Brief Report

CLINICAL TRIALS AND OBSERVATIONS

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

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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 Organization[1] 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, Huether 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.

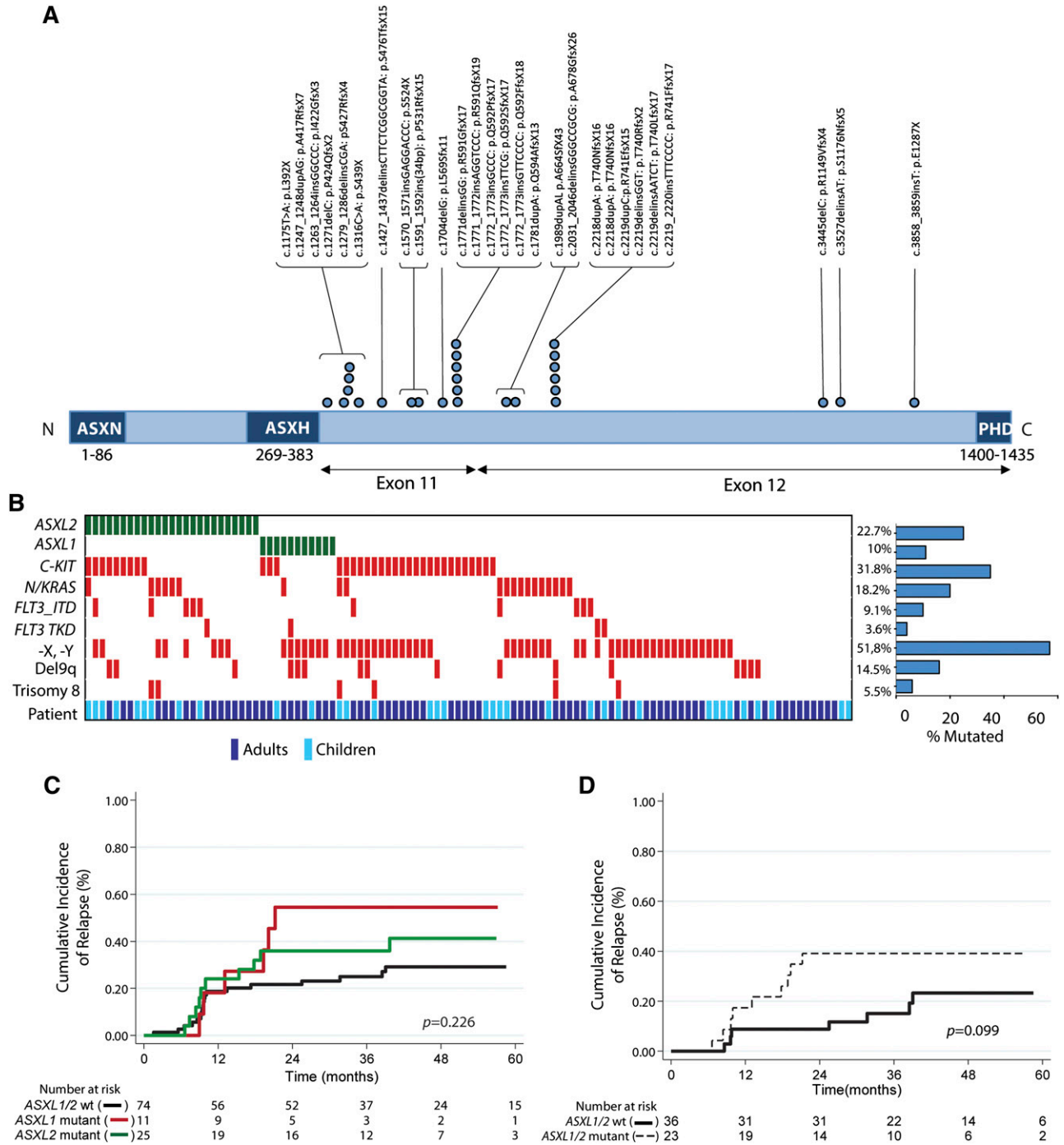There is an Inside *Blood* Commentary on this article in this issue.

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 *ASXL*1 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.

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[13] (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[14] [A Randomized Study of Gemtuzumab Ozogamicin (GO) with Daunorubicine 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

Table 1. Characteristics and outcome of t(8;21) AML patients according to *ASXL2* and *ASXL1* mutational status

Patient characteristics	All patients (n = 110)	*ASXL1/2* wild-type (n = 74)	*ASXL1* mutant (n = 11)	*ASXL2* mutant (n = 25)	P*	P†
Age, median [range] (years)	31 [4-60]	33 [4-59]	35 [7-47]	22 [4-60]	.265	.227
Sex (M/F)	59/51	43/31	5/6	11/14	.400	.223
WBC, median [range] (G/L)	12.8 [1.3-163]	10.7 [1.3-94.5]	15.8 [3.4-48.2]	20.3 [2.7-163]	.029	.011
Blasts, [range] %	55 [17-98]	58 [17-98]	45 [28-81]	50 [25-94]	.140	.047
Additional cytogenetic abnormalities						
Loss of Y	36/109 (33%)	27/74 (36%)	4/10 (40%)	5/25 (20%)	.289	.286
Del(9q)	16/109 (15%)	10/74 (14%)	3/10 (30%)	3/25 (12%)	.351	.773
+8	6/109 (6%)	4/74 (5%)	0/10 (0%)	2/25 (8%)	.801	.999
Gene mutations						
FLT3-TKD	4/110 (4%)	2/74 (3%)	1/11 (9%)	1/25 (4%)	.380	.596
FLT3-ITD	10/110 (9%)	5/74 (7%)	0/11 (0%)	5/25 (20%)	.156	.291
KIT-Ex8	12/110 (11%)	6/74 (8%)	2/11 (18%)	4/25 (16%)	.294	.202
KIT-Ex17	24/110 (22%)	17/74 (23%)	1/11 (9%)	6/25 (24%)	.629	.807
KIT (total)	35/110 (32%)	22/74 (31%)	3/11 (27%)	9/25 (36%)	.902	.830
K-RAS or *N-RAS*	20/110 (18%)	13/74 (18%)	1/11 (9%)	6/24 (24%)	.589	.798
Any receptor tyrosine kinase mutation	47/110 (43%)	29/74 (39%)	4/11 (36%)	14/25 (56%)	.355	.310
Early response and outcome						
Day 16 bone marrow (>5% blasts)‡	12/75 (16%)	11/50 (22%)	0/9 (0%)	1/16 (6.25%)	.156	.052
Complete remission	110/110	74/74	11/11	25/25	NA	NA
Patients achieving more than 3 log-fold reduction in *RUNX1-RUNX1T1* transcripts at MRD2¶	74/101 (73%)	47/65 (72%)	9/11 (82%)	18/25 (72%)	.330	.193
3-year cumulative incidence of relapse, % [95% confidence interval]	30.1 [22.9-40.7]	25.0 [16.3-37.2]	54.6 [29.3-83.3]	36.0 [20.6-57.8]	.226	.103
3-year overall survival, % [95% confidence interval]	80.1 [72.0-87.3]	79.8 [68.1-87.5]	81.8 [44.7-95.1]	84.0 [62.8-93.7]	.881	.620

*P value comparing *ASXL1* mutant versus *ASXL2* mutant versus *ASXL1/2* wild-type patients.
†P value comparing *ASXL1* or *ASXL2* mutant patients versus *ASXL1/2* wild-type patients.
‡Day 16 bone marrow data only available for adult patients.
¶MRD2 represents time before initiation of second consolidation.

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 1; supplemental Methods). All additional molecular and cytogenetic analyses are described in the supplemental Methods.

Statistical methods

Details of statistical analysis are located in supplemental Methods.

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) 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*,[15] *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.

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,[16] *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 at presentation than their wild-type counterparts (20.3 vs 15.8 vs 10.7×10^9 cells/μL, 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-RUNX1T1* transcript before initiation of second consolidation (minimal residual disease 2 [MRD2]) as the sole factor significantly influencing outcome in multivariate analysis of CBF patients.[13] 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.[12] 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[17-21] 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).[13] 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*,[22] suggests a shared mechanism of transformation for mutations in these 2 genes. Indeed, 32.7% of t(8;21) patients harbor an *ASXL1* or *ASLX2* 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-RUNX1T1* translocation and mutations in *ASXL1* or *ASXL2* may be a critical step in furthering our knowledge of CBF AML pathogenesis and therapy.

Authorship

Contribution: E.J. was the principal investigator of the CBF-2006 study; G.L. is the principal investigator of the Elam02 study; H.D. and C.P created the patient database; N.B. performed statistical analysis; E.J., N.B., J.-B.M, N.I., H.D., G.L., and A.P. enrolled patients in the studies; N.D., O.N., S.G., C.P., C.L., H.L, P.E., A.R., and S.C. performed genetic analysis and analyzed mutational data; M.F. interpreted next-generation sequencing results; and J.-B.M., N.D., N.B., and O.A.-W. wrote the manuscript, which was approved by all the authors.

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