Frequent ASXL2 mutations in acute myeloid leukemia patients with t(8;21)/RUNX1-RUNX1T1 chromosomal translocations

Jean-Baptiste Micot,1,2 Nicolas Duployez,3 Nicolas Boissel,4 Arnaud Petit,5 Sandrine Geoffry,3 Olivier Nibouret,3 Catherine Lacombe,6 Helene Lapillonne,7 Pascaleine Etancelin,7 Martin Figeac,8 Aline Renneville,3 Sylvie Castaigne,9 Guy Leverger,5 Norbert Ifrah,10 Hervé Dombret,9 Claude Preudhomme,3 Omar Abdel-Wahab,2 and Eric Jourdan11

1Hematology Department, INSERM Unité Mixte de Recherche 1009, Gustave Roussy Cancer Campus Grand Paris, Villejuif, Paris-Sud University, Orsay, France; 2Human Oncology and Pathogenesis Program and Leukemia Service, Memorial Sloan-Kettering Cancer Center and Weill Cornell Medical College, New York, NY; 3Laboratory of Hematology and Tumor Bank, INSERM U837 Team 3, Cancer Research Institute of Lille, Centre Hospitalier Régional Universitaire de Lille, University Lille Nord de France, Lille, France; 4Department of Hematology and EA3518, Hôpital Saint-Louis (Assistance Publique–Hôpitaux de Paris), University Paris Diderot, Paris, France; 5Department of Pediatric Hematology, Hôpital Armand Trousseau (Assistance Publique–Hôpitaux de Paris), Paris, France; 6Laboratory of Hematology, Hôpital Armand Trousseau (Assistance Publique–Hôpitaux de Paris), Groupe Hospitalier Universitaire de l’Est Parisien, Paris, France; 7Laboratory of Hematology, Hôpital Armand Trousseau (Assistance Publique–Hôpitaux de Paris), Groupe Hospitalier Universitaire de l’Est Parisien, Paris, France; 8Functional and Structural Genomic Platform, Lille 2 University, IFR-114, Lille, France; 9Department of Hematology, Versailles Hospital, Le Chesnay, France; 10Department of Hematology, INSERM U892, Centre Hospitalier Universitaire d’Angers, Angers, France; and 11Department of Hematology and Oncology, Centre Hospitalier Universitaire de Nîmes, University Montpellier-Nîmes, Nîmes, France

Key Points

- **ASXL2 was mutated in 22.7% (25/110) of adult and pediatric t(8;21)/RUNX1-RUNX1T1 acute myeloid leukemia patients.**
- **ASXL2 mutations are mutually exclusive with ASXL1 mutations and occur in t(8;21) but not inv(16)/t(16;16) or RUNX1-mutant AML.**

**Acute myeloid leukemia (AML) with t(8;21) (q22;q22) is considered to have favorable risk; however, nearly half of t(8;21) patients are not cured, and recent studies have highlighted remarkable genetic heterogeneity in this subset of AML. Here we identify somatic mutations in additional sex combs-like 2 (ASXL2) in 22.7% (25/110) of patients with t(8;21), but not in patients with inv(16)/t(16;16) (0/60) or RUNX1-mutated AML (0/26). ASXL2 mutations were similarly frequent in adults and children t(8;21) and were mutually exclusive with ASXL1 mutations. Although overall survival was similar between ASXL1 and ASXL2 mutant t(8;21) AML patients and their wild-type counterparts, patients with ASXL1 or ASXL2 mutations had a cumulative incidence of relapse of 54.6% and 36.0%, respectively, compared with 25% in ASXL1/2 wild-type counterparts (P = .226).**

These results identify a high-frequency mutation in t(8;21) AML and identify the need for future studies to investigate the clinical and biological relevance of ASXL2 mutations in this unique subset of AML. (*Blood*. 2014;124(9):1445-1449)

Introduction

Acute myeloid leukemia (AML) with t(8;21) (q22;q22) is recognized by the World Health Organization1 as a unique subtype of AML within the category of “AML with recurrent genetic abnormalities.” Compared with other cytogenetic subsets of AML, patients with t(8;21) are considered a favorable risk group according to their high remission and survival rates.2 At the same time, nearly half of t(8;21) AML patients are not cured,3,4 and there is a need for markers to identify patients unlikely to respond to current therapies and develop novel therapeutic approaches based on better understanding of the pathophysiology of this subset of AML.

The t(8;21) results in fusion of RUNX1 with RUNX1T1, and considerable experimental evidence reveals that full-length RUNX1-RUNX1T1 is not sufficient to induce leukemic transformation on its own.5 It is therefore posited that additional genetic alterations cooperate with RUNX1-RUNX1T1 translocations to induce overt leukemia. Identification of these additional genetic abnormalities in patients with core-binding factor (CBF) translocations has been helpful in predicting outcome and hopefully serving as novel therapeutic targets for CBF AML. Indeed, more than 50% of t(8;21) AML patients have been shown to have a mutation in KIT, FLT3, N-RAS, or K-RAS,6-10 all of which have been pursued as potential therapeutic targets. Moreover, work by Krauth et al identified that mutations in the polycomb-associated gene ASXL1 occur in 11.5% of t(8;21) adult AML patients and are associated with adverse event-free survival.11 Intriguingly, Huerer et al recently identified mutations in additional sex combs-like 2 (ASXL2) in several pediatric leukemia patients.12 Here we identify a high frequency of ASXL2 mutations among CBF AML patients specifically bearing RUNX1-RUNX1T1 translocations and use data from uniformly treated CBF AML adult and pediatric patient populations to identify clinical correlates and mutational cooccurrence of ASXL2 mutations in this unique subset of AML.


J.-B.M., N.D., N.B., O.A.-W., and E.J. contributed equally to this study.

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Methods

Patients and treatments

One hundred ten t(8;21) and 60 inv(16)/t(16;16) AML patients were included, including 75 t(8;21) and 32 inv(16)/t(16;16) adults from the CBF-2006 trial (A Phase 3 Trial of Systematic Versus Response-adapted Timed-Sequential Induction in Patients with Core Binding Factor [CBF] Acute Myeloid Leukemia [AML]; EudraCT 2006 005163-26; ClinicalTrials.gov NCT00428558), as well as 35 t(8;21) and 28 inv(16)/t(16;16) children from the ELAM02 trial (Treating Patients with Childhood Acute Myeloid Leukemia with Interleukin-2; ClinicalTrials.gov NCT00149162). Twenty-six de novo adult AML patients with \( RUNX1 \) mutations were also included (from the ALFA-0701 trial [A Randomized Study of Gemtuzumab Ozogamicin (GO) with Daunorubicin and Cytarabine in Untreated AML Aged of 50-70 Years Old]). Studies were approved by the Ethics Committee of Nimes University Hospital and by the Institutional Review Board of the French Regulatory Agency and were

Figure 1. \( ASXL2 \) mutations are frequent in AML patients bearing the t(8;21) translocation and are associated with a trend for increased risk for relapse. (A) Gene diagram depicting \( ASXL2 \) mutations in adult and pediatric patients with t(8;21) AML. (B) Pattern of molecular and cytogenetic lesions in patients with t(8;21) AML. Each column represents 1 of the 110 t(8;21) AML samples sequenced here. Patient demographics (adult vs pediatric sample) are also included. Cumulative incidence of relapse according to enrollment \( ASXL2 \) and \( ASXL1 \) mutation status in (C) the entire t(8;21) AML cohort and (D) adult patients who achieved more than 3 log-fold reduction in \( RUNX1 \)-\( RUNX1T1 \) transcripts before initiation of second consolidation course.
conducted in accordance with the Declaration of Helsinki protocol. Outcome data were updated as of December 2013, for a median follow-up of 44.8 months.

Mutational and minimal residual disease analysis

Whole-exome sequencing of DNA was performed from pretreatment as well as paired remission bone marrow mononuclear cells from 3 t(8;21) AML patients (supplemental Methods, available on the Blood Web site). All coding exons of ASXL2 were sequenced by next-generation sequencing for all patients at a median depth of 1153× (supplemental Table 1; supplemental Figure 3). Likewise, although ASXL1 mutations (supplemental Table 3) were mutually exclusive with ASXL2 mutations (Figure 1B). Analysis of mutational cooccurrences revealed a significant cooccurrence of ASXL2 and FLT3-ITD mutations ($P = .046$), a feature not shared by ASXL1 mutant t(8;21) patients.

No ASXL1 or ASXL2 mutations were found in 60 inv(16)/t(16;16) AML patients (supplemental Figure 3). Likewise, although ASXL1 mutations were seen in 30.8% of RUNX1-mutated de novo AML patients, 0/26 RUNX1-mutant AML samples had an ASXL2 mutation (supplemental Figure 3).

Implications of ASXL2 mutations

Unlike mutations in ASXL1, which have been associated with advanced age in AML, ASXL2 mutations were similarly frequent among pediatric and adult t(8;21) AML patients (9/35 vs 16/75, respectively; $P = .6$; supplemental Figure 2C). Patients with ASXL2 or ASXL1 mutations had a significantly higher median white blood cell count (173,000 vs 52,500 per microliter; $P < .0001$; supplemental Figure 3A).

Results and discussion

Discovery and recurrence of ASXL2 mutations

To identify novel mutations cooperating with RUNX1-RUNX1T1 fusions, we performed whole-exome sequencing on 3 diagnostic t(8;21) AML samples paired with a remission sample for each patient. This analysis revealed a somatic ASXL2 p.R741EfsX15 mutation restricted to the diagnosis sample of 1 patient with a variant allele frequency of 39.5% (supplemental Figure 2A) and a somatic ASXL1 p.L823X mutation in a second patient (variant allele frequency, 39%; supplemental Table 2). After validating the mutation in ASXL2 by Sanger sequencing (supplemental Figure 2B), we next performed targeted sequencing of ASXL2 across 110 pediatric and adult t(8;21) AML patients. Overall, 22.7% of t(8;21) patients (25/110) harbored an ASXL2 mutation (Figure 1A). The majority of ASXL2 mutations (85%) were out-of-frame frameshift mutations (supplemental Table 3). The somatic nature of these mutations in ASXL2 was verified by sequencing DNA from time of presentation and at remission in 6 patients total. Similar to previously described mutations in ASXL1, ASXL2 mutations were exclusively heterozygous, with the exception of a single patient with 2 different truncating ASXL2 mutations (supplemental Table 3), and were enriched in the 3' region of the gene, occurring exclusively in exons 11 and 12. Interestingly, ASXL1 mutations (supplemental Table 3) were mutually exclusive with ASXL2 mutations (Figure 1B). Analysis of mutational cooccurrences revealed a significant cooccurrence of ASXL2 and FLT3-ITD mutations ($P = .046$), a feature not shared by ASXL1 mutant t(8;21) patients.

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cell count at presentation than their wild-type counterparts (20.3 vs 15.8 × 10^9 cells/L, respectively; \( P = .029 \)) (Table 1). Data for adult and pediatric t(8;21) AML patients are reported separately in supplemental Table 4.

Although overall survival was similar between ASXL1 or ASXL2 mutant t(8;21) AML patients and wild-type counterparts (supplemental Figure 4A-C), patients with ASXL1 or ASXL2 mutations had a 3-year cumulative incidence of relapse of 54.6% and 36.0%, respectively, compared with 25% in ASXL1/2 wild-type counterparts (\( P = .226 \); Figure 1C and supplemental Figure 4D). Previous analysis of the CBF-2006 trial identified achievement of more than a 3 log-fold reduction in the RUNX1-RUNXIT1 transcript before initiation of second consolidation (minimal residual disease 2 [MRD2]) as the sole factor significantly influencing outcome in multivariate analysis of CBF patients. Among adults achieving more than 3 log-fold reduction at MRD2, the 3-year cumulative incidence of relapse was 44.4%; it was 35.7% for ASXL1 and ASXL2 mutated patients, respectively, compared with 15.2% in ASXL1/2 wild-type patients (\( P = .128 \); Figure 1D and supplemental Figure 4E).

Huether et al first noted ASXL2 mutations in 7.2% of pediatric CBF AML patients. Here we extend those findings and identify that ASXL2 mutations are specifically recurrent in adult as well as pediatric CBF AML patients bearing t(8;21), occurring in nearly 25% of such patients compared with 0% of inv(16)/t(16;16) patients (\( P = .00005 \)). The fact that ASXL2 mutations were not identified in prior whole-exome/genome sequencing studies in AML suggests that genomic studies focused on specific clinical and genetic subsets of AML may identify additional novel mutations enriched within defined subsets of AML patients. Moreover, the discovery that t(8;21) AML patients who achieve a more than 3 log-fold reduction at MRD2 but harbor ASXL1 or ASXL2 mutations have a potentially higher risk for relapse suggests the need for further studies addressing the prognostic relevance of ASXL2 mutations in t(8;21). The fact that ASXL1 and ASXL2 mutations were mutually exclusive with one another, combined with the multiple unique domains in common between ASXL1 and ASXL2, suggests a shared mechanism of transformation for mutations in these 2 genes. Indeed, 32.7% of t(8;21) patients harbor an ASXL1 or ASXL2 mutation, making ASXL gene family alterations among the most common genetic alterations in t(8;21) AML patients. Understanding the functional basis for this frequent cooccurrence of RUNX1-RUNXIT1 translocation and mutations in ASXL1 or ASXL2 may be a critical step in furthering our knowledge of CBF AML pathogenesis and therapy.

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Authorship

Correspondence: Omar Abdel-Wahab, Memorial Sloan-Kettering Cancer Center, Zuckerman 802, 408 East 69th Street, New York, NY 10065; e-mail: abdelwaa@mskcc.org; and Eric Jourdan, Hôpital Universitaire Carémeau, Place du Pr. Robert Debré, 30029 Nîmes Cedex 9, France; e-mail: eric.jourdan@chu-nimes.fr.

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Correspondence: Omar Abdel-Wahab, Memorial Sloan-Kettering Cancer Center, Zuckerman 802, 408 East 69th Street, New York, NY 10065; e-mail: abdelwaa@mskcc.org; and Eric Jourdan, Hôpital Universitaire Carémeau, Place du Pr. Robert Debré, 30029 Nîmes Cedex 9, France; e-mail: eric.jourdan@chu-nimes.fr.


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