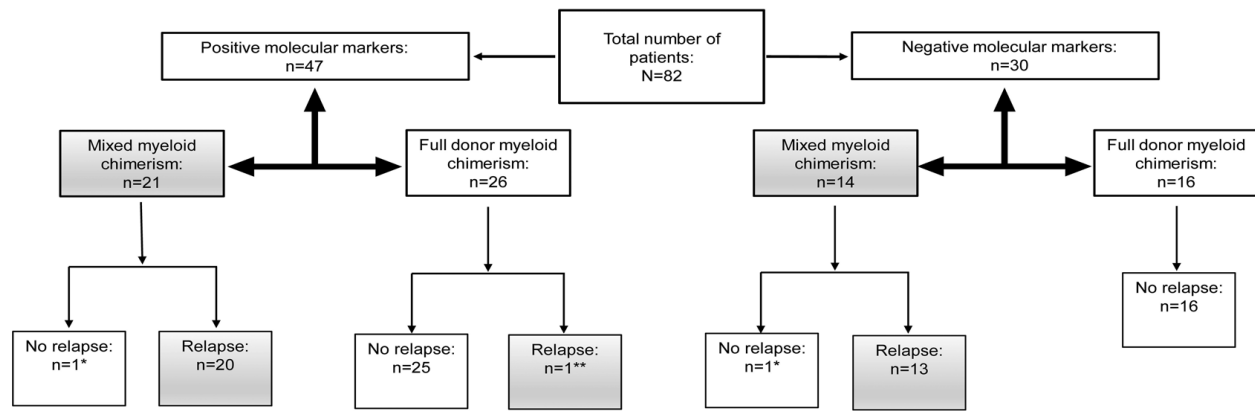


Figure 1. Flow chart of myelofibrosis patient cohorts and outcomes according to myeloid chimerism and molecular disease status. \*These two patients underwent immunosuppression reduction before any evidence of relapse. One was converted to full donor chimerism, and the other had progressive loss of donor myeloid chimerism and graft failure. \*\*This patient with mixed T-cell chimerism had a molecular relapse (recurrent *JAK2* mutation) that responded to a tacrolimus dose reduction (converted to full donor chimerism and had molecular remission).

leukemia and one patient had accelerated MF with 19% blasts. One patient died from complications of graft-versus-host disease (GvHD) (after immunosuppression reduction) and persistent disease. The other four patients died while in complete remission (CR); two patients achieved CR with immunosuppression reduction alone (died from GvHD complications), and one patient each achieved CR after donor leukocyte infusion and second allogeneic SCT (both died of second malignancy). For the 17 surviving patients, nine responded to immunosuppression reduction alone and converted to full donor myeloid chimerism and complete remission, seven patients were salvaged with second allogeneic SCT, and one patient was not evaluable at time of last follow-up.

The majority of the patients underwent molecular testing for both chimera and clonal molecular markers on the same date. MMC and molecular relapse were concurrently detected in all but five patients. Among these five patients, molecular relapse was preceded by MMC in three patients, and one patient had an initially positive *JAK2* V617F molecular relapse followed shortly by MMC. The fifth patient did not undergo concomitant clonal molecular testing at the initial presentation when found to have MMC but had a molecular relapse 6 months later.

We assessed survival in the whole patient group and according to MMC status. With a median follow-up of 49 months (range: 3-105), the 4-year PFS and OS rates in all study patients were 32% and 51%, respectively. When stratified according to chimerism status, the 4-year PFS rate was 4% in those with MMC versus 60% in those with full donor myeloid chimerism ( $P < 0.0001$ ) (Online Supplementary Figure S3). Similarly, patients with MMC had a 4-year OS of 47% compared to 59% in those with full chimerism. However, this difference was not statistically significant, likely because of the small sample size and salvage treatment interventions.

Highly sensitive molecular testing is increasingly being used for early detection of relapse and guidance of therapies. Approximately 60% of patients with MF harbor the *JAK2* mutation.<sup>21</sup> Other molecular markers (MPL and CALR) are increasingly being used, but not yet validated as strong predictors of relapse after allo-SCT. Hence, the need for a better universal marker to predict relapse because morphologic findings are not helpful in many

cases. Loss of donor chimerism has long been correlated with increased relapse incidence after allo-SCT in various hematologic neoplasms. Furthermore, Thiede *et al.*<sup>17</sup> proved that MMC not only predicts clinical relapse of chronic myeloid leukemia but is also associated with reappearance of BCR-ABL1 translocation transcripts.

The present study is one of the largest to demonstrate a strong association between MMC and morphologic and molecular relapse in patients with MF. The finding in a few patients that loss of myeloid chimerism may precede early molecular relapse is worth further investigation. Patients who never developed MMC rarely relapse. We propose that myeloid chimerism testing alone or in combination with other clonal molecular markers can be one of the earliest and most accurate methods of predicting relapse, particularly early after transplant where clinical and morphologic bone marrow findings are frequently not useful in confirming relapse. Our findings suggest the unmet need for a revised definition of MF relapse after allogeneic SCT to account for the role of MMC and molecular data in the posttransplant setting. Early intervention with immunosuppression reduction alone in this high-risk population with MMC is feasible and worth further investigation.

Samer A. Srouf,<sup>1</sup> Amanda Olson,<sup>1</sup> Stefan O. Ciurear,<sup>1</sup> Parth Desai,<sup>2</sup> Qaiser Bashir,<sup>1</sup> Betul Oran,<sup>1</sup> Prithviraj Boser,<sup>3</sup> Rohit Mehtar,<sup>1</sup> Keyur P. Patel,<sup>4</sup> Naveen Pemmarajur,<sup>3</sup> Naval Daver,<sup>3</sup> Srdan Verstovsek,<sup>3</sup> Richard E. Champlin<sup>1</sup> and Uday R. Popat<sup>1</sup>

<sup>1</sup>Department of Stem Cell Transplantation and Cellular Therapy, The University of Texas MD Anderson Cancer Center, Houston;

<sup>2</sup>Department of Medicine, The University of Texas Health Science Center at San Antonio, San Antonio;

<sup>3</sup>Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston and

<sup>4</sup>Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Correspondence: UDAY R. POPAT

upopat@mdanderson.org

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