# Donor cell-derived myelofibrosis relapse after allogeneic stem cell transplantation

Primary myelofibrosis (PMF) is a rare myeloproliferative neoplasm characterized by clonal proliferation of mature myeloid lineages derived from stem cells (erythrocytes, leukocytes and magakaryocytes) with variable megakaryocyte atypia associated with reticulin and / or collagen bone marrow (BM) fibrosis, osteosclerosis, ineffective erythropoiesis, angiogenesis, extramedullary hematopoiesis and abnormal expression of cytokines.

Allogeneic hemopoietic stem cell transplantation (alloHSCT) is currently the only curative approach for patients with myelofibrosis, and for this reason the number of allografts for these indications have been growing over the past years.

Unfortunately relapse of myelofibrosis (MF) after an alloHSCT occurs in 10-40% of cases: patients usually present with a declining donor chimerism, and a reappearance of driver mutations if present; BM biopsy is usually consistent with typical megakaryocyte abnormalities and stromal fibrosis. Ultimately BM cells exhibit progressive loss of donor chimerism, and the relapse is therefore of recipient origin. Here we report two allografted MF patients who relapsed in donor cells.

**Case 1**: A 58-year-old male patient with triple negative myelofibrosis, heavy transfusion burden and with no hematological malignancies present in the family. He was transplanted with BM hematopoietic stem cells from his 32-year-old haploidentical son in February 2016. The conditioning used was thiotepa 10 mg/kg, busulfan 6.4 mg/kg and fludarabine 150 mg/m$^2$ (TBF). Graft-*versus*-host disease (GvHD) prophylaxis was post-transplant cyclophosphamide (PTCY) 50 mg, cyclosporin A and mycophenolate. The patient engrafted and exhibited 100% donor chimerism, without GvHD. His blood counts were normal thereafter. In January 2019 he experienced a gradual decline in blood counts and a BM biopsy was unremarkable. A new BM biopsy in March 2019 showed initial return of BM fibrosis with megakaryocyte abnormalities: chimerism was full donor (100% donor). In January 2020 a BM biopsy showed an evident return of fibrosis and megakariocyte abnormalities; the patient was anemic and thrombocytopenic and required red cell and platelet transfusions again. He received a second alloHSCT from another haploidentical donor, but he remained cytopenic: chimerism showed full second donor chimerism on day +60, but then there was a gradual return to first donor chimerism and relapse. He died of progressive disease in March 2022. Therefore the (first) donor relapse dominated also after the second transplant.

Next-generation sequencing (NGS) was performed before transplant and identified a mutation in *SF3B1* (variant allele frequency [VAF] 8%; at relapse the latter was not present, but NGS identified a polymorphism in *IDH2* c.315C>T, p.(Gly105=) which was not present prior to transplant.

**Case 2**: A 60-year-old female patient with *CALR1*-positive MF, with complex cytogenetics (del6, del7, del13). A first degree relative had chronic lymphocytic leukemia (CLL) and a second degree relative had acute myeloid leukemia (AML). She received peripheral blood alloHSCT from her 48- year-old HLA-identical sister, in September 2018, with the same conditioning regimen (TBF) and GvHD prophylaxis as in case 1. Engraftment was rapid and complete: 6 months later the patient showed 100% donor chimerism: *CALR1*-negative, 46 XX. In March 2021 the patient became pancytopenic, with elevated lactate dehydrogenase >1,000 IU/L, and a BM biopsy showed relapse of myelofibrosis with MF3. The patient remained *CALR1*-negative and showed 100% donor chimerism and normal 46 XX cytogenetics. She underwent a second alloHSCT from an unrelated donor and died of transplant-associated microangiopathy.

NGS before transplant identified *CALR* as the only mutation which was not present at relapse. Donor chimerism was studied in unfractionated BM mononuclear cells (MNC), and in colony-forming unit granulocyte/ erythroid/ macrophage/ megakaryocyte colonies (CFU-GEMM), granulocyte macrophage colonies (CFU-GM), and erythroid colonies (CFU-E) isolated from peripheral blood. In addition, we analyzed the donor chimerism on mesenchymal cells from BM and in circulating endothelial progenitor cells. Results from these studies highlighted a full donor chimerism in MNC BM cells, CFU-GM, CFU-GEMM, CFU-E and CD3+ selected T cells. Endothelial and mesenchymal cells remained of recipient origin (Figures 1 and 2).

Endothelial colony-forming cell (ECFC) assay was performed according to Ingram *et al.*[1] After 2 days, non-adherent cells were removed and residual adherent cells were grown in EGM-2 medium for 21 days, with medium replacement every 3 days. Well-circumscribed monolayers of cells with cobblestone appearance, growing from day 9 to day 21, were counted as ECFC. Mesenchymal stromal cells (MSC) were isolated from BM samples as previously described.[2]

CFU assay on peripheral blood and BM MNC were performed as previously reported. After 2 weeks, colonies were enumerated according to their morphology as burst-

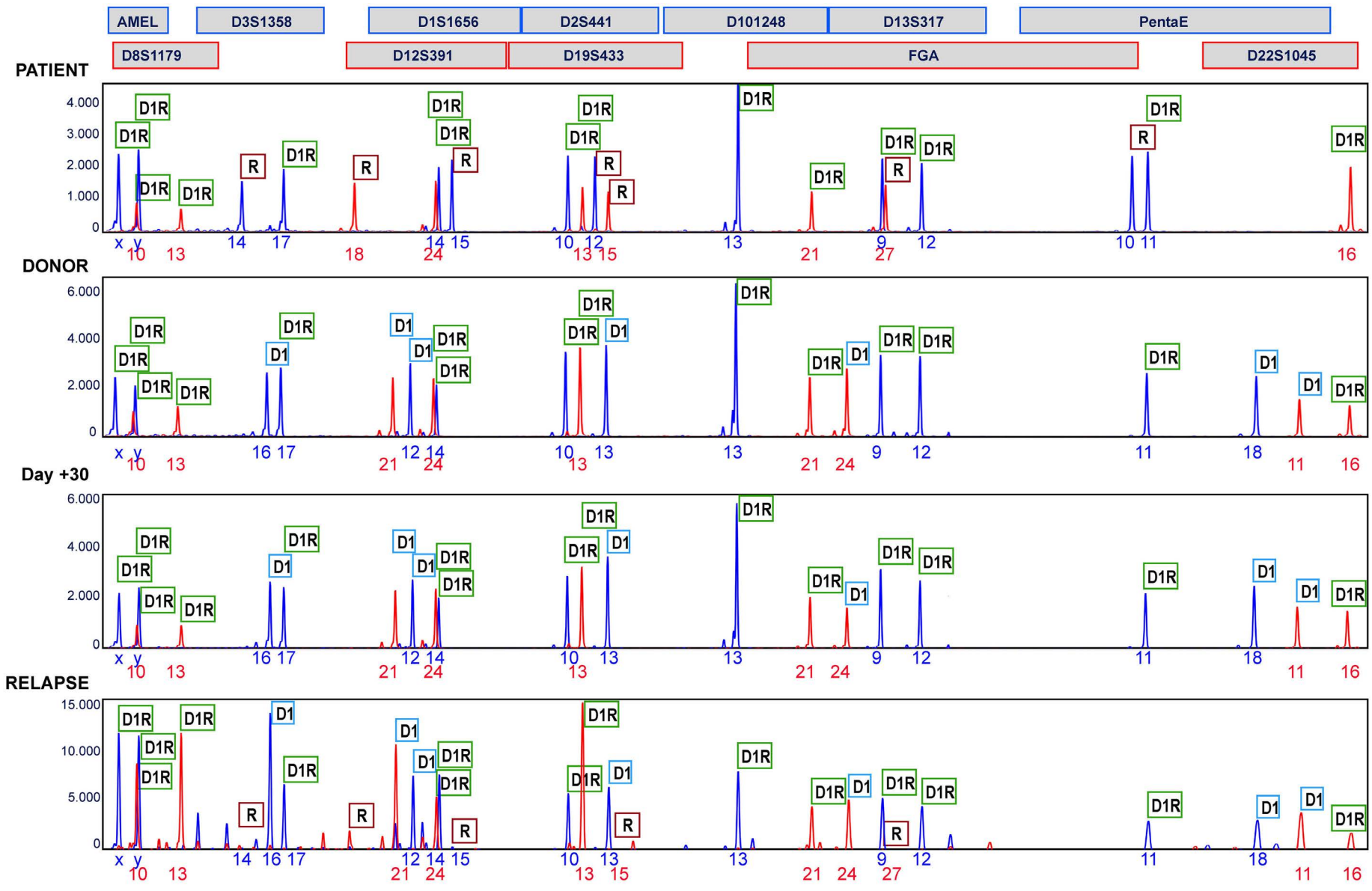


**Figure 1. Chimerism analysis of donor and patient 1 before transplant.** After transplant (day +30) the proportion of donor chimerism was 98,9% on 12 evaluable microsatellites. At the time of relapse the proportion of donor chimerism on unfractionated bone marrow cells was 98.1% on 12 evaluable microsatellites.

forming unit erythroid (BFU-E), CFU-GM and CFU-GEMM.[3] The different CFU were collected and stored at -20°C for chimerism studies.

We compared the chimerism pattern in all donor and patient samples: patients' and donors' baseline, follow-up after transplant, at relapse, on CFU-GM, CFU-GEMM, ECFC and MSC. As previously described, chimerism was assessed by polymerase chain reaction analysis of short tandem repeats (STR). The proportion of donor recipient chimerism was calculated using the PowerPlex Fusion System (Promega srl, Italy) on 24 STR loci. Full-donor chimerism (F-DC) was defined as having >95% donor alleles.[4]

Recurrence of disease in donor cells have been described as a rare entity in acute leukemia. Several characteristics have been described like lower incidence in sex-matched transplantation, variable latency between alloHSCT and recurrence of disease, higher prevalence in transplant from family donors as compared from unrelated donors, a donor age older than the recipient, conditioning regimen including TBI.[5] A survey from the European Society of Blood and Marrow Transplantation has estimated a prevalence of donor relapse of 80.5 cases per 100,000 transplants, and a cumulative incidence of 0.067%, 0.132% and 0.363%, respectively, at 5, 10, and 25 years after alloHSCT,[6] but perhaps the real incidence of relapse in donor cells is underestimated. Its diagnosis depends on the demonstration of the donor origin of neoplastic cells. Due to the heterogeneity of the diseases the most used approach is the indirect demonstration of donor derivation through the confirmation of full-donor chimerism performed by STR analysis. This approach has some limitations related to the low sensitivity of the method. In fact despite its applicability to both sex-matched and -mismatched transplants, in practice, the co-amplification of alleles generally reduces the detection sensitivity of current STR polymerase chain reaction techniques to almost 1% of the minor component. Therefore, STR would not be able to detect residual recipient hematopoiesis, but a morphological relapse involves, if not the totality, a large proportion of cells in excess of the detection limit of STR and is, hence, easily detectable by STR.

We present here two cases of MF who relapsed in donor cells. The marker used to identify donor cells, was chim-

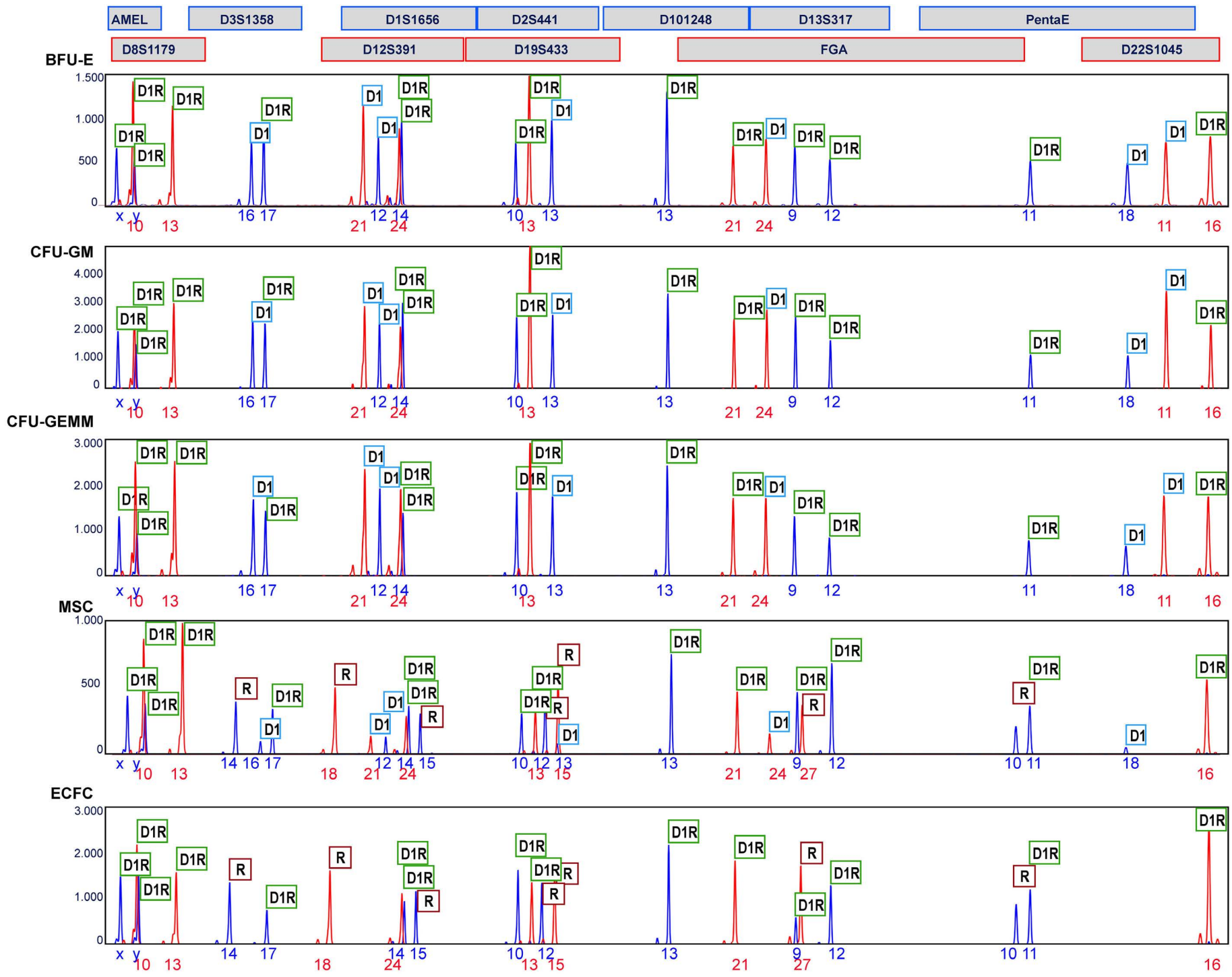


**Figure 2. Chimerism analysis of *in vitro* colonies from patient 1.** Donor short tandem repeats (STR) were detected in different cell colonies (CFU-GM 99.7%, CFU-GEMM 99.4%, BFU-E 99.6%, and CD3+ 98.1% [not shown]). The proportion of donor STR in endothelial colony-forming cell (ECFC) colonies was 1.8% and 2.8% for mesenchymal stromal cell (MSC) colonies. CFU: clony-forming unit; CFU-GM: CFU granulocyte macrophage; BFU-E: burst-forming unit erythroid; CFU-GEMM: CFU granulocyte/ erythroid/ macrophage/ megakaryocyte.

erism performed by STR; indeed the first patient was a triple-negative MF, and the second patient relapsed without the original *CALR* mutations or the original complex cytogenetics. We could prove 100% donor chimerism in all cell lineages grown in cell culture (GM, GEMM, erythroid), and in CD3+ selected cells, whereas mesenchymal and endothelial colonies remained of recipient origin. Both patients had a hematologic relapse, with systemic symptoms and progression, which ultimately lead to a fatal outcome. Both donors remain healthy with normal blood counts years after donation.

The pathogenesis of relapse in donor cells remains poorly understood: genetic predisposition, viral transfection, a permissive BM microenvironment and defective immunosurveillance, have all been hypothesized. A genetic predisposition could be the reason for the relapse in the second patient who had two relatives with leukemia. In addition, in the setting of MF, an intriguing factor could be an abnormal microenvironment, leading to a neoplastic transformation of donor hematopoietic stem cells. Epigenetic changes in the BM microenvironment, which lead to β-catenin activation and disease progression of MDS, have been described.[7] Aldoss *et al.*[8] speculated that the BM microenvironment plays an important role in maintaining homeostasis of hematopoietic stem cells. Moreover preclinical studies have demonstrated that changes in marrow BM mesenchymal stem cells, stromal cells, endothelial cells, adipocytes, osteogenic cells, and osteoblasts, have been associated with the onset or progression of leukemia, as well as with chemotherapy

resistance.[9-13] Some studies have shown *JAK2*-positive endothelial cells,[14] while others have not,[15] suggesting we still do not understand the cause-effect relationship of the microenvironment and the myeloproliferative neoplasm. In our two patients the microenvironment was of host origin. Thus a defective (permissive) microenvironment may induce or allow leukemic transformation also in healthy normal donor cells.

To our knowledge, these are the first two MF patients reported with a relapse in donor cells, compared to several cases reported for acute leukemia.[6] One reason for the discrepancy could be that MF remains a rare indication (2-5% of allogeneic transplants), and, with a 0.13% incidence of donor relapse at 10 years, the number of long-term surviving MF patients, may still be not numerous enough. On the other hand, the number of allografted MF patients is rapidly increasing, and more cases may be detected in the future: for this reason we should continue to study chimerism, in case of reappearance of driver mutations or hematologic relapse in MF after an alloHSCT.

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**Contributions**

PC, LT and AB designed the work. NO, SG, MR and LMR performed research and analyzed data. EM, SL, GM, CGV, MB, FF, CP, FS and SS treated the patients. PC, LT and AB wrote the paper and all co-authors critically reviewed the manuscript and gave their final approval.

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**Data-sharing statement**

Data regarding these two patients, are available on request to the corresponding author.

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