Acute myeloid leukemia with translocation t(3;5): new molecular insights

AML with translocation t(3;5) belongs to the "AML with myelodysplasia-related changes" defined in the 2008 WHO classification.[1] The incidence of this balanced abnormality is less than 0.5% of AML.[2] The identified breakpoints occur at 3q25.1 on chromosome 3 and at 5q34 on chromosome 5, where the nucleolar phosphoprotein nucleophosmin 1 (*NPM1*) is located.[3] At locus 3q25.1, Yoneda-Kato *et al.* identified a new gene, myeloid/myelodysplastic leukemia factor 1 (*MLF1*), and thus highlighted the fusion transcript *NPM1-MLF1*.[4] The physiological role of MLF1 has not been well characterized. In hematologic diseases, *MLF1* was found to be overexpressed in more than 25% of myelodysplastic syndrome (MDS) in transformation phase and MDS-associated AML.[5] *NPM1*, the partner gene of *MLF1* in the t(3;5)(q25.1;q34) translocation, is better known for being affected by a 4 bp insertion in exon 12 that occurs in 30-35% of all AML cases.[6]

To better characterize AML with *NPM1-MLF1*, we report morphological, immunophenotypic, cytogenetic features, and the first description of gene mutations analysis and gene expression profiling (GEP) in this cytogenetic entity.

This study included 7 cases diagnosed between 2002 and 2011 in France; case n. 1 has been reported previously.[7] The main clinical and biological characteristics of the patients studied are shown in Table 1. Molecular analysis was performed on cryo-preserved bone marrow mononuclear cells. The RT-PCR for the detection of *NPM1-MLF1* fusion transcript was carried out using the primers described by Yoneda-Kato *et al.*[4] The screening for mutations in *NPM1*, *FLT3*, *CEBPA*, *WT1*, *IDH1/2*, *DNMT3A* was performed as previously reported.[8] GEP was performed in 4 patients (UPN 2, 3, 6 and 7), according to standard protocol with Genechip Affymetrix HG 133 plus 2.0 array.[9]

The analysis of the Acute Leukemia French Association (ALFA) trials database showed a very low incidence (3 of 1333; 0.23%) of AML with *NPM1-MLF1* among adult AML patients with available karyotype. This incidence is consistent with that reported by Grimwade *et al.* (<0.5%).[10] The mean age of patients with *NPM1-MLF1* was 24 years. Cytological characteristics showed that a 3-lineage dysplasia was present in the great majority of cases, both in peripheral blood and bone marrow smears. Dysmegakaryopoiesis was constantly observed, dysgranulopoiesis was associated with peroxidase deficiency in 3 cases, and dyserythropoiesis occurred in 5 of 7 cases. Flow cytometry analysis showed that blast cells were positive for the myeloid antigens CD117, CD13, and CD33. CD34 was negative in 6 of 7 patients, as frequently observed in *NPM1* mutated AML.[11] Cytogenetic analysis showed that the translocation t(3;5)(q25.1;q34) was present as the sole abnormality at diagnosis in all cases. The presence of the chimeric fusion transcript *NPM1-MLF1* was confirmed in each case. Furthermore, all 7 cases harbored *WT1* overexpression and at least one mutation (*WT1* exon 7, *FLT3-ITD* and/or *IDH2R140* mutation) was identified in the 4 adult cases (Table 1). In contrast, no mutation was found in the 3 pediatric cases. Considering age at AML diagnosis, associated gene mutations in *NPM1-MLF1* positive AML seem to be similar to those in *NPM1* mutated AML. *DNMT3A* mutations, that occur in 50-60% of *NPM1* mutated AML, were not found in our cohort. GEP of AML with *NPM1-MLF1* was performed searching for a specific signature associated with this translocation. In order to do this, we compared 4 patients with *NPM1-MLF1* to a reference

Table 1. Clinico-biological characteristics of the 7 AML patients with t(3;5)(q25.1;q34).

Patient number	Age (years)	Gender	Type of AML	FAB classification	WBC (G/L)	Hemoglobin (g/dL)	Platelets (G/L)	PB blasts (%)	BM blasts (%)	Flow cytometry	Karyotype	NPM-MLF1 transcript	Additional molecular abnormalities identified	Treatment	CR after induction	Relapse (months)	CR2	ABMT (months)	OS (months)
1	3	F	*de novo*	M2	80.4	3	20	30	32	CD34-	46,XX,t(3;5)(q25;q34)[12]/ 46,XX[8]	Positive	none	LAME 91	yes	no		no	2
2	45	F	*de novo*	M2	4.3	8.8	127	8	69	CD34-	46,XX,t(3;5)(q25;q34) [9]	Positive	*WT1* Exon 7 mutation	ALFA-9802	yes	yes (70)	yes	yes (6) CR2	84+
3	49	F	*post MDS*	M2	35.5	9.6	61	55	57	CD34-	46,XX,t(3;5)(q24;q34)[10]. additionnal abnormality at relapse t(7;19)	Positive	FLT3-ITD	according to ALFA-9802	yes	yes (12)	no	no	18
24	34	F	*de novo*	M2	24.9	8.4	85	85	75	CD34-	46,XX,t(3;5)(q25;q34) [20]	Positive	*FLT3*-ITD *IDH2*-R140Q	ALFA-0702	yes	yes (9)	no	no	14
5	15	F	*de novo*	M2	4.3	11.2	46	9	22	CD34-	46,XX,t(3;5)(q25;q34) [20]	Positive	none	ELAM02	yes	no		no	16+
6	2	M	*de novo*	M4	28.1	13.4	54	4	25	CD34-	46,XX,t(3;5)(q25;q34) [12]	Positive	none	ELAM02	yes	no		yes (4)	14+
7	22	F	*de novo*	M2	17	6.5	257	31	45	CD34+	46,XX,t(3;5)(q25;q34) [13]	Positive	*IDH2*-R140Q	ALFA-0702	yes	no		yes (6)	7

AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; FAB: French-American-British; WBC: white blood cell; CR: complete remission, CR2: second CR; ABMT: allogeneic bone marrow transplantation; OS: overall survival; +: alive.

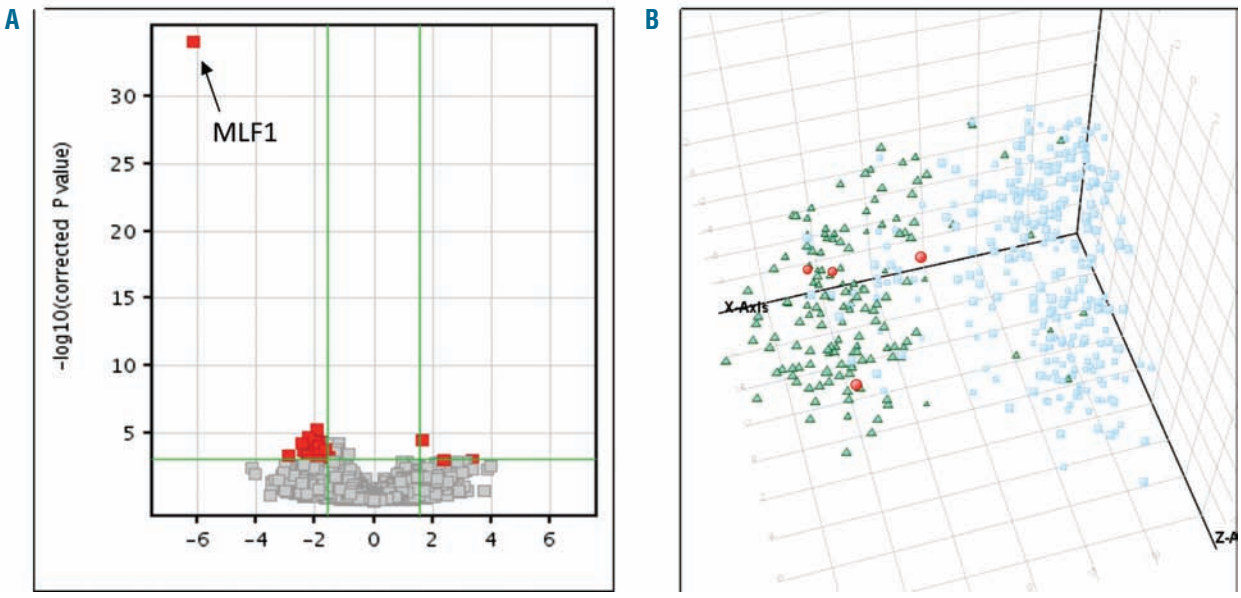

Figure 1. Results of gene expression profiling. (A) Volcano plot representing probes with statistically significant changes in gene expression among 189 probes used in 126 AML *NPM1* mutated without *FLT3*-ITD as control group and 4 AML with t(3;5)(q25.1;q34). The arrows indicate *MLF1* probes. The horizontal line and the vertical lines show respectively *P*=0.001 and fold-change >3. (B) Principal component analysis representing 436 patients with AML separated on a robust *NPM1* mutated signature and 4 AML with t(3;5)(q25.1;q34). Blue squares represent *NPM1* wild-type patients; green triangles represent *NPM1* mutated patients; red circles represent patients with t(3;5)(q25.1;q34).

group consisting of 436 patients with AML without t(3;5). Only two probes targeting *MLF1* were significantly overexpressed ($P<10^{-10}$) (Figure 1A). In a second step, we performed principal component analysis using the same patient cohort and a set of 189 probes defining a robust *NPM1* mutated signature (*Online Supplementary Table S1*). This analysis revealed that patients with AML with *NPM1-MLF1* co-segregated with the group of AML with *NPM1* mutations (Figure 1B). Thus, our results suggest that *NPM1-MLF1* positive AML and *NPM1* mutated AML may share common signaling pathways critical for leukemogenesis.

Regarding clinical outcome, all patients achieved complete remission (Table 1). Four out of 7 patients died, 2 from a cause other than leukemia. The 3 patients alive are in first complete remission, and minimal residual disease based on *WT1* expression and *NPM1-MLF1* transcript still remains negative at last follow up. Overall, the clinical outcome of the 7 patients studied appears consistent with the intermediate prognosis recently reassessed from poor prognosis by Grimwade *et al.*[10]

In conclusion, the comparison of AML with *NPM1-MLF1* and AML with *NPM1* mutations showed similar immunophenotypical and molecular features, including gene mutation patterns and GEP. Our findings suggest that these two types of AML may share common signaling pathways critical for leukemogenesis. However, this hypothesis needs to be confirmed in larger patient cohorts.

Florent Dumézy,[1,2,3] Aline Renneville,[1,2,3] Caroline Mayeur-Rousse,[4] Olivier Nibourel,[1,2,3] Elise Labis,[5] and Claude Preudhomme[1,2,3]

[1]Laboratoire d'Hématologie, CHU de Lille; [2]Université de Lille 2, Lille; [3]IRCL Inserm U837, Lille; [4]Laboratoire d'Hématologie, CHU de Hautepierre, Strasbourg; and [5]Laboratoire de Cytogénétique, CHU de Lille, France

Correspondence: claude.preudhomme@chru-lille.fr
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