Published in final edited form as:

Leukemia. 2020 February ; 34(2): 358–368. doi:10.1038/s41375-019-0560-3.

Clinical and molecular characterization of patients with acute myeloid leukemia and sole trisomies of chromosomes 4, 8, 11, 13 or 21

Bhavana Bhatnagar^{#1,2}, Ann-Kathrin Eisfeld^{#3}, Jessica Kohlschmidt^{2,4}, Krzysztof Mrózek², Deedra Nicolet^{2,4}, Dimitrios Papaioannou³, Christopher J. Walker², Shelley Orwick^{1,2}, James S. Blachly^{1,2}, Jonathan E. Kolitz⁵, Bayard L. Powell⁶, Andrew J. Carroll⁷, Richard M. Stone⁸, John C. Byrd^{1,2}, Clara D. Bloomfield^{1,2}

¹Division of Hematology, Department of Internal Medicine, The Ohio State University, Columbus, OH, USA

²The Ohio State University Comprehensive Cancer Center, Columbus, OH, USA

³Department of Internal Medicine, The Ohio State University, Columbus, OH, USA

⁴Alliance Statistics and Data Center, The Ohio State University, Columbus, OH, USA

⁵Monter Cancer Center, Hofstra Northwell School of Medicine, Lake Success, NY, USA

⁶Wake Forest Baptist Comprehensive Cancer Center, Winston Salem, NC, USA

⁷University of Alabama at Birmingham, Birmingham, AL, USA

⁸Dana-Farber Cancer Institute, Boston, MA, USA

[#] These authors contributed equally to this work.

Abstract

Sole trisomies of chromosomes 4, 8, 11, 13 and 21 account for 89–95% of all sole trisomies in adult AML patients. We analyzed clinical and molecular characteristics of 138 de novo AML patients with sole +4, +8, +11, +13 or +21, and compared them with AML patients with those trisomies occurring in addition to other chromosome abnormalities (non-sole trisomy) and with cytogenetically normal AML (CN-AML) patients. Mutations in methylation-related genes were most commonly observed within each sole trisomy group (+4, 55%; +8, 58%; +11, 71%; +13, 71%; +21, 75% of patients). Patients with sole trisomies, excluding +4, also had frequent mutations in spliceosome genes (+8, 43%; +11, 65%; +13, 65%; +21, 45% of patients). In contrast, +4 patients frequently had mutations in transcription factor genes (44%) and *NPM1*

*Bhavana Bhatnagar, Bhavana.Bhatnagar@osumc.edu.

Author contributions BB, A-KE, JK, KM, DN, CJW, JCB, and CDB contributed to the design and analysis of the study and the writing of the manuscript. A-KE, DP, and SO performed laboratory-based research. JSB performed the data processing. JK and DN performed statistical analysis. KM, JEK, BLP, AJC, RMS, JCB, 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 that they have no conflict of interest.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Supplementary information The online version of this article (<https://doi.org/10.1038/s41375-019-0560-3>) contains supplementary material, which is available to authorized users.

(36%). While 48% of patients with sole trisomies harbored mutations in a spliceosome gene, spliceosome mutations were observed in only 24% of non-sole trisomy ($n = 131$, $P < 0.001$) and 19% of CN-AML patients ($n = 716$, $P < 0.001$). Our data suggest that mutations affecting methylation-related genes are a molecular hallmark of sole trisomies. Mutations in spliceosome genes were also commonly observed in many sole trisomy patients and represent a novel finding in this cytogenetic subgroup.

Introduction

Acute myeloid leukemia (AML) is a clinically and biologically heterogeneous hematologic malignancy characterized by genetic and epigenetic alterations that result in differentiation arrest and clonal expansion of leukemic blasts in the blood and bone marrow (BM).

Recurrent chromosomal abnormalities are identified in 55–60% of newly diagnosed adults with AML and provide powerful prognostic information [1-3]. The acquisition of an additional, third copy, of an intact chromosome (trisomy) occurs as a sole abnormality in 7–8% of adult patients with AML [4, 5]. However, the molecular features of AML patients with these numerical abnormalities are not well understood.

Among sole trisomies, those involving chromosomes 4, 8, 11, 13 and 21 account for 89–95% of all sole trisomies in AML patients [4, 5], and each associates with variable disease outcomes [4-11]. For instance, patients with sole trisomy 8 (+8), the most frequently detected trisomy, have been reported to have an intermediate outcome according to some [2], and an adverse outcome according to other studies [9]. AML patients with isolated +4, +11, +13 and +21 had a poor prognosis in most [4, 7, 10-12], but not all [5], reports. Patients with any sole trisomy are categorized as having intermediate-risk disease according to the 2017 European LeukemiaNet (ELN) classification, provided that no other coexisting gene mutations lead to their reclassification into the favorable- or adverse-risk groups [13].

As the critical importance of gene mutations for disease biology and therapy response has been acknowledged by the inclusion of several mutations into the classifications of the World Health Organization and the ELN, the frequencies and impact of those mutations have been most extensively studied in patients with cytogenetically normal AML (CN-AML) [14-16]. However, more recently, research on the incidence and prognostic impact of gene mutations in patients with abnormal cytogenetics continues to emerge [16-20].

At present there are limited data pertaining to the mutational spectrum of sole trisomies in AML [21-27]. Moreover, there is limited information regarding clinical and molecular features of patients who harbor a trisomy among other chromosomal abnormalities (non-sole trisomy). In this study, we analyzed the mutational status of 138 newly diagnosed AML patients with sole +4, +8, +11, +13 or +21, treated on Cancer and Leukemia Group B (CALGB)/Alliance for Clinical Trials in Oncology (Alliance) trials, and compared clinical and molecular features among the sole trisomy groups, as well as with those of 131 non-sole trisomy and 716 CN-AML newly diagnosed patients.

Patients and methods

Patients and treatment

We examined 138 adult patients with de novo AML, median age 61 years (range, 19–84), with sole +4 ($n = 11$), +8 ($n = 65$), +11 ($n = 21$) +13 ($n = 21$) or +21 ($n = 20$). We also analyzed 131 patients who harbored trisomies of chromosomes 4, 8, 11, 13 and/or 21 and one or more additional chromosome abnormalities (hereafter referred to as non-sole trisomy), as well as 716 adult patients with CN-AML. Patients with acute promyelocytic leukemia were excluded from analysis. Almost all patients received intensive cytarabine and daunorubicin or idarubicin-based induction treatment on CALGB/Alliance trials [28–41]. Details regarding these trials are provided in the Supplementary Information.

Twenty-four of the sole trisomy patients underwent allogeneic hematopoietic stem cell transplantation in first complete remission (CR) and were excluded from outcome analyses. Institutional review board approval of all protocols was obtained before any research was performed. In accordance with the Declaration of Helsinki, patients provided study-specific written informed consent to participate in treatment studies (see Supplementary Information), CALGB 8461 (cytogenetic studies), CALGB 9665 (leukemia tissue bank), and CALGB 20202 (molecular studies), which involved collection of pretreatment BM aspirates and blood samples.

Cytogenetic analyses

Pretreatment cytogenetic analyses of BM and/or blood samples were performed on CALGB companion protocol 8461 in CALGB institutional cytogenetics laboratories using unstimulated short-term (24 and/or 48 h) cultures, and the results were confirmed by central karyotype review as previously reported [42]. In each patient with CN-AML, 20 BM metaphase cells were analyzed and the karyotype found to be normal. Viable cryopreserved BM or blood cells of patients enrolled onto the CALGB 9665 tissue bank protocol were stored for future analyses prior to starting treatment.

Mutational profiling

Mononuclear cells were enriched through Ficoll-Hypaque gradient centrifugation and cryopreserved until use. Genomic DNA was extracted using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany). The mutational status of 77 protein-coding genes was determined centrally at the Ohio State University by targeted amplicon sequencing using the MiSeq platform (Illumina, San Diego, CA). Additional details regarding molecular analyses are provided in the Supplementary Information [43–48].

Clinical endpoints and statistical analysis

Definitions of clinical endpoints, i.e., CR, disease-free survival (DFS) and overall survival (OS), are provided in the Supplementary Information. The main objective of this study was to provide comprehensive mutation analysis of de novo AML patients with sole +4, +8, +11, +13 or +21, and to compare the mutational landscapes among these patients. In addition, we also compared mutational landscape of all patients with sole trisomies combined into one group with those of AML patients with non-sole trisomies and of those with CN-AML.

Demographic and clinical features of these groups were also compared using the Fisher's exact for categorical variables and Wilcoxon rank-sum tests for two way comparisons or Kruskal–Wallis test for comparison of more than two groups for continuous variables [49]. Estimated probabilities of DFS and OS were calculated using the Kaplan–Meier method [50], and the log-rank test evaluated differences between survival distributions. All analyses were performed by the Alliance Statistics and Data Center on a database locked on January 9, 2017 using SAS 9.4.

Results

Clinical characteristics and molecular features of patients with sole trisomies

A comparison of pretreatment clinical and demographic characteristics for 138 de novo AML patients with sole +4, +8, +11, +13 and +21 is provided in Table 1. We detected a total of 444 gene mutations (median, three mutations/ patient, range, 0–9 mutations) in the 138 patients with a sole trisomy. Out of the 78 genes tested, 31 were mutated in 2% or more of sole trisomy patients, indicating a broad mutational spectrum (Supplementary Table S1). When grouped according to gene function [16], the identified gene mutations were found in all functional groups. However, the mutational profile was predominated by mutations in methylation-related genes (64% of sole trisomy patients; Supplementary Table S2), spliceosome-encoding genes (48%), and genes encoding kinases (37%) and transcription factors (36%; Fig. 1a).

Within the spliceosome-encoding genes, *SRSF2* was the most frequently mutated, accounting for more than half (56%) of all spliceosome gene mutations (Fig. 1b). Mutations in *U2AF1* (28%) accounted for the second most frequently mutated spliceosome gene (Fig. 1b).

We observed some differences in the mutation frequencies of specific spliceosome genes depending on the trisomy type (Figs. 1a and 2). *SRSF2* mutations were commonly detected in +13 patients, observed in 50% of patients, and were also frequent in +21 patients (35%) and +8 patients (28%), but not as often in +4 (18%) or +11 (15%) patients. *U2AF1* was mutated in 43% of +11 patients and in 15% of +8 patients (Fig. 2). Other genes that were commonly mutated in several of the trisomy groups included *DNMT3A*, *FLT3* (*FLT3*-ITD), *IDH2*, *RUNX1*, and *TET2* (Fig. 1a).

Frequencies of gene mutations in some functional groups differed among patients with specific sole trisomies (Table 2). Patients with +4 lacked mutations in chromatin remodeling genes which were observed in 14–30% of patients in the other sole trisomy groups (+8, 29%; +11, 14%; +13, 29%; and +21, 30% of patients). In addition, spliceosome mutations were less frequent in +4 patients, detected in 18% of patients, compared with the other sole trisomy groups (+8, 43%; +11, 65%; +13, 65%; and +21, 45% of patients). This suggests that the disease biology of sole +4 patients may differ from that of patients with other sole trisomies. *NPM1* mutations, which were found in patients with +4 (36%), +8 (15%), +13 (15%) and +21 (35%) groups, were absent in patients with +11 (Table 2). A comparison of clinical outcome of patients with sole +4, +8, +13 and +21 who harbored *NPM1* mutations with outcome of those without *NPM1* mutations revealed that patients with *NPM1* mutations

had a higher CR rate (94% vs 48%; $P < 0.001$) and a trend for a longer OS ($P = 0.06$; 3-year rates, 47% vs 19%). However, there was no significant difference in DFS between the groups ($P = 0.21$; 3-year rates, 31% vs 11 %).

Based on the assumption that mutations occurring with higher variant allele frequencies (VAFs) may represent earlier events during leukemogenesis than mutations with lower VAFs that presumably occur in a fraction of leukemic blasts at later stages [17], we examined the VAFs of cooccurring mutations in those sole trisomy patients who harbored two or more mutations. Among the most common gene mutations in patients with a sole trisomy, those that had high VAFs (>0.3) [27] in all or a vast majority of patients with these mutations, were *STAG2*, *TET2*, *NPM1*, *IDH2*, *TYK2*, *U2AF1*, *ETV6*, *SRSF2*, *DNMT3A*, *RUNX1*, and *BCOR* mutations. In contrast, low VAFs were found in a majority of patients harboring *FLT3*-TKD and mutations in the *NRAS* and *WT1* genes.

There were no significant differences in CR rates or OS among the five sole trisomy groups (Table 1), whereas there were too few patients who achieved a CR to evaluate DFS.

Molecular characteristics of younger and older sole trisomy patients

We also analyzed mutation patterns of patients with +8, +11, +13 and +21 separately in younger (<60 years) and older (≥ 60 years) patients. The group of +4 patients was too small to allow a similar analysis.

Patients with sole +8 constituted the majority of patients in this analysis ($n = 65$). Younger +8 patients ($n = 33$) had higher presenting white blood cell (WBC) counts (median, 20.7 vs $6.6 \times 10^9/l$, $P = 0.02$) and higher median percentage of blood blasts (37% vs 13%, $P = 0.004$) than older +8 patients ($n = 32$; Supplementary Table S3). There were several notable differences in the mutational landscape between younger and older sole +8 patients. Older +8 patients had more mutations in *SRSF2* (50% vs 6%, $P < 0.001$), *RUNX1* (34% vs 9%, $P = 0.02$) and *TET2* (25% vs 6%, $P = 0.04$) than younger +8 patients (Supplementary Table S4). Older +8 patients also had a higher number of mutations in spliceosome-encoding genes (69% vs 18%, $P < 0.001$), transcription factors (55% vs 9%, $P < 0.001$) and methylation-related genes (72% vs 45%, $P = 0.04$), and less mutations in genes affecting protein kinase function (21% vs 60%, $P = 0.004$) than younger +8 patients (Supplementary Table S5). Despite these notable molecular differences, there was no significant difference between older and younger +8 patients with respect to outcome (Supplementary Table S6).

Within the sole +13 group, younger patients ($n = 8$) had a lower pretreatment hemoglobin level (median, 7.7 vs 9.1 g/dl, $P = 0.03$), and higher percentages of blood (median, 73% vs 8%, $P = 0.009$) and BM (median, 87% vs 61%, $P < 0.001$) blasts compared with +13 patients aged ≥ 60 years ($n = 13$) (Supplementary Table S7). The only significant molecular difference between the groups was that no older patient harbored *FLT3*-ITD whereas 38% of the younger patients did ($P = 0.04$, data not shown). Notably, we observed *RUNX1* mutations in only 38% of our sole +13 patients, which is considerably less than percentages reported previously [22, 23, 25]. There were too few patients to compare outcomes between older and younger +13 patients.

There were no significant differences in clinical features (Supplementary Table S8) or mutation patterns (Supplementary Table S9) between younger ($n = 5$) and older ($n = 16$) patients with sole +11 or younger ($n = 12$) and older ($n = 8$) sole +21 patients (Supplementary Tables S10, S11). There were too few patients to perform outcome analyses.

Clinical features, mutation patterns, and outcome of sole trisomy patients compared with non-sole trisomy patients

We next analyzed pretreatment characteristics, mutation frequencies, and outcome of 131 non-sole trisomy patients and compared these features with those of patients with sole trisomies 4, 8, 11, 13 and 21 combined into one group.

Each AML patient in the non-sole trisomy group had one or more of the five most frequent trisomies (i.e., +4, +8, +11, +13 and +21). The most frequent was trisomy 8, detected in 80 (61%) of 131 patients, followed by trisomy 13 found in 27 (21%) patients, trisomy 21 in 26 (20%) patients, trisomy 4 in 20 (15%) patients, and trisomy 11 detected in 18 (14%) patients (since several patients harbored more than one trisomy, the aforementioned percentages add up to more than 100%). In addition, trisomies of other chromosomes were detected in 48 patients (37%) in the non-sole trisomy group, with trisomy 22 found in 15 (11%) patients, and trisomies of chromosomes 2, 3, 15 and 16 and an extra copy of chromosome Y detected in single patients each. Structural abnormalities were detected in 100 (76%) of the non-sole trisomy patients, and 67 (51%) patients had complex karyotypes (Supplementary Table S12).

Clinically, patients with non-sole trisomy had higher percentages of blood (median, 48% vs 37%, $P = 0.03$) and BM (median, 72% vs 65%, $P = 0.05$) blasts compared with patients with sole trisomy (Table 3). In addition, patients with non-sole trisomy more often presented with extramedullary disease (26% vs 14%, $P = 0.03$).

With respect to their molecular features, we observed both commonalities and differences between sole and non-sole trisomy patients. There were several genes that were mutated in 5% of patients in both sole trisomy and non-sole trisomy groups; including *ASXL1*, *BCOR*, *DNMT3A*, *FLT3-ITD*, *FLT3-TKD*, *IDH1*, *IDH2*, *NPM1*, *NRAS*, *RUNX1*, *SRSF2*, *STAG2*, *TET2*, and *ZRSR2* (Supplementary Table S1). Mutations in *FLT3-ITD* and *IDH2* were significantly more frequent in sole trisomy patients than in non-sole trisomy patients (*FLT3-ITD*, 25% vs 9%, $P < 0.001$; *IDH2*, 33% vs 8%, $P < 0.001$). Mutations in the *ETV6* gene were rare, affecting only 4% of sole trisomy patients, but they were absent in non-sole trisomy patients ($P = 0.03$). We observed a higher frequency of mutations in spliceosome genes, including *SRSF2* (29% vs 14%, $P = 0.003$) and *U2AF1* (14% vs 4%, $P = 0.003$, Supplementary Table S1), in sole trisomy patients compared with non-sole trisomy patients. In contrast, *TP53* mutations were recurrently observed in non-sole trisomy patients, but were rare in sole trisomy patients (17% vs 1%, $P < 0.001$). Of the 22 patients in the non-sole trisomy group who harbored *TP53* mutations, 21 (95%) had a complex karyotype, whereas the remaining patient (5%) had two abnormal clones, one with +9 and +13 and another with a sole +13.

Patients with a sole trisomy had a higher number of mutations compared with non-sole trisomy patients [median mutation number, 3 (range, 0–9) vs 2 (range, 0–8), $P < 0.001$]. Most of the mutations in the sole trisomy group occurred in genes encoding kinases, methylation-related, spliceosome, and transcription factors, whereas patients with non-sole trisomy had more mutations in genes involved in the RAS pathway as well as in tumor suppressor genes (Supplementary Table S2). There were no significant differences between the sole and non-sole trisomy patients with respect to CR rates, relapse rates, DFS, and OS (Supplementary Table S13).

Clinical features and mutation patterns of patients with sole trisomies compared with those of cytogenetically normal AML patients

We next compared clinical and molecular characteristics between sole trisomy patients and 716 CN-AML patients. Clinically, CN-AML patients, younger than patients with sole trisomy (median age, 53 vs 61 years, $P < 0.001$), were less often men (52% vs 67%, $P < 0.001$) and had higher presenting hemoglobin levels (median, 9.3 vs 8.9 g/dl, $P = 0.02$), WBC counts (median, 29.5 vs $9.6 \times 10^9/l$, $P < 0.001$), and blood blast percentages (median, 57% vs 37%, $P < 0.001$), and more often presented with extramedullary disease (29% vs 14%, $P < 0.001$; Table 3).

There were several differences in the mutation spectrum between sole trisomy patients and those with CN-AML. Genes that were more commonly mutated in sole trisomy patients compared with CN-AML patients included *ASXL1* (15% vs 6%, $P < 0.001$), *BCOR* (10% vs 4%, $P = 0.004$), *IDH2* (33% vs 13%, $P < 0.001$), *IKZF1* (4% vs 1%, $P = 0.04$), *RUNX1* (23% vs 10%, $P < 0.001$), *SRSF2* (29% vs 9%, $P < 0.001$), *STAG2* (8% vs 4%, $P = 0.04$), and *U2AF1* (14% vs 2%, $P < 0.001$; Supplementary Table S1). In contrast, we observed higher frequencies of *FLT3*-ITD (35% vs 25%, $P = 0.02$), biallelic mutations in *CEBPA* (12% vs 4%, $P = 0.01$), and mutations in *NPM1* (57% vs 18%, $P < 0.001$) and *SMC3* (4% vs 0%, $P = 0.006$) in CN-AML patients than in sole trisomy patients (Supplementary Table S1). Collectively, spliceosome-encoding genes were more frequently mutated in sole trisomy patients compared with CN-AML patients (48% vs 19%, $P < 0.001$; Supplementary Table S2) as expected.

There were also notable differences in disease outcomes and survival in CN-AML patients compared with sole trisomy patients. CN-AML patients had significantly better CR rates (79% vs 56%, $P < 0.001$) and longer DFS (1.1 vs 0.8 years, $P = 0.008$) and OS (1.5 vs 1.3 years, $P < 0.001$) (Supplementary Table S13) than sole trisomy patients.

Discussion

Sole trisomies are relatively frequent in patients with AML and are known to associate with inferior outcomes, particularly in older and nontransplanted patients [4, 51]. Among the sole trisomies, +4, +8, +11, +13 and +21 account for the vast majority of all sole trisomies in AML. The elucidation of both common and contrasting clinical and molecular features between the different recurrent trisomy groups may provide insights regarding disease biology within this specific subset of AML patients. To our knowledge, this is the largest series of patients with sole trisomies for whom comprehensive mutation analysis has been

performed, and also the first study that compares these molecular features with those of non-sole trisomy patients and patients with CN-AML.

We observed only a few clinical differences between patients within each trisomy group and although there were a number of mutations that occurred in at least one patient across all trisomy groups, specifically, *DNMT3A*, *FLT3*-ITD, *IDH2*, *NRAS*, *RUNX1*, *SRSF2*, and *TET2* mutations, there were a few notable molecular differences. Interestingly, mutations in the chromatin remodeling genes *ASXL1* and *BCOR* were found in all trisomy groups except for +4 patients, and mutations in spliceosome genes were less frequent in patients with +4 compared with the other trisomy groups, suggesting that +4 patients may have a separate disease biology. Another series of 13 AML patients with +4 reported the mutation status of a limited number of genes, but *FLT3* mutations were observed in five (four *FLT3*-ITD and one *FLT3*-TKD) out of 10 (50%) +4 patients [24], compared with a single patient in our cohort of +4 patients with *FLT3*-ITD. Otherwise, the mutation landscape was similar to our patients with +4.

With respect to their outcomes, our +4 patients appeared to have a lower CR rate compared with reports from Lazarevic et al. [5], Gupta et al. [7], and Bains et al. [23] (67% vs 86%, 76.7%, and 85%, respectively), but a longer median OS of 33.8 months, which is more consistent with results from Lazarevic et al. [5] and Bains et al. [24] (median OS, 30 and 28 months, respectively), and much higher than the median OS of 9 months reported by Gupta et al. [7]. The small numbers of patients in each of these studies precludes definitive interpretation of these data.

Patients with +11 had a distinct molecular profile (including frequent *U2AF1*, *IDH2*, *DNMT3A* mutations and the presence of *FLT3*-ITD and *MLL*-PTD) that has been previously described [27]. In contrast to the other trisomy groups, +11 patients also lacked mutations in the *NPM1* gene, which was mutated in 15–36% of the patients in the other trisomy groups. Absence of *NPM1* or *KIT* mutations in patients with +11 in our analysis is consistent with a previous study [52].

There were no major clinical or molecular differences between younger and older +13 patients. In contrast to other studies that have reported a high frequency of *RUNX1* mutations, ranging between 80 and 100%, in AML patients with +13, mainly in AML-M0 [22, 23], we observed *RUNX1* mutations in only 38% of our sole +13 patients.

As a unifying molecular feature of all patients with sole trisomy other than those with sole +4, the high frequency of methylation-related (65%) and spliceosome (50%) mutations may be one of the most significant findings from our analysis. A smaller analysis of patients with isolated trisomy 13 previously reported the high frequency (81%) of *SRSF2* mutations in this specific subgroup [25] compared with 50% in our study. Mutations in methylation-related genes are frequently observed in multiple cytogenetic groups as previously reported [19]. Spliceosome gene mutations, in contrast, are known to commonly occur in patients with myelodysplastic syndromes and myeloproliferative neoplasms [53–55] but have been infrequently reported in de novo AML patients [19]. Therefore, we consider the relatively high frequency of spliceosome gene mutations as a striking feature of our patient cohort.

Overall, there were not many differences in clinical characteristics between younger and older AML patients with sole trisomies, with the exception of patients with +8. As expected, patients with isolated trisomy 8 constituted the majority of sole trisomy patients in this analysis. Older +8 patients have been previously shown to have inferior outcomes compared with younger +8 patients [9]. We also examined the effect of age on molecular changes in patients with sole trisomies. In this series, older +8 patients were found to have a higher frequency of *SRSF2*, *RUNX1*, and *TET2* mutations compared with younger +8 patients. Notably, from a functional standpoint, the majority of mutations in older +8 patients occurred in methylation-related genes, spliceosome genes and genes-encoding transcription factors while younger +8 patients presented with gene mutations that most often affected kinase function. These molecular differences between older and younger +8 patients did not translate into significant differences in their CR rates, DFS, or OS.

When we compared the mutation landscape of sole trisomy patients with non-sole trisomy patients, we observed significant differences in the frequencies of *FLT3*-ITD and mutations in the *ETV6* and *IDH2* genes between patients with sole trisomy and those with non-sole trisomy. The main limitation of this comparison is the heterogeneous nature of the cytogenetics of the non-sole trisomies group. However, the high frequency of mutations in spliceosome-encoding genes was not seen in patients with additional chromosomal aberrations.

Compared with patients with CN-AML, sole trisomy patients more commonly displayed mutations in spliceosome-encoding genes, as well as *ASXL1*, *BCOR*, *IDH2*, *IKZF1*, *RUNX1*, and *STAG2* mutations. CN-AML patients, as expected, had higher frequencies of *CEBPA*, *FLT3*-ITD, *NPM1*, and *SMC3* mutations [15, 16, 56]. CN-AML patients in our series also had significantly higher CR rates and longer DFS and OS compared with those of sole trisomy patients.

In summary, this study is, to our knowledge, the largest to characterize the clinical and molecular features of AML patients with a sole trisomy. We observed a high frequency of mutations in methylation-related genes as well as in spliceosome-encoding genes in sole trisomy patients. With the exception of sole +4 patients, mutations in spliceosome genes were significantly more common in sole trisomy patients compared with non-sole trisomy AML and CN-AML patients and this represents the most novel finding of this analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors are grateful to the patients who consented to participate in the 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 Lisa J. Sterling for data management. This research was supported by the National Institutes of Health (NIH) grants R35 CA197734, 5P30 CA016058, U10 CA180861, CA101140, CA140158, CA196171, CA180821, CA180882, CA077658, UG1 CA233338, the Coleman Leukemia Research Foundation, the Pelotonia Fellowship Program (A-KE), and the D. Warren Brown Foundation.

References

1. Byrd JC, Mrózek K, Dodge RK, Carroll AJ, Edwards CG, Arthur DC, et al. Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). *Blood*. 2002;100:4325–36. [PubMed: 12393746]
2. Grimwade D, Walker H, Oliver F, Wheatley K, Harrison C, Harrison G, et al. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1612 patients entered into the MRC AML 10 trial. *Blood*. 1998;92:2322–33. [PubMed: 9746770]
3. Mrózek K, Heerema NA, Bloomfield CD. Cytogenetics in acute leukemia. *Blood Rev*. 2004;18:115–36. [PubMed: 15010150]
4. Farag SS, Archer KJ, Mrózek K, Vardiman JW, Carroll AJ, Pettenati MJ, et al. Isolated trisomy of chromosomes 8, 11, 13 and 21 is an adverse prognostic factor in adults with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B 8461. *Int J Oncol*. 2002;21:1041–51. [PubMed: 12370753]
5. Lazarevic VL, Rosso A, Juliusson G, Antunovic P, Derolf ÅR, Deneberg S, et al. Incidence and prognostic significance of isolated trisomies in adult acute myeloid leukemia: a population-based study from the Swedish AML registry. *Eur J Haematol*. 2017;98:493–500. [PubMed: 28152233]
6. Strati P, Daver N, Ravandi F, Pemmaraju N, Pierce S, Garcia-Manero G, et al. Biological and clinical features of trisomy 21 in adult patients with acute myeloid leukemia. *Clin Lymphoma Myeloma Leuk*. 2013;13:S276–S281. [PubMed: 23969309]
7. Gupta V, Minden MD, Yi Q-L, Brandwein J, Chun K. Prognostic significance of trisomy 4 as the sole cytogenetic abnormality in acute myeloid leukemia. *Leuk Res*. 2003;27:983–91. [PubMed: 12859991]
8. Schoch C, Haase D, Fonatsch C, Haferlach T, Löffler H, Schlegelberger B, et al. The significance of trisomy 8 in de novo acute myeloid leukaemia: the accompanying chromosome aberrations determine the prognosis. *Br J Haematol*. 1997;99:605–11. [PubMed: 9401073]
9. Byrd JC, Lawrence D, Arthur DC, Pettenati MJ, Tantravahi R, Qumsiyeh M, et al. Patients with isolated trisomy 8 in acute myeloid leukemia are not cured with cytarabine-based chemotherapy: results from Cancer and Leukemia Group B 8461. *Clin Cancer Res*. 1998;4:1235–41. [PubMed: 9607582]
10. Heinonen K, Mrózek K, Lawrence D, Arthur DC, Pettenati MJ, Stamberg J, et al. Clinical characteristics of patients with de novo acute myeloid leukaemia and isolated trisomy 11: a Cancer and Leukemia Group B study. *Br J Haematol*. 1998;101:513–20. [PubMed: 9633896]
11. Baer MR, Bloomfield CD. Trisomy 13 in acute leukemia. *Leuk Lymphoma*. 1992;7:1–6.
12. Lu Y, Yuan J, Wang H, Pei R, Chen Z, Jin J. [Clinical features and prognosis of acute myeloid leukemia with acquired trisomy 21]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi*. 2017;34:554–8. [PubMed: 28777858]
13. Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Buchner T, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129:424–47. [PubMed: 27895058]
14. Mrózek K, Marcucci G, Paschka P, Whitman SP, Bloomfield CD. Clinical relevance of mutations and gene-expression changes in adult acute myeloid leukemia with normal cytogenetics: are we ready for a prognostically prioritized molecular classification? *Blood*. 2007;109:431–48. [PubMed: 16960150]
15. Schlenk RF, Döhner K, Krauter J, Fröhling S, Corbacioglu A, Bullinger L, et al. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. *New Engl J Med*. 2008;358:1909–18. [PubMed: 18450602]
16. Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *New Engl J Med*. 2013;368:2059–74. [PubMed: 23634996]
17. Metzeler KH, Herold T, Rothenberg-Thurley M, Amler S, Sauerland MC, Görlich D, et al. Spectrum and prognostic relevance of driver gene mutations in acute myeloid leukemia. *Blood*. 2016;128:686–98. [PubMed: 27288520]

18. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, et al. Genomic classification and prognosis in acute myeloid leukemia. *New Engl J Med*. 2016;374:2209–21. [PubMed: 27276561]
19. Eisfeld A-K, Mrózek K, Kohlschmidt J, Nicolet D, Orwick S, Walker CJ, et al. The mutational oncoprint of recurrent cytogenetic abnormalities in adult patients with de novo acute myeloid leukemia. *Leukemia*. 2017;31:2211–8. [PubMed: 28321123]
20. Mrózek K, Eisfeld AK, Kohlschmidt J, Carroll AJ, Walker CJ, Nicolet D et al. Complex karyotype in de novo acute myeloid leukemia: typical and atypical subtypes differ molecularly and clinically. *Leukemia*. 2019;33:1620–34. [PubMed: 30737482]
21. Caligiuri MA, Strout MP, Schichman SA, Mrózek K, Arthur DC, Herzig GP, et al. Partial tandem duplication of *ALL1* as a recurrent molecular defect in acute myeloid leukemia with trisomy 11. *Cancer Res*. 1996;56:1418–25. [PubMed: 8640834]
22. Dicker F, Haferlach C, Kern W, Haferlach T, Schnittger S. Trisomy 13 is strongly associated with *AML1/RUNX1* mutations and increased *FLT3* expression in acute myeloid leukemia. *Blood*. 2007;110:1308–16. [PubMed: 17485549]
23. Silva FPG, Lind A, Brouwer-Mandema G, Valk PJM, Giphart-Gassler M. Trisomy 13 correlates with *RUNX1* mutation and increased FLT3 expression in AML-M0 patients. *Haematologica*. 2007;92:1123–6. [PubMed: 17650443]
24. Bains A, Lu G, Yao H, Luthra R, Medeiros LJ, Sargent RL. Molecular and clinicopathologic characterization of AML with isolated trisomy 4. *Am J Clin Pathol*. 2012;137:387–94. [PubMed: 22338050]
25. Herold T, Metzeler KH, Vosberg S, Hartmann L, Röllig C, Stölzel F, et al. Isolated trisomy 13 defines a homogeneous AML subgroup with high frequency of mutations in spliceosome genes and poor prognosis. *Blood*. 2014;124:1304–11. [PubMed: 24923295]
26. Becker H, Maharry K, Mrózek K, Volinia S, Eisfeld A-K, Radmacher MD, et al. Prognostic gene mutations and distinct gene- and microRNA-expression signatures in acute myeloid leukemia with a sole trisomy 8. *Leukemia*. 2014;28:1754–8. [PubMed: 24651097]
27. Eisfeld A-K, Kohlschmidt J, Mrózek K, Blachly JS, Nicolet D, Kroll K, et al. 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*. *Leukemia*. 2016;30:2254–8. [PubMed: 27435003]
28. Kolitz JE, George SL, Marcucci G, Vij R, Powell BL, Allen SL, et al. P-glycoprotein inhibition using valspodar (PSC-833) does not improve outcomes for patients under age 60 years with newly diagnosed acute myeloid leukemia: Cancer and Leukemia Group B study 19808. *Blood*. 2010;116:1413–21. [PubMed: 20522709]
29. Blum W, Sanford BL, Klisovic R, DeAngelo DJ, Uy G, Powell BL, et al. Maintenance therapy with decitabine in younger adults with acute myeloid leukemia in first remission: a phase 2 Cancer and Leukemia Group B study (CALGB 10503). *Leukemia*. 2017;31:34–9. [PubMed: 27624549]
30. Kolitz JE, George SL, Dodge RK, Hurd DD, Powell BL, Allen SL, et al. Dose escalation studies of cytarabine, daunorubicin, and etoposide with and without multidrug resistance modulation with PSC-833 in untreated adults with acute myeloid leukemia younger than 60 years: final induction results of Cancer and Leukemia Group B study 9621. *J Clin Oncol*. 2004;22:4290–301. [PubMed: 15514371]
31. Moore JO, George SL, Dodge RK, Amrein PC, Powell BL, Kolitz JE, et al. Sequential multiagent chemotherapy is not superior to high-dose cytarabine alone as postremission intensification therapy for acute myeloid leukemia in adults under 60 years of age: Cancer and Leukemia Group B study 9222. *Blood*. 2005;105:3420–7. [PubMed: 15572587]
32. Moore JO, Dodge RK, Amrein PC, Kolitz J, Lee EJ, Powell B, et al. Granulocyte-colony stimulating factor (filgrastim) accelerates granulocyte recovery after intensive postremission chemotherapy for acute myeloid leukemia with aziridinyl benzoquinone and mitoxantrone: Cancer and Leukemia Group B study 9022. *Blood*. 1997;89:780–8. [PubMed: 9028308]
33. Stone RM, Berg DT, George SL, Dodge RK, Paciucci PA, Schulman P, et al. Granulocyte-macrophage colony-stimulating factor after initial chemotherapy for elderly patients with primary acute myelogenous leukemia. *N Engl J Med*. 1995;332:1671–7. [PubMed: 7760868]

34. Mayer RJ, Davis RB, Schiffer CA, Berg DT, Powell BL, Schulman P, et al. Intensive postremission chemotherapy in adults with acute myeloid leukemia. *N Engl J Med*. 1994;331:896–903. [PubMed: 8078551]
35. Schiffer CA, Davis RB, Schulman P, Cooper B, Coyle T, Lee E, et al. Intensive post remission therapy of acute myeloid leukemia (AML) with cytoxan/etoposide (CY/VP16) and diazaquone/mitoxantrone (AZQ/MITO). *Blood*. 1991;78:460. (abstract 1829)
36. Attar EC, Johnson JL, Amrein PC, Lozanski G, Wadleigh M, DeAngelo DJ, et al. Bortezomib added to daunorubicin and cytarabine during induction therapy and to intermediate-dose cytarabine for consolidation in patients with previously untreated acute myeloid leukemia age 60 to 75 years: CALGB (Alliance) study 10502. *J Clin Oncol*. 2013;31:923–9. [PubMed: 23129738]
37. Lee EJ, George SL, Caligiuri M, Szatrowski TP, Powell BL, Lemke S, et al. Parallel phase I studies of daunorubicin given with cytarabine and etoposide with or without the multidrug resistance modulator PSC-833 in previously untreated patients 60 years of age or older with acute myeloid leukemia: results of Cancer and Leukemia Group B study 9420. *J Clin Oncol*. 1999;17:2831–9. [PubMed: 10561359]
38. Baer MR, George SL, Caligiuri MA, Sanford BL, Bothun SM, Mrózek K, et al. Low-dose interleukin-2 immunotherapy does not improve outcome of patients age 60 years and older with acute myeloid leukemia in first complete remission: Cancer and Leukemia Group B study 9720. *J Clin Oncol*. 2008;26:4934–9. [PubMed: 18591543]
39. Marcucci G, Moser B, Blum W, Stock W, Wetzler M, Kolitz JE, et al. A phase III randomized trial of intensive induction and consolidation chemotherapy ± oblimersen, a pro-apoptotic Bcl-2 antisense oligonucleotide in untreated acute myeloid leukemia patients >60 years old. *J Clin Oncol*. 2007;25:360s. (abstract 7012)
40. Roboz GJ, Mandrekar SJ, Desai P, Laumann K, Walker AR, Wang ES, et al. A randomized trial of 10 days of decitabine alone or with bortezomib in previously untreated older patients with acute myeloid leukemia: CALGB 11002 (Alliance). *Blood Adv*. 2018;2:3608–17. [PubMed: 30567725]
41. Uy GL, Mandrekar SJ, Laumann K, Marcucci G, Zhao W, Levis MJ, et al. A phase 2 study incorporating sorafenib into the chemotherapy for older adults with FLT3-mutated acute myeloid leukemia: CALGB 11001. *Blood Adv*. 2017;1:331–40. [PubMed: 29034366]
42. Mrózek K, Carroll AJ, Maharry K, Rao KW, Patil SR, Pettenati MJ, et al. Central review of cytogenetics is necessary for cooperative group correlative and clinical studies of adult acute leukemia: the Cancer and Leukemia Group B experience. *Int J Oncol*. 2008;33:239–44. [PubMed: 18636143]
43. Cibulskis K, Lawrence MS, Carter SL, Sivachenko A, Jaffe D, Sougnez C, et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nat Biotechnol*. 2013;31:213–9. [PubMed: 23396013]
44. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, et al. A framework for variation discovery and geno-typing using next-generation DNA sequencing data. *Nat Genet*. 2011;43:491–8. [PubMed: 21478889]
45. Kroll KW, Eisfeld A-K, Lozanski G, Bloomfield CD, Byrd JC, Blachly JS. MuCor: mutation aggregation and correlation. *Bioinformatics*. 2016;32:1557–8. [PubMed: 26803155]
46. Robinson JT, Thorvaldsdottir H, Winckler W, Guttman M, Lander ES, Getz G, et al. Integrative genomics viewer. *Nat Biotechnol*. 2011;29:24–6. [PubMed: 21221095]
47. Marcucci G, Maharry K, Radmacher MD, Mrózek K, Vukosavljevic T, Paschka P, et al. Prognostic significance of, and gene and microRNA expression signatures associated with, *CEBPA* mutations in cytogenetically normal acute myeloid leukemia with high-risk molecular features: a Cancer and Leukemia Group B Study. *J Clin Oncol*. 2008;26:5078–87. [PubMed: 18809607]
48. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127:2391–405. [PubMed: 27069254]
49. Vittinghoff E, Glidden DV, Shiboski SC, McCulloch CE. Regression methods in biostatistics: linear, logistic, survival and repeated measures models. New York, NY, USA: Springer; 2005.
50. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc*. 1958;53:457–81.

51. Chevallier P, Labopin M, Nagler A, Ljungman P, Verdonck LF, Volin L, et al. Outcome after allogeneic transplantation for adult acute myeloid leukemia patients exhibiting isolated or associated trisomy 8 chromosomal abnormality: a survey on behalf of the ALWP of the EBMT. *Bone Marrow Transplant.* 2009;44:589–94. [PubMed: 19349953]
52. Alseraye FM, Zuo Z, Bueso-Ramos C, Wang S, Medeiros LJ, Lu G. Trisomy 11 as an isolated abnormality in acute myeloid leukemia is associated with unfavorable prognosis but not with an *NPM1* or *KIT* mutation. *Int J Clin Exp Pathol.* 2011;4:371–7. [PubMed: 21577323]
53. Je EM, Yoo NJ, Kim YJ, Kim MS, Lee SH. Mutational analysis of splicing machinery genes *SF3B1*, *U2AF1* and *SRSF2* in myelodysplasia and other common tumors. *Int J Cancer.* 2013;133:260–5. [PubMed: 23280334]
54. Malcovati L, Papaemmanuil E, Bowen DT, Boulwood J, Della Porta MG, Pascutto C, et al. Clinical significance of *SF3B1* mutations in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms. *Blood.* 2011;118:6239–46. [PubMed: 21998214]
55. Papaemmanuil E, Cazzola M, Boulwood J, Malcovati L, Vyas P, Bowen D, et al. Somatic *SF3B1* mutation in myelodysplasia with ring sideroblasts. *New Engl J Med.* 2011;365:1384–95. [PubMed: 21995386]
56. Thota S, Viny AD, Makishima H, Spitzer B, Radivoyevitch T, Przychodzen B, et al. Genetic alterations of the cohesin complex genes in myeloid malignancies. *Blood.* 2014;124:1790–8. [PubMed: 25006131]

Fig. 1.
a Oncoprint of mutations found in patients with de novo acute myeloid leukemia and sole trisomies of chromosomes 4, 8, 11, 13 and 21. Only mutations that were found in at least three patients (or 2% of patients) in at least one sole trisomy group are included in the oncoprint. Rows represent individual patients, who are arranged with respect to their cytogenetic findings. Columns correspond to single genes that are clustered into the previously described functional groups [16]. The mutation status of each gene is indicated by color: red = mutated, white = wild type, gray = data missing. **b** Pie chart illustrating

percentages of specific spliceosome gene mutations in patients with sole trisomies of chromosomes 4, 8, 11, 13 and 21

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 2. Oncoprint of mutations found in patients with de novo acute myeloid leukemia and sole trisomy of chromosomes 4, 8, 11, 13 or 21. Mutated genes are clustered into the previously described functional groups [16]. Only genes found mutated in at least three patients (or 2% of patients) in at least one of the trisomy groups are included in the oncoprint. The frequency of each mutation detected within a given cytogenetic subset is indicated by color as shown in the legend

Table 1

Summary of clinical characteristics for trisomy patient groups

Characteristic	Sole + 4 <i>n</i> = 11	Sole + 8 <i>n</i> = 65	Sole + 11 <i>n</i> = 21	Sole + 13 <i>n</i> = 21	Sole + 21 <i>n</i> = 20	<i>P</i> ^a
Age, years						0.004
Median	48	59	69	66	51	
Range	19–79	20–81	25–84	36–79	21–77	
Sex, <i>n</i> (%)						0.11
Male	5 (45)	45 (69)	16 (76)	17 (81)	10 (50)	
Race, <i>n</i> (%)						0.89
White	10 (91)	57 (88)	20 (95)	19 (90)	17 (85)	
Non-white	1 (9)	8 (12)	1 (5)	2 (10)	3 (15)	
Hemoglobin, g/dl						0.30
Median	8.7	9.1	8.2	8.7	8.5	
Range	8.0–10.1	5.0–14.1	4.3–11.0	5.2–11.2	6.4–13.3	
Platelet count, ×10 ⁹ /l						0.30
Median	65	54	51	74	58	
Range	34–177	8–233	11–673	4–256	17–341	
WBC count, ×10 ⁹ /l						0.30
Median	8.2	9.5	25.3	5.4	21.5	
Range	0.6–81.9	0.6–268.0	0.7–131.4	1.1–144.6	1.4–41.0	
Blood blasts, %						0.14
Median	57	26	41	55	35	
Range	10–93	0–97	0–96	0–95	4–82	
Bone marrow blasts, %						0.26
Median	60	65	62	77	54	
Range	15–88	18–94	21–88	30–95	24–90	
Extramedullary involvement, <i>n</i> (%)	1 (10)	9 (15)	2 (11)	4 (19)	2 (12)	0.98
CR rate, <i>n</i> (%) ^b	6 (67)	27 (55)	5 (31)	11 (65)	10 (71)	0.19
Relapse rate, <i>n</i> (%) ^b	5 (83)	21 (78)	4 (80)	9 (82)	8 (80)	1.00
Disease-free survival ^b						ND ^c

Characteristic	Sole + 4 n = 11	Sole + 8 n = 65	Sole + 11 n = 21	Sole + 13 n = 21	Sole + 21 n = 20	P ^a
Median, years	0.6	0.6	1.0	0.8	1.0	
% disease-free at 1 year (95% CI)	17 (1–52)	41 (23–58)	60 (13–88)	45 (17–71)	50 (18–75)	
% disease-free at 3 years (95% CI)	0	15 (5–30)	0	18 (3–44)	30 (7–58)	
Overall survival ^b						0.12
Median, years	2.8	1.0	0.7	0.9	1.9	
% alive at 1 year (95% CI)	75 (31–93)	75 (31–93)	38 (15–60)	47 (23–68)	71 (41–88)	
% alive at 3 years (95% CI)	50 (15–77)	50 (15–77)	0	29 (11–51)	43 (18–66)	

CI confidence interval, CR complete remission, ND not done, WBC white blood cell

^a P-values presented are for the comparison of the five trisomy groups. P-values for categorical variables are from Fisher's exact test. P-values for continuous variables are from Kruskal-Wallis test and P-values for the time to event variables are from the log-rank test

^b Patients who underwent allogeneic stem cell transplantation in first CR are not included in outcome analyses

^c P-value was not calculated because too few patients achieved a CR

Table 2

Summary of molecular functional groupings for trisomy patient groups

Functional groups	Sole + 4 <i>n</i> = 11	Sole + 8 <i>n</i> = 65	Sole + 11 <i>n</i> = 21	Sole + 13 <i>n</i> = 21	Sole + 21 <i>n</i> = 20	<i>P</i> ^a
Chromatin remodeling, <i>n</i> (%)						0.17
Mutated	0 (0)	19 (29)	3 (14)	6 (29)	6 (30)	
Wild type	11 (100)	46 (71)	18 (86)	15 (71)	14 (70)	
Cohesin complex, <i>n</i> (%)						0.39
Mutated	1 (9)	10 (15)	3 (14)	3 (14)	0 (0)	
Wild type	10 (91)	55 (85)	18 (86)	18 (86)	20 (100)	
Kinases, <i>n</i> (%)						0.25
Mutated	3 (27)	24 (41)	11 (52)	4 (21)	6 (30)	
Wild type	8 (73)	34 (59)	10 (48)	15 (79)	14 (70)	
Methylation-related, <i>n</i> (%)						0.53
Mutated	6 (55)	38 (58)	15 (71)	15 (71)	15 (75)	
Wild-type	5 (45)	27 (42)	6 (29)	6 (29)	5 (25)	
<i>NPM1</i> , <i>n</i> (%)						0.01
Mutated	4 (36)	10 (15)	0 (0)	3 (15)	7 (35)	
Wild type	7 (64)	55 (85)	21 (100)	17 (85)	13 (65)	
RAS pathway, <i>n</i> (%)						0.77
Mutated	2 (18)	9 (14)	5 (24)	3 (14)	2 (10)	
Wild type	9 (82)	56 (86)	16 (76)	18 (86)	18 (90)	
Spliceosome, <i>n</i> (%)						0.06
Mutated	2 (18)	28 (43)	13 (65)	13 (65)	9 (45)	
Wild type	9 (82)	37 (57)	7 (35)	7 (35)	11 (55)	
Transcription factors, <i>n</i> (%)						0.55
Mutated	4 (44)	20 (32)	3 (25)	8 (47)	7 (47)	
Wild type	5 (56)	43 (68)	9 (75)	9 (53)	8 (53)	
Tumor suppressor, <i>n</i> (%)						0.87
Mutated	1 (9)	7 (11)	1 (5)	1 (5)	1 (5)	
Wild type	10 (91)	58 (89)	20 (95)	20 (95)	19 (95)	

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Mutation status of the functional groups is defined by 1 gene mutation detected in 1 of the respective genes assigned to the functional groups [16]: chromatin remodeling (*ASXL1*, *BCOR*, *BCORL1*, *EZH2*, and *SMARCA2*), cohesin complex (*RAD21*, *SMC1A*, *SMC3* and *STAG2*), kinases (*AXL*, *FLT3* internal tandem duplications [*FLT3*-ITD], *FLT3* tyrosine kinase domain mutations [*FLT3*-TKD], *KIT*, and *TYK2*), methylation-related (*DNMT3A*, *IDH1/2*, and *TET2*), NPM1 (*NPM1*), the RAS pathway (*CBL*, *KRAS*, *NRAS*, and *PTPN11*), spliceosome (*SF3B1*, *SRSF2*, *U2AF1*, and *ZRSR2*), transcription factors (*CEBPA*, *ETV6*, *GATA2*, *IKZF1*, *NOTCH1*, and *RUNX1*), and tumor suppressors (*PHF6*, *TP53*, and *WT1*)

^a *P*-values are from Fisher's exact test comparing all five trisomy groups

Table 3

Summary of clinical characteristics for sole trisomy versus non-sole trisomy patients or sole trisomy versus CN-AML patients

Characteristic	Sole trisomies <i>n</i> = 138	Non-sole trisomies <i>n</i> = 131	<i>P</i> ^{<i>a,b</i>}	CN-AML <i>n</i> = 716	<i>P</i> ^{<i>a,c</i>}
Age, years			0.07		<0.001
Median	61	58		53	
Range	19–84	17–84		17–83	
Sex, <i>n</i> (%)			0.70		<0.001
Male	93 (67)	85 (65)		370 (52)	
Female	45 (33)	46 (35)		346 (48)	
Race, <i>n</i> (%)			0.46		0.64
White	123 (89)	108 (86)		638 (91)	
Non-white	15 (11)	18 (14)		66 (9)	
Hemoglobin, g/dl			0.11		0.02
Median	8.9	9.2		9.3	
Range	4.3–14.1	3.0–14.7		4.2–25.1	
Platelet count, ×10 ⁹ /l			0.59		0.93
Median	60	57		57	
Range	4–673	4–376		5–850	
WBC count, ×10 ⁹ /l			0.09		<0.001
Median	9.6	14.6		29.5	
Range	0.6–268.0	0.8–320.0		0.6–475.0	
Blood blasts, %			0.03		<0.001
Median	37	48		57	
Range	0–97	0–99		0–99	
Bone marrow blasts, %			0.05		0.07
Median	65	72		68	
Range	15–95	4–98		0–99	
Extramedullary involvement, <i>n</i> (%)	18 (14)	31 (26)	0.03	200 (29)	<0.001

WBC white blood cell

^{*a*} *P*-values for categorical variables are from Fisher's exact test and for continuous variables are from Wilcoxon rank-sum test

^{*b*} *P*-values are comparing sole trisomy vs non-sole trisomy patients

^{*c*} *P*-values are comparing sole trisomy vs CN-AML patients