were excluded because der(16)t(8;16) encoding *CBP-MOZ* was not generated in this three-way translocation. Translocation t(8;18;16)(p11;q21;p13), another three-way translocation variant of t(8;16)(p11;p13) found in a 15-month-old boy with AML M5b, also has a structure that could not generate *CBP-MOZ* but *MOZ-CBP*, while RT-PCR was not performed in this case.[7] Moreover, Murati *et al*. reported a 72-year-old man with AML M5a having a complex t(8;16)(p11;p13), in whom only *MOZ-CBP* but not *CBP-MOZ* was detected as expected because of the insertion of 8q material between 16p and 8p on the der(16).[2] These results clearly show that the *CBP-MOZ* fusion transcripts are not essential for the development of AML with t(8;16)(p11;p13) and its variants.

We find for the first time that *MOZ-CBP* fusion transcripts are expressed in a case of infant leukemia with a variant of t(8;16)(p11;p13), and the fusion transcripts were shown to be two novel variants. Although predicted *MOZ-CBP* fusion proteins lack a protein interaction domain, C/H1 (cysteine/histidine-rich domain 1), compared to previously reported ones, these fusion proteins retain most of the other functional domains of CBP, including the bromodomain and the HAT domain (Figure 2c) which were reported to be important for *MOZ-CBP* to inhibit the Runx1-mediated transcription and myeloid cell differentiation.[8] This suggests that the novel *MOZ-CBP* proteins are also leukemogenic and contribute to leukemic development in this case. While the reason for the favorable outcome of infant leukemia with t(8;16)(p11;p13) is not clear, secondary mutations may be required to develop aggressive disease as observed in adult patients. The difference of *MOZ-CBP* structures between infant and adult patients might also be related to the different outcomes. To clarify these questions, more cases of both infant and adult AML with t(8;16)(p11;p13) need to be analyzed.

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References

1. Schmidt HH, Strehl S, Thaler D, Strunk D, Sill H, Linkesch W, et al. RT-PCR and FISH analysis of acute myeloid leukemia with t(8;16)(p11;p13) and chimeric MOZ and CBP transcripts: breakpoint cluster region and clinical implications. Leukemia 2004;18:1115-21.
2. Murati A, Adélaïde J, Quilichini B, Rémy V, Sainty D, Stoppa AM, et al. New types of MYST3-CBP and CBP-MYST3 fusion transcripts in t(8;16)(p11;p13) acute myeloid leukemias. Haematologica 2007;92:262-3.
3. Sainati L, Bolcato S, Cocito MG, Zanesco L, Basso G. Transient acute monoblastic leukemia with reciprocal (8;16)(p11;p13) translocation. Pediatr Hematol Oncol 1996; 13:151-7.
4. Weintraub M, Kaplinsky C, Amariglio N, Rosner E, Brok-Simoni F, Rechavi G. Spontaneous regression of congenital leukaemia with an 8;16 translocation. Br J Haematol 2000;111:641-3.
5. Classen CF, Behnisch W, Reinhardt D, Koenig M, Möller P, Debatin KM. Spontaneous complete and sustained remission of a rearrangement CBP (16p13)-positive disseminated congenital myelosarcoma. Ann Hematol 2005; 84:274-5.
6. Schmidt HH. MYST3/CREBBP (MOZ/CBP) and CREBBP/MYST3 (CBP/MOZ) transcripts in AML with t(8;16)(p11;p13). Genes Chromosomes Cancer 2005;42: 207-8.
7. Mo J, Lampkin B, Perentesis J, Poole L, Bao L. Translocation (8;18;16)(p11;q21;p13). A new variant of t(8;16)(p11;p13) in acute monoblastic leukemia: case report and review of the literature. Cancer Genet Cytogenet 2006; 165:75-8.
8. Kitabayashi I, Aikawa Y, Nguyen LA, Yokoyama A, Ohki M. Activation of AML1-mediated transcription by MOZ and inhibition by the MOZ-CBP fusion protein. EMBO J 2001;20:7184-96.

Pulmonary extramedullary hematopoiesis in patients with myelofibrosis undergoing allogeneic stem cell transplantation

We examined the lung function of 11 patients with intermediate/high risk myelofibrosis undergoing allogeneic hematopoietic stem cell transplantation (HSCT). In 3 patients, chest computerized tomography (CT) scans revealed multiple pulmonary nodules with extramedullary hematopoiesis that disappeared after transplantation. Pulmonary extramedullary hematopoiesis in patients with myelofibrosis rapidly regresses after allogeneic HSCT.

Primary myelofibrosis (PMF) or myelofibrosis secondary to polycythemia vera (PV-MF) or essential thrombocythemia (ET-MF) are clonal myeloproliferative disorders often characterized by pancytopenia, bone marrow fibrosis, leukoerythrocytosis, teardrop poikilocytosis and splenomegaly.[1] Splenomegaly, in particular, is the result of extramedullary hematopoiesis. Due to the extramedullary hematopoiesis, patients with myelofibrosis can develop pulmonary hypertension secondary to hematopoietic infiltration, portal hypertension, thrombocytosis, hypercoagulability, and left ventricular failure.[2,3] Radiographic findings such as ground glass appearance, effusions, septal thickening on chest computerized tomography have been also described in PMF patients.[4] In patients with intermediate/high risk myelofibrosis,[5] allogeneic hematopoietic stem cell transplant (HSCT) is the only known curative therapy and the development of reduced intensity conditioning has allowed a decrease in transplant related mortality while inducing long-term remission, especially in older patients[6-9] In particular, HSCT can restore a normal hematopoiesis, and allows the resolution of marrow fibrosis[10] as well as the progressive reduction of splenomegaly.[11]

In this study, we evaluated the chest computerized tomography (CT) and pulmonary function tests (PFTs) of 11 consecutive patients with primary myelofibrosis (n=5), PV-MF (n=3), or ET-MF (n=3) who received an allogeneic hematopoietic stem cell transplantation (HSCT) to assess the presence of pulmonary extramedullary hematopoiesis. An informed consent was signed prior to transplant and the transplants were performed according to the protocol approved by the University of Illinois at Chicago Institutional Review Board. All patients received an HLA matched graft from related (n=7) or unrelated (n=4)

donors. Prior to transplantation, patients underwent cardiac and pulmonary testing in addition to other standard laboratory testing. Cardiac testing was performed by either a transthoracic echocardiogram (TTE) or by nuclear multiple gated acquisition scan (MUGA). Pulmonary evaluation was made by CT of the chest and functional tests. In particular, values of corrected diffusion limiting capacity of oxygen (DLCO), forced expiratory volume in one second (FEV1), forced vital capacity (FVC), and FEV1/FVC ratio were determined within 30 days prior to transplant. Patients were conditioned with a fludarabine based regimen including melphalan (n=10) or targeted i.v. busulfan (n=1) as previously described[12] Graft-versus-host disease (GVHD) prophylaxis consisted of standard methotrexate and tacrolimus (started on day -2). Tacrolimus levels were maintained between 5 and 15 ng/mL until day +180 unless GVHD or recurrence of disease occurred. A total of 4 patients who received a transplant from unrelated donors received thymoglobulin (rabbit antithymocyte globulin, ATG, Genzyme Inc.) at 7 mg/kg total dose as additional GVHD prophylaxis on day -3 to day -1. Myeloid growth factors were not used in the peri-transplant setting. Post transplantation, patients' pulmonary function was re-evaluated at three, six, and 12 months. Of 11 patients, 3 (27%) showed bilateral small pulmonary nodules, which varied in size from 5 mm to 15 mm by chest CT. The nodules were contrast enhancing and were located diffusely throughout the lungs. Due to the limited size of the nodules and the lack of symptoms suggestive of an infectious etiology, in 2 patients biopsy was not performed. In one patient, who showed a progressively increasing number of these nodules in the two months prior to transplant, an open biopsy of one nodule was performed to rule out an infectious or extra-hematologic neoplastic process (Figure 1A). The biopsy revealed areas of hematopoiesis with scattered megakaryocytes consistent with extramedullary hematopoiesis (Figure 1B). We then analyzed the pulmonary function testing in all the 11 patients in this study. The analysis of pulmonary function in the 3 patients with nodules in the lung showed a reduced DLCO (Figure 1C, left panel) (p=0.04) and a trend for reduced FEV1 and FEV1/ FVC ratio (Figure 1C, central and right panel) compared to the other 8 patients. The age of the patients also correlated with DLCO values since 4 out of 4 patients (2 with nodules) older than 60 years of age had a median DLCO of 56% (range 52-59%) compared to 7 patients younger than 60 years (one with nodules) who had a median DLCO of 72% (range 59-83) (p=0.006). However, older patients did not have a significantly longer duration of disease compared to the younger ones (p=0.2). None of the patients had clinical symptoms of pulmonary hypertension and a transthoracic echocardiogram (TTE) was performed in 7 of them showing a median pulmonary systolic pressure of 24 mmHg (range:20-34). We analyzed whether the degree of splenomegaly in the 3 patients with nodules differed from that of the patients without nodules. The patients with pulmonary nodules had extensive splenomegaly with spleen size of 25, 30.5, and 34 cm. In the 8 patients without pulmonary nodules or pulmonary disease, the median spleen size was 21 cm (range 12.5-34 cm) (n=7 patients) (p=0.2) with one patient who had previously undergone splenectomy. Lung CT scan was repeated within three months after an allogeneic HSCT and the nodules resolved in all of the 3 patients. This also correlated with a reduction in spleen size, which occurred in all the 10 patients with initial splenomegaly. Pulmonary hematopoiesis has rarely been reported in patients with primary or secondary MF. Nevertheless pulmonary findings at the time of autopsy are quite common.[4] In addition, the clinical course when pulmonary hematopoiesis is present is unknown and one study suggested that pulmonary hematopoiesis correlated with progression of the disease.[4] In our series, 27% of patients with PMF, ET-MF, or PV-MF and with a Lille score ≥1 had multiple nodules in the lungs. In one case, these nodules were biopsied and showed extramedullary hematopoiesis. The other two cases had similar nodules and although biopsies were not performed, we believe they also represented alveolar foci of hematopoiesis due to the rapid clearance after allogeneic HSCT. Our findings are consistent with the observation of progressive reduction of bone marrow fibrosis and splenomegaly following reduced intensity conditioning

Table 1. Characteristics of patients with primary myelofibrosis or myelofibrosis secondary to polycythemia vera or essential thrombocythemia in the study.

Number	11
Male/Female	5/6
Median Age (range) (years)	57 (range 54-63)
Diagnosis	
PMF	5
ET-MF	3
PV-MF	3
Time (Dx-Tx) (years)	5.4 (0.7-15)
Median WBC (range) ($\times 10^9$/L)	5.2 (3.1-68.4)
Median hemoglobin (range) (g/dL)	9.9 (8.2-12.5)
Median platelet count (range) ($\times 10^9$/L)	158 (40-348)
Prior Therapy*	6/11

*PMF: primary myelofibrosis; Dx-Tx: diagnosis to transplant; ET-MF: myelofibrosis secondary to essential thrombocythemia; PV-MF: myelofibrosis secondary to polycythemia vera; WBC: white blood cell count. *hydroxyurea, corticosteroids, anagrelide, or thalidomide.*

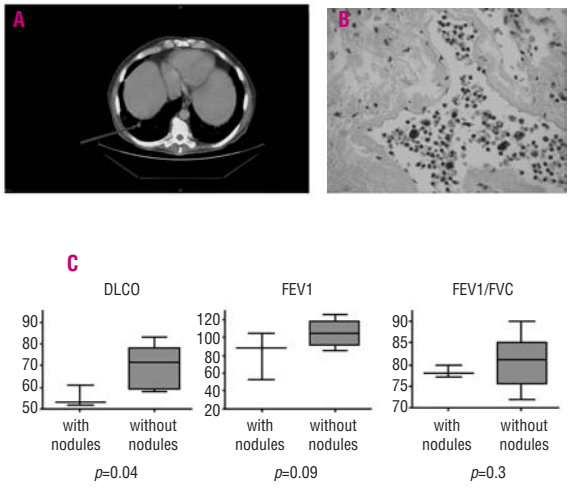

Figure 1. Pulmonary extramedullary hematopoiesis in patients with intermediate/high risk myelofibrosis undergoing allogeneic HSCT. (A) Pulmonary nodule in the right lung of a patient with PMF detected by CT scan (see arrow). (B) Section of lung showing hematopoietic precursors (nucleated erythroid and myeloid precursors and megakaryocytes) in the alveolar space. Some pigment laden macrophages are also noted. (H&E stain; original magnification x 400). (C) Differences in pulmonary function testing (DLCO, left panel; FEV1, central panel; FEV1/FVC, right panel) in patients with (n=3) or without (n=8) pulmonary nodules. The difference in DLCO was statistically different (p=0.04) by two-tailed Mann Whitney test.

allogeneic HSCT.[1,10,11] In conclusion, pulmonary extramedullary hematopoiesis may be a more common finding in patients with myelofibrosis than has been previously reported. However, it is not a contraindication for allogeneic HSCT.

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References

1. Hoffman R, Prchal JT, Samuelson S, Ciurea SO, Rondelli D. Philadelphia chromosome-negative myeloproliferative disorders: biology and treatment. Biol Blood Marrow Transplant 2007;13:64-72.
2. Einsfelder BM, Muller KM. [Pulmonary hypertension in chronic myeloproliferative disorders]. Pathologe 2005;26: 169-77.
3. Garcia-Manero G, Schuster SJ, Patrick H, Martinez J. Pulmonary hypertension in patients with myelofibrosis secondary to myeloproliferative diseases. Am J Hematol 1999;60:130-5.
4. Ozbudak IH, Shilo K, Hale S, Aguilera NS, Galvin JR, Franks TJ. Alveolar airspace and pulmonary artery involvement by extramedullary hematopoiesis: a unique manifestation of myelofibrosis. Arch Pathol Lab Med 2008;132:99-103.
5. Dupriez B, Morel P, Demory JL, Lai JL, Simon M, Plantier I, et al. Prognostic factors in agnogenic myeloid metaplasia: a report on 195 cases with a new scoring system. Blood 1996;88:1013-8.
6. Guardiola P, Anderson JE, Bandini G, Cervantes F, Runde V, Arcese W, et al. Allogeneic stem cell transplantation for agnogenic myeloid metaplasia: a European Group for Blood and Marrow Transplantation, Societe Francaise de Greffe de Moelle, Gruppo Italiano per il Trapianto del Midollo Osseo, and Fred Hutchinson Cancer Research Center Collaborative Study. Blood 1999;93:2831-8.
7. Deeg HJ, Gooley TA, Flowers ME, Sale GE, Slattery JT, Anasetti C, et al. Allogeneic hematopoietic stem cell transplantation for myelofibrosis. Blood 2003;102:3912-8.
8. Rondelli D, Barosi G, Bacigalupo A, Prchal JT, Popat U, Alessandrino EP, et al. Allogeneic hematopoietic stem-cell transplantation with reduced-intensity conditioning in intermediate- or high-risk patients with myelofibrosis with myeloid metaplasia. Myeloproliferative Diseases-Research Consortium. Blood 2005;105:4115-9.
9. Kröger N, Zabelina T, Schieder H, Panse J, Ayuk F, Stute N, et al. Pilot study of reduced-intensity conditioning followed by allogeneic stem cell transplantation from related and unrelated donors in patients with myelofibrosis. Br J Haematol 2005;128:690-7.
10. Ni H, Barosi G, Rondelli D, Hoffman R. Studies of the site and distribution of CD34+ cells in idiopathic myelofibrosis. Am J Clin Pathol 2005;123:833-9.
11. Ciurea SO, Sadegi B, Wilbur A, Alagiozian-Angelova V, Gaitonde S, Dobogai LC, et al. Effects of extensive splenomegaly in patients with myelofibrosis undergoing a reduced intensity allogeneic stem cell transplantation. Br J Haematol 2008;141:80-3.
12. Chunduri S, Dobogai LC, Peace D, Saunthararajah Y, Chen HY, Mahmud N, et al. Comparable kinetics of myeloablation between fludarabine/full-dose busulfan and fludarabine/melphalan conditioning regimens in allogeneic peripheral blood stem cell transplantation. Bone Marrow Transplant 2006;38:477-82.

Exon 8 splice site mutations in the gene encoding the E3-ligase *CBL* are associated with core binding factor acute myeloid leukemias

Acute myeloid leukemia (AML) is a heterogeneous disease with diverse genetic abnormalities and variable response to treatment. In the last decade the diverse genetic abnormalities have refined risk-stratification of AML.[1] Recently, mutations in the Casitas B-cell lymphoma gene encoding the E3-ligase CBL[2] were identified in *de novo* AML.[3,4] In one study, a single case with an inactivating point mutation in exon9 of the *CBL* gene was identified in a cohort of 150 *de novo* AML cases.[3] In a second study, exon8 missense mutations were demonstrated in 3 out of 12 randomly selected AML cases.[4] In an additional AML case, a DNA insertion/deletion mutation in intron7 of the *CBL* gene resulted in the expression of a *CBL* splice variant, i.e., a *CBL* mRNA lacking exon8.[4]

All published *CBL* mutations are located within the conserved linker region (LR) and ring finger (RF) of the CBL protein.[2] In fact, the mutant *CBL* splice variant without exon8 results in an in-frame deletion, which encodes a CBL protein lacking part of the LR, including two essential tyrosine residues, and almost the entire RF, which is critical for E3 activity.[2] This suggests that mutant CBL may act as a dominant negative protein by inhibiting proper downregulation of critical activated tyrosine kinases, such as KIT and FLT3 in AML.[5]

It is still not clear how frequently mutations in the *CBL* gene occur in newly diagnosed AML. In a diverse population of primary AML (n=319, Table 1) we assessed the frequency of CBL mutations, i.e., point mutations in exon8[4] and exon9[3], and mutations affecting proper splicing of CBL exon8.[4]

Patients had a diagnosis of primary AML, confirmed by cytological examination of blood and bone marrow, and were treated according to the HOVON protocols (*http://www.hovon.nl*). After obtaining patients' informed consent, bone marrow aspirates or peripheral blood samples were taken at diagnosis (n=319). CBL exon8 mRNA splice variants, as well as point mutations in exon8, were determined by cDNA amplification using the primer set CBLe6F 5'-AAACCTCTCTTCCAAGCACTG-3' and CBLe9R 5'-TCCCTCTAGGATCAAACGGA-3' or CBL-exon7-FOR 5'-GTGAACCAACTCCCCAAGAC-3' and CBL-exon9-REV 5'-GGACAGCCCTGACCTTCTG-3'. Mutations in genomic DNA were determined by amplification using CBL-intron7-FOR 5'-GGACCCAGACTA-GATGCTTTC-3' and CBL-exon8-REV 5'-GTGCACAT-GAGGTGTCCACAG-3' (mutations 5' of exon8) or CBL-exon7-FOR and CBL-exon9-REV (mutations 3' of exon8). (0.25 mM dNTP, 15 pmol primers, 2 mM $MgCl_2$, Taq polymerase and 1xbuffer (Invitrogen Life Technologies, Breda, The Netherlands); 1 cycle at 94°C for 5 mins., 35 cycles at 94°C for 1 min, 60°C for 1 min., 72°C for 1 min., and 1 cycle at 72°C for 7 mins.). Samples showing aberrant patterns were sequenced by using forward and reverse primers on the ABI PRISM3100 genetic analyzer