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Donor cell-derived myelofibrosis relapse after allogeneic stem cell transplantation

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Data sharing: data of these 2 patients, are available to share , on request to the corresponding Author.
To the Editor

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

Allogeneic hematopoietic stem cell transplantation (alloHSCT) is currently the only curative approach for patients with myelofibrosis, and for this reason the number of allografts for this indication 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; a bone marrow (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, and heavy transfusion burden. No hematological malignancies in the family. He was transplanted with bone marrow 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) 50mg/kgx2, 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 bone marrow (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 evident return of fibrosis and megakaryocyte abnormalities; the patient was anemic and thrombocytopenic and required again red cell and platelet transfusions. 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 (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 pre-transplant.
Case 2: A 60 year old female patient with myelofibrosis CALR1+, 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 that patient showed 100% donor chimerism. CALR1 negative, 46 XX. In March 2021 the patient became pancytopenic, with elevated LDH >1000 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, but she died early 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 bone marrow mononuclear cells (MNC), and in CFU-GEMM colonies, granulocyte macrophage (GM) colonies, and erythroid colonies (CFU-E) isolated from peripheral blood. In addition, we analyzed the donor chimerism on mesenchymal cells from bone marrow 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 (Fig. 1 and 2).

Endothelial Colony Forming Cells (ECFC) assay was performed according to Ingram et al. 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 cobblestone-appearing cells, growing from day 9 to day 21, were counted as ECFCs. Mesenchymal stromal cells (MSC) were isolated from BM samples as previously described.

Colony forming unit (CFU) assay on PB and BM MNCs, were performed as previously reported. After weeks, colonies were enumerated according to their morphology as burst forming unit-erythroid (BFU-E), CFU-granulocyte/ macrophage (CFU-GM) and CFU-granulocyte/ erythroid/ macrophage/ mega-karyocyte (CFU-GEMM). The different CFUs were collected and stored at -20°C for chimerism studies.

We compared the chimerism pattern in all donor and patients' 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 PCR 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.

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 disease, higher prevalence in transplant from
family donors as compared from unrelated donors, a donor age older than the recipient, conditioning regimen including TBI. A survey from the European Society of Blood and Marrow Transplantation (EBMT) 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. But perhaps the real incidence of relapse in donor cells is underestimated. Its diagnosis depends on demonstration of 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 it is applicable to both sex-matched and -mismatched transplants, in practice, the coamplification of alleles generally reduces the detection sensitivity of current STR PCR 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.

We present here two cases of myelofibrosis who relapsed in donor cells. The marker used to identify donor cells, was chimerism performed by STR; 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 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 bone marrow microenvironment and defective immunosurveillance, have all been hypothesized. A genetic predisposition could be the reason for relapse in the second patient who had two relatives with leukemia. In addition, in the setting of myelofibrosis an intriguing factor could be an abnormal microenvironment, leading to a neoplastic transformation of donor hematopoietic stem cells. Epigenetic changes in the bone marrow microenvironment, which lead to β-catenin activation and disease progression of MDS, have been described. Aldoss et al speculated that the marrow microenvironment plays an important role in maintaining homeostasis of hematopoietic stem cells. Moreover preclinical studies have demonstrated that changes in marrow 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. Some studies have shown JAK2 positive endothelial cells, but others have not, suggesting we still do not understand the cause-effect relation 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 myelofibrosis remains a rare indication (2-5% of allogeneic transplants), and, with a 0.13% incidence of donor relapse at 10 years, the number of surviving MF patients long term, 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 studying chimerism, in case of reappearance of driver mutations or hematologic relapse in myelofibrosis after an allogeneic HSCT.
REFERENCES

**Legend to figures**

**Figure 1:** Chimerism analysis in donor and patient N.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 BM cells was 98.1% on 12 evaluable microsatellites.

**Figure 2:** Chimerism analysis in patient N.1, on in vitro colonies. Donor STR were detected in different cell colonies (CFU-GM-99.7%, CFU-GEMM-99.4%, BFU-E99.6%, and CD3 not shown - 98.1%). The proportion of donor STR in ECFC colonies was 1.8% and 2.8% for MSC colonies. (abbreviations in text).