Published in final edited form as:

Leukemia. 2016 November ; 30(11): 2254–2258. doi:10.1038/leu.2016.196.

Adult acute myeloid leukemia with trisomy 11 as the sole abnormality is characterized by the presence of five distinct gene mutations: *MLL*-PTD, *DNMT3A*, *U2AF1*, *FLT3*-ITD and *IDH2*

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Trisomy of chromosome 11 (+11) is the second most common isolated trisomy in acute myeloid leukemia (AML) patients.¹ The presence of +11 is associated with intermediate^{2–3} or poor patient outcomes.^{4–6} Whereas the clinical characteristics of solitary +11 have been well established,^{4–6} relatively little is known about the mutational landscape of sole +11 AML in the age of next-generation sequencing techniques that allow examination of multiple genes relevant to AML pathogenesis.⁶ So far, the most common molecular feature in AML with isolated +11 is the presence of a partial tandem duplication of the *MLL* (*KMT2A*) gene (*MLL*-PTD), which is detectable in up to 90% of patients.⁷ Furthermore, a frequent co-occurrence of the *FLT3* internal tandem duplication (*FLT3*-ITD) with *MLL*-PTD has been reported.⁸ The aim of our study was to better characterize the mutational landscape of adult AML patients with sole +11.

Bone marrow (BM) or blood samples containing 20% leukemic blasts were obtained from 23 AML patients with isolated +11 identified among 1625 adult AML patients with

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The authors declare no conflicts of interest.

AUTHORSHIP CONTRIBUTIONS

A-KE, JK, KM, JCB, and CDB contributed to the study design; A-KE, JK, KM, AdIC and CDB contributed to the data interpretation, A-KE, JK, KM and CDB wrote the manuscript; A-KE and SO performed laboratory-based research; JSB and KK performed the data processing; JK and DN performed statistical analysis; AJC, RMS, KM and CDB were involved directly or indirectly in the care of patients and/or sample procurement. All authors read and agreed on the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

Supplementary Information is available at *Leukemia*'s website.

diagnostic material available for molecular studies. Thus, the frequency of sole +11 in our AML patient cohort was 1.4%. Pretreatment cytogenetic analyses of BM and/or blood samples were performed by Cancer and Leukemia Group B (CALGB)/Alliance for Clinical Trials in Oncology (Alliance)-approved institutional laboratories and the results centrally reviewed. Trisomy 11 (found in 2 metaphase cells; Supplementary Table S1) was the only clonal chromosome abnormality detected in each patient in pretreatment specimens subjected to unstimulated 24- and/or 48-hour culture. The patients were treated on CALGB/Alliance trials (for details, see Supplementary Information). Study protocols were in accordance with the Declaration of Helsinki and approved by the institutional review boards at each center, and all patients provided written informed consent.

Definitions of the clinical endpoints [complete remission (CR), disease-free (DFS) and overall (OS) survival] and statistical methods are provided in the Supplementary Information. Data collection and statistical analyses were performed by the Alliance for Clinical Trials in Oncology Statistics and Data Center. The study database was locked on December 8, 2015.

The mutational status of 80 protein coding genes (see Supplementary Information for experimental procedures, analyses algorithm and achieved sequencing depth) was determined by targeted amplicon sequencing using the MiSeq platform (Illumina, San Diego, CA). Testing for the presence or absence of *FLT3*-ITD, *MLL*-PTD and mutations in *CEBPA* were performed as previously described (see Supplementary Information). Thus, since *FLT3* and *MLL* were also part of the 80 gene targeted amplicon panel, our approach allowed the assessment of the mutational status of 81 genes.

Additionally, RNA from all patients with available material (n=18) was used for RNA sequencing studies (RNAseq; Supplementary Information). Mutations detected in the targeted sequencing approach at the DNA level were also analyzed at the RNA level by visual inspection of the BAM files.

The clinical characteristics and outcomes of 23 patients with sole +11 are summarized in Table 1. The patients were predominantly older, with 18 patients (78%) over the age of 60 years. They were older than the remaining patients in the total patient cohort who did not have sole +11 (median, 71 vs 52 years; $P<0.001$). Only 13% of the isolated +11 patients had signs of extramedullary involvement of their leukemia. The outcomes of sole +11 AML patients were very poor. Only 11 (48%) patients achieved a CR, and 54% of those who did eventually relapsed. Both DFS and OS were short, with medians of 12.5 and 11.7 months and 36-month rates of 14% and 11%, respectively (Table 1). However, three of four patients who received allogeneic stem cell transplantation in first CR were alive and disease-free for more than 3 years.

Results of mutational analyses performed using the targeted sequencing panel are shown in Table 1 and Figure 1A. Ninety-two mutations were found in 28 of the 81 tested genes, with a median of four mutations per patient (range, 1–6 mutations). All mutations detected at the DNA level were also validated at the RNA level if the gene was adequately covered in the RNAseq (Supplementary Table S1). Strikingly, mutations in only five genes accounted for

62% of the mutational load (57/92 mutations) of the patients: *MLL*-PTD (detected in 70% of patients), *U2AF1* (48%) and *DNMT3A* (48%; R882, n=7 and non-R882, n=4) mutations, *FLT3*-ITD (43%) and *IDH2* (39%) mutations. All sole +11 patients harbored at least one of these five gene mutations. Other gene mutations were found in only a few patients. *FLT3*-TKD, *SRSF2* and *ZRSR2* mutations were each detected in three patients, and *IDH1*, *MED12*, *PTPN11*, *RUNX1*, *STAG2* and *TET2* mutations each in two patients in our cohort. Fourteen genes were mutated in single cases only (Table 1). With the exception of *IDH2* and *U2AF1*, which were mutually exclusive, the five dominating gene mutations (*MLL*-PTD, *DNMT3A*, *U2AF1*, *FLT3*-ITD and *IDH2*) frequently co-occurred. Most patients harbored more than one of these five mutations, with a median of three (range: 1–4).

Whereas *DNMT3A* and *IDH2* are among the most frequently mutated genes in AML patients,⁹ *U2AF1* mutations are rare in AML.^{10,11} Initially discovered in approximately 10% of patients with myelodysplastic syndrome,¹⁰ they occur in roughly 20% of chronic myelomonocytic leukemia patients,¹¹ but have not been hitherto associated with any specific AML subset. *U2AF1* mutations are considered as early (possibly disease initiating) leukemogenic events since they were detected at the earliest presentation of chronic myelomonocytic leukemia.¹¹ The effects of mutant *U2AF1* on hematopoiesis and myeloid neoplasms are profound; they include significantly altering hematopoiesis and pre-mRNA splicing *in vitro* and *in vivo*.¹⁰ Notably, while *U2AF1* mutations can usually be found in two hotspot positions of the gene (S34 and Q84),¹⁰ all sole +11 patients were mutated only at the S34 hotspot. Interestingly, sole +11 is now the second isolated trisomy associated with a specific spliceosome gene mutation. Previously, Herold *et al.*¹² found ~80% of AML patients with isolated trisomy 13 to harbor mutations in *SRSF2*.

Our data confirm *MLL*-PTD and, to a lesser extent, *FLT3*-ITD as common events in +11 AML.^{6–8} However, the high mutation frequencies of *U2AF1* and genes involved in methylation (*DNMT3A*, *IDH2*) have hitherto not been reported in +11 AML. It has been shown that in cases with +11 and *MLL*-PTD, only one chromosome contains the *MLL*-PTD and the remaining two chromosomes harbor wild-type *MLL* alleles.¹³ Yet, despite having two wild-type alleles, the *MLL* wild-type transcript is not expressed in primary blasts harboring *MLL*-PTD.¹³ This phenomenon can be reversed with DNA methyltransferase and histone deacetylase inhibitor treatment, suggesting epigenetic modifications to be responsible for the allele-specific expression.¹⁴ It is tempting to speculate that mutations in *DNMT3A* and/or *IDH2* may contribute to the epigenetic changes in +11 AML, including differential activation of the wild-type *MLL* transcript.

Notably, *DNMT3A*, *U2AF1* and *IDH2* mutations (which were determined by the targeted sequencing panel) were always present at high VAFs (>30%), suggesting that they represent early mutational events. The percentages of cells with +11 in pretreatment samples (based on cytogenetic analysis in all cases and fluorescence *in situ* hybridization in two) were also high in most cases (Supplementary Table 1). The vast majority of patients (19 of 23, 83%) had 60% of cells with sole +11 (including 11 patients with 100% of +11-positive cells), whereas in only 4 patients (17%), cells with sole +11 constituted a minority, 10% to 30% of the analyzed cells. Thus, it seems that in most instances gain of chromosome 11 represents

an important, early alteration, whereas in a smaller subset of patients, +11 might represent a cooperating rather than an initiating event.

We tested whether any of the clinical or molecular pretreatment parameters influenced the probability of achieving CR, DFS or OS of sole +11 patients. Since patients younger than 60 years and those aged 60 years or more were treated differently, and there were only five younger patients, the outcome analyses were performed in patients aged ≥ 60 years. We found that the presence of *DNMT3A* mutations (R882 and non-R882 combined) associated with a lower probability to achieve a CR ($P=0.02$, Supplementary Table S2), with only one of eight (13%) patients with a *DNMT3A* mutation achieving a CR compared with seven of 10 (70%) patients with wild-type *DNMT3A*. Moreover, the presence of *DNMT3A* mutations was also associated with shorter OS ($P=0.004$; median, 6.8 vs 22.5 months; Figure 1B and Supplementary Table S2). No other gene mutation associated with any outcome endpoint in our analyses. Our findings are consistent with the recent report by Kao *et al.*¹⁵ who identified co-occurring *DNMT3A* mutations as an unfavorable prognostic factor in AML patients harboring *MLL*-PTD, most of whom had a normal karyotype. However, because of the relatively small number of patients analyzed, our results concerning the prognostic impact of *DNMT3A* mutations in sole +11 AML are preliminary and warrant further studies.

Taken together, although our analyses are limited by a relatively small sample size, our study sheds new light on the mutational background of this prognostically adverse subset of AML patients with sole +11.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Support:

This work was supported in part by the National Cancer Institute (grants CA180821 and CA180882 [to the Alliance for Clinical Trials in Oncology], CA140158, CA180861, CA196171, CA180850, CA16058 and CA77658), the Coleman Leukemia Research Foundation, the Warren D. Brown Foundation, the Pelotonia Fellowship Program (A-KE), and by an allocation of computing resources from The Ohio Supercomputer Center.

The authors are grateful to the patients who consented to participate in these clinical trials and the families who supported them; to Donna Bucci and the CALGB/Alliance Leukemia Tissue Bank at The Ohio State University Comprehensive Cancer Center, Columbus, OH, for sample processing and storage services, and to Lisa J. Sterling and Chris Finks for data management.

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Figure 1. Distribution of recurrent mutations, and prognostic impact of mutations in the *DNMT3A* gene in patients with acute myeloid leukemia (AML) and sole +11. **(a)** Oncoprint of recurrent mutations found in 23 sole +11 patients analyzed. Red indicates the presence of a gene mutation, grey, wild-type status, and white, mutation status not determined. Depicted separately at the top of the figure are the five genes, which we found commonly mutated in sole +11 patients. Only genes mutated in two or more patients are included in the oncoprint. s-AML denotes secondary AML; t-AML, therapy-related AML. **(b)** Overall survival of older (aged ≥ 60 years) AML patients with sole +11 according to the presence or absence of mutations in the *DNMT3A* gene. The presence of a *DNMT3A* mutation was the only factor significantly associated with reduced overall survival in our analysis.

Table 1

Characteristic^d	<i>Sole +11 AML (n=23)</i>
<i>Age, years</i>	
Median	71
Range	25–84
<i>Age group, n (%)</i>	
<60 years	18 (78)
60 years	5 (22)
Female sex, <i>n (%)</i>	5 (22)
<i>Race, n (%)</i>	
White	22 (96)
Nonwhite	1 (4)
<i>Hemoglobin, g/dl</i>	
Median	8.3
Range	4.3–11.0
<i>Platelet count, $\times 10^9/l$</i>	
Median	67
Range	11–673
<i>WBC, $\times 10^9/l$</i>	
Median	25.1
Range	0.7–131.4
<i>Bone marrow blasts, %</i>	
Median	52
Range	21–88
<i>Blood blasts, %</i>	
Median	38
Range	0–96
<i>s-AML, n (%)</i>	
Yes	1 (4)
No	22 (96)
<i>t-AML, n (%)</i>	
Yes	2 (9)
No	21 (91)
<i>Extramedullary involvement, n (%)</i>	
Total	3 (13)
Central nervous system	0 (0)
Gum hypertrophy	1 (4)
Hepatomegaly	0 (0)
Lymphadenopathy	3 (13)

Characteristic^a	<i>Sole +11 AML (n=23)</i>
Mediastinal mass	0 (0)
Skin infiltrates	0 (0)
Splenomegaly	1 (4)
<i>Transplantation, n (%)</i>	
Allogeneic in 1st CR	4 (17)
No allogeneic in 1st CR	19 (83)
<i>ASXL1, n (%)</i>	
Mutated	1 (4)
Wild-type	22 (96)
<i>AXL, n (%)</i>	
Mutated	1 (4)
Wild-type	22(96)
<i>BCOR, n (%)</i>	
Mutated	1 (4)
Wild-type	22 (96)
<i>BCORL1, n (%)</i>	
Mutated	1 (4)
Wild-type	22 (96)
<i>BIRC6, n (%)</i>	
Mutated	1 (4)
Wild-type	22(96)
<i>CBL, n (%)</i>	
Mutated	1 (4)
Wild-type	22(96)
<i>CSNK1A, n (%)</i>	
Mutated	1 (4)
Wild-type	22 (96)
<i>DNMT3A, n (%)</i>	
Mutated	11 (48)
R882	7
Non-R882	4
Wild-type	12 (52)
<i>ETV6, n (%)</i>	
Mutated	1 (4)
Wild-type	22 (96)
<i>FLT3-ITD, n (%)</i>	
Present	10 (43)
Absent	13 (57)
<i>FLT3-TKD, n (%)</i>	

Characteristic^a	<i>Sole +11 AML (n=23)</i>
Present	3 (14)
Absent	19 (86)
<i>IDH1, n (%)</i>	
Mutated	2 (9)
Wild-type	21 (91)
<i>IDH2, n (%)</i>	
Mutated	9 (39)
Wild-type	14 (61)
<i>JAK1, n (%)</i>	
Mutated	1 (4)
Wild-type	22 (96)
<i>KRAS, n (%)</i>	
Mutated	1 (4)
Wild-type	22 (96)
<i>MED12, n (%)</i>	
Mutated	2 (9)
Wild-type	21 (91)
<i>MLL-PTD, n (%)</i>	
Present	16 (70)
Absent	7 (30)
<i>PRKCB, n (%)</i>	
Mutated	1 (4)
Wild-type	22 (96)
<i>PTPN11, n (%)</i>	
Mutated	2 (9)
Wild-type	21 (91)
<i>RUNX1, n (%)</i>	
Mutated	2 (9)
Wild-type	21 (91)
<i>SMC1A, n (%)</i>	
Mutated	1 (4)
Wild-type	22 (96)
<i>SRSF2, n (%)</i>	
Mutated	3 (14)
Wild-type	19 (86)
<i>STAG2, n (%)</i>	
Mutated	2 (9)
Wild-type	21 (91)
<i>TET2, n (%)</i>	

Characteristic ^a	<i>Sole +11 AML</i> (n=23)
Mutated	2 (9)
Wild-type	21 (91)
<i>U2AF1</i> , n (%)	
Mutated	11 (48)
Wild-type	12 (52)
<i>WT1</i> , n (%)	
Mutated	1 (4)
Wild-type	22 (96)
<i>ZRSR2</i> , n (%)	
Mutated	3 (13)
Wild-type	20 (87)
Complete remission, <i>n</i> (%)	11 (48)
Disease-free survival ^b	
Median, months	12.5
% Disease-free at 12 months (95% CI)	57 (17–84)
% Disease-free at 36 months (95% CI)	14 (1–46)
Overall survival	
Median, months	11.7
% Alive at 12 months (95% CI)	48 (27–66)
% Alive at 36 months (95% CI)	11 (1–35)

Abbreviations: s-AML, secondary AML; t-AML, therapy-related AML; CI, confidence interval; *FLT3*-ITD, internal tandem duplication of the *FLT3* gene; *FLT3*-TKD, tyrosine kinase domain mutation in the *FLT3* gene; *MLL*-PTD, partial tandem duplication of the *MLL* (*KMT2A*) gene; *n*, number; WBC, white blood count.

^aOnly those genes that have been found mutated in at least one patient are listed in the Table in alphabetical order. No mutations were detected in the following genes: *AKT1*, *ARAF*, *ATM*, *BCL2*, *BRAF*, *BRD4*, *BRINP3*, *BTK*, *CCND1*, *CCND2*, *CEBPA* (biallelic), *CTNNB1*, *EZH2*, *FBXW7*, *GATA1*, *GATA2*, *GSK3B*, *HIST1H1E*, *HNRNPK*, *IKZF1*, *IKZF3*, *IL7R*, *JAK2*, *JAK3*, *KIT*, *KLHL6*, *KMT2A* (other than PTD), *MAPK1*, *MAPK3*, *MYD88*, *NOTCH1*, *NPM1*, *PHF6*, *PIK3CD*, *PIK3CG*, *PLCG2*, *PLEKHG5*, *PRKD3*, *PTEN*, *RAD21*, *RAF1*, *SAMHD1*, *SETBP1*, *SFI*, *SF3A1*, *SF3B1*, *SMARCA2*, *SMC3*, *SYK*, *TGM7*, *TP53*, *TYK2* *U2AF2* *XPO1* and *ZMYM3*

^bPatients who received an allogeneic hematopoietic stem cell transplantation in first CR (*n*=4) were excluded from the disease-free survival analysis.