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ORIGINAL ARTICLE

The mutational oncoprint of recurrent cytogenetic abnormalities in adult patients with *de novo* acute myeloid leukemia

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Recurrent chromosomal abnormalities and gene mutations detected at the time of diagnosis of acute myeloid leukemia (AML) are associated with particular disease features, treatment response and survival of AML patients, and are used to denote specific disease entities in the World Health Organization classification of myeloid neoplasms and acute leukemia. However, large studies that integrate cytogenetic and comprehensive mutational information are scarce. We created a comprehensive oncoprint of mutations associated with recurrent cytogenetic findings by combining the information on mutational patterns of 80 cancer- and leukemia-associated genes with cytogenetic findings in 1603 adult patients with *de novo* AML. We show unique differences in the mutational profiles among major cytogenetic subsets, identify novel associations between recurrent cytogenetic abnormalities and both specific gene mutations and gene functional groups, and reveal differences in cytogenetic and mutational features between patients younger than 60 years and those aged 60 years or older. The identified associations between cytogenetic and molecular genetic data may help guide mutation testing in AML, and result in more focused application of targeted therapy in patients with *de novo* AML.

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INTRODUCTION

Acute myeloid leukemia (AML) is classified based on the morphology of the leukemic blasts and associated dysplasia, surface and intracellular protein expression, and specific chromosomal abnormalities.[1] In addition, gene mutations contribute to disease features, treatment response and survival, and promising examples show that some of those mutations might be used as therapeutic targets.[1–4] The recent sequencing efforts of The Cancer Genome Atlas (TCGA)[2] and other studies[3–5] have begun to uncover the mutational landscape of AML. It is widely acknowledged that some gene mutations are strongly enriched in certain cytogenetic subsets of AML,[6] such as, *NPM1* and biallelic *CEBPA* mutations in cytogenetically normal AML (CN-AML),[2,6,7] *TP53* mutations in complex karyotype AML (CK-AML)[8,9] and *KIT* mutations in core-binding factor AML (CBF-AML).[6,10,11] These gene mutations in some cases contribute to disease features, treatment response and overall survival.[6,9,11,12] Furthermore, certain gene mutations may be used as therapeutic targets in cytogenetic subgroups, as demonstrated by the integration of tyrosine kinase inhibitors into clinical trials in patients with CBF-AML with *KIT* mutations.[1] Despite these promising examples, there is a paucity of sufficiently large studies that correlate cytogenetics with extensive mutational information in AML patients.[1–3,6]

To depict the wealth of genomic information in a graphical way, the concept of an oncoprint has recently been developed.[13,14] The oncoprint is a concise and compact graphical summary of genomic alterations (that is, gene mutations and cytogenetic abnormalities) in multiple genes across a set of tumor samples.[14] Consequently, we created an oncoprint of 80 mutations associated with recurrent cytogenetic findings in one of the largest cohorts of 1603 adult patients with *de novo* AML other than acute promyelocytic leukemia.

MATERIALS AND METHODS

Patients, treatment and cytogenetic studies

Pretreatment bone marrow (BM) or peripheral blood (PB) samples containing $\geqslant$20% leukemic blasts were obtained from 1603 adult patients diagnosed with *de novo* AML, 1080 of whom were aged $<$60 years and 523 were aged 60 years or older. All patients were enrolled onto Cancer and Leukemia Group B (CALGB) companion protocols CALGB 8461 (cytogenetic studies), CALGB 9665 (leukemia tissue bank) and CALGB 20202 (molecular studies), and were similarly treated on CALGB/Alliance for Clinical Trials in Oncology (Alliance) trials.[15–25] Patients with acute

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promyelocytic leukemia, AML evolving from an antecedent hematologic malignancy or treatment-related AML were not included in the study. Cytogenetic analyses of pretreatment BM and/or PB samples were performed by institutional laboratories approved by the CALGB/Alliance using unstimulated short-term (24- to 48-h) cultures. Twenty or more BM metaphase cells were analyzed in each patient designated as having a normal karyotype. Cytogenetic results were confirmed by central karyotype review.[26] All patients provided written informed consent, and study protocols were in accordance with the Declaration of Helsinki and approved by the Institutional Review Boards at each participating center.

Molecular analyses

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 79 protein coding genes was determined centrally at The Ohio State University by targeted amplicon sequencing using the MiSeq platform (Illumina, San Diego, CA, USA). In brief, DNA library preparations were performed according to the manufacturer's directions. Samples were pooled and run on the MiSeq machine using the Illumina MiSeq Reagent Kit v3. Sequenced reads were aligned to the hg19 genome build using the Illumina Isis Banded Smith-Waterman aligner. Single nucleotide variant and indel calling were performed using, respectively, MuTect and VarScan.[27,28] All called variants underwent visual inspection of the aligned reads using the Integrative Genomics Viewer (Broad Institute, Cambridge, MA, USA).[29] Variant filtering was done using the MuCor algorithm.[30] The variant allele fraction (VAF) cut-off was set to 0.10 for inclusion into the analyses. To distinguish between driver and passenger mutations, the analysis was repeated with a VAF cut-off of 0.30 (Supplementary Figure S1). Variants (missense, nonsense or frameshift) were considered to be mutations if they were not reported in the 1000 Genome database, dbSNP137 or dbSNP142. In the instances when <15 reads were present, the gene mutation status was determined not to be evaluable. Testing for the presence or absence of *FLT3* internal tandem duplication (*FLT3*-ITD) was performed using the Pindel algorithm on the targeted sequencing data. In addition to the 79 gene sequencing panel, testing for *CEBPA* mutations was performed with Sanger sequencing methods as previously described,[7] thus resulting in a total of 80 genes whose mutational status was assessed in our study. In accordance with the revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia,[31] only patients with biallelic *CEBPA* mutations were considered as *CEBPA* mutated. Gene mutations were assigned to nine previously described[2] functional groups: (1) chromatin remodeling (*ASXL1*, *BCOR*, *BCORL1*, *EZH2* and *SMARCA2*); (2) cohesin complex (*RAD21*, *SMC1A*, *SMC3* and *STAG2*); (3) kinases {*AXL*, *FLT3* [both *FLT3*-ITD and tyrosine kinase domain mutations (*FLT3*-TKD)], *KIT* and *TYK2*}; (4) methylation-related (*DNMT3A*, *IDH1*, *IDH2*, and *TET2*); (5) *NPM1* (*NPM1*); (6) RAS pathway (*CBL*, *KRAS*, *NRAS* and *PTPN11*); (7) spliceosome (*SF3B1*, *SRSF2*, *U2AF1* and *ZRSR2*); (8) transcription factors (*CEBPA*, *ETV6*, *IKZF1*, *GATA2*, *NOTCH1* and *RUNX1*); and (9) tumor suppressors (*PHF6*, *TP53* and *WT1*).

Statistical analyses

Baseline clinical characteristics among patients belonging to five major cytogenetic groups (please see below) were compared using Fisher's exact test for categorical variables and the Wilcoxon rank sum test for continuous variables (Supplementary Table S1). Fisher's exact test was used for comparisons of frequencies of specific mutations (Supplementary Table S2) and frequencies of gene mutations assigned to the functional groups (Table 1) among the five major cytogenetic groups. Fisher's exact test was also used for comparisons of frequencies of gene mutations assigned to the functional groups between all younger and older AML patients (Table 2), and between the younger and older AML patients classified into five major cytogenetic groups (Supplementary Tables S3–S8). We adjusted *P*-values to control for per family error rate and a *P*-value of $\leqslant 0.05$ was considered as statistically significant. With power of 80% we can detect up to a 10% difference in gene mutations assigned to a functional group between groups. The dataset was locked on 21 January, 2016. Data collection and statistical analyses were performed by the Alliance Statistics and Data Center.

RESULTS AND DISCUSSION

Cytogenetically, the AML patients were classified into 34 specific karyotype subgroups[32,33] (listed in individual lines in Figure 1), which were then assigned to one of five major cytogenetic groups: (1) CN-AML, indicated by green color in Figures 1–4 and Supplementary Figure S1; (2) CK-AML, indicated by red (defined by the presence of $\geqslant 3$ chromosome aberrations that did not include balanced (reciprocal) translocations or inversions,[34,35] and subdivided into 'typical' complex karyotype, that is, containing abnormalities resulting in loss of chromosome material from 5q, 7q and/or 17p, and 'atypical' complex karyotype that does not contain the aforementioned abnormalities[35]); (3) CBF-AML, indicated by gray [that is, patients with t(8;21)(q22;q22) or inv (16)(p13.1q22)/t(16;16)(p13.1;q22)]; (4) AML with balanced translocations or inversions other than those associated with CBF-AML (irrespective of karyotype complexity), indicated by yellow; and (5) AML with unbalanced chromosomal abnormalities detected in non-complex karyotypes, indicated by blue color (that is, trisomies, monosomies and deletions). Using a targeted next-generation sequencing panel supplemented by Sanger sequencing, we analyzed the mutational status of 80 cancer- and leukemia-associated genes. The mutated genes were assigned to the nine aforementioned functional groups, which were defined based on the genes' biologic functions.[2] This comprehensive framework enabled us to characterize the mutational features of most recurrent cytogenetic abnormalities seen in AML patients (Figures 1 and 2; Table 1; Supplementary Table S2).

We detected a total of 4390 gene mutations in our patient cohort, with a median of three mutations (range, 0–9 mutations) detected per patient. Among all 1603 patients, the most frequently affected functional groups were the methylation-related group, with 45% of patients harboring mutations in at least one methylation-related gene, kinases (36% of patients) and *NPM1* (31% of patients), and the least frequently affected were the cohesin complex (12%) and tumor suppressors (14%) (Table 1).

We also investigated the chronology of mutations during clonal evolution stratified by functional groups: based on mutation VAFs we analyzed whether they were the first, second, third, fourth, fifth or later mutational event within a given patient sample. Our results indicate that mutations in tumor suppressor genes, the cohesin complex or the spliceosome (if present) are most commonly the first mutational events (in 47%, 43% and 41% of mutated patients, respectively), whereas kinase mutations and mutations in RAS genes were only rarely the mutations with the highest VAF in a patient sample (21% and 22%, respectively), indicating that they might arise later during clonal evolution (Supplementary Figure S2).

The mutational oncoprint revealed key differences in gene mutation frequencies among the five major cytogenetic groups (Figures 1 and 2; Table 1). Among the most striking was the high incidence of mutations in methylation-related genes in patients with CN-AML, CK-AML or unbalanced abnormalities (cytogenetic subgroups #1, #2–3 and #19–34, respectively), in whom methylation-related genes constituted a functional group that was either the most or second most often affected by mutations. In contrast, these mutations were almost absent in CBF-AML patients (cytogenetic subgroups #4–5) or rather rare in patients harboring non-CBF-AML-related balanced translocations or inversions (cytogenetic subgroups #6–18). Furthermore, mutations in spliceosome genes were frequent in patients with unbalanced abnormalities (cytogenetic subgroups #19–34), especially those with sole trisomy of chromosomes 4, 8, 11, 13 or 21 (cytogenetic subgroups #19-23).

CN-AML patients had a broad mutational spectrum, involving all functional groups (green color; subgroup #1, Figures 1 and 2; Table 1). Mutations in methylation-related genes (61% of patients),

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Table 1. Frequencies of gene mutations assigned to the nine functional groups detected in patients with *de novo* acute myeloid leukemia, listed for the total patient cohort and separately for the five major cytogenetic groups

Functional group[a]	*All* n = *1603*	*CN-AML* n = *716*	*Complex karyotype* n = *137*	*CBF-AML* n = *177*	*Balanced rearrangements* n = *224*	*Unbalanced abnormalities* n = *349*	*P*[b]
Chromatin remodeling, n *(%)*							< 0.001
Mutated	265 (17)	105 (15)	17 (12)	16 (9)	42 (19)	85 (24)	
Wild-type	1338 (83)	611 (85)	120 (88)	161 (91)	182 (81)	264 (76)	
Cohesin complex, n *(%)*							< 0.001
Mutated	191 (12)	110 (15)	9 (7)	10 (6)	21 (9)	41 (12)	
Wild-type	1412 (88)	606 (85)	128 (93)	167 (94)	203 (91)	308 (88)	
Kinases, n *(%)*							< 0.001
Mutated	533 (36)	318 (46)	19 (15)	56 (32)	45 (22)	95 (30)	
Wild-type	968 (64)	369 (54)	106 (85)	118 (68)	156 (78)	219 (70)	
Methylation-related, n *(%)*							< 0.001
Mutated	714 (45)	438 (61)	43 (31)	4 (2)	41 (18)	188 (54)	
Wild-type	889 (55)	278 (39)	94 (69)	173 (98)	183 (82)	161 (46)	
NPM1, n *(%)*							< 0.001
Mutated	492 (31)	407 (57)	6 (5)	0 (0)	11 (5)	68 (20)	
Wild-type	1086 (69)	305 (43)	123 (95)	177 (100)	207 (95)	274 (80)	
RAS pathway, n *(%)*							0.008
Mutated	389 (24)	174 (24)	19 (14)	51 (29)	65 (29)	80 (23)	
Wild-type	1214 (76)	542 (76)	118 (86)	126 (71)	159 (71)	269 (77)	
Spliceosome, n *(%)*							< 0.001
Mutated	321 (20)	136 (19)	21 (16)	11 (6)	41 (18)	112 (32)	
Wild-type	1271 (80)	578 (81)	113 (84)	165 (94)	182 (82)	233 (68)	
Transcription factors, n *(%)*							< 0.001
Mutated	301 (21)	131 (21)	22 (18)	8 (5)	39 (21)	101 (33)	
Wild-type	1122 (79)	502 (79)	101 (82)	169 (95)	143 (79)	207 (67)	
Tumor suppressors, n *(%)*							< 0.001
Mutated	223 (14)	84 (12)	63 (46)	12 (7)	26 (12)	38 (11)	
Wild-type	1380 (86)	632 (88)	74 (54)	165 (93)	198 (88)	311 (89)	

Abbreviations: CBF-AML, core-binding factor AML; CN-AML, cytogenetically normal acute myeloid leukemia; *n*, number. [a]Chromatin remodeling is mutated if *ASXL1*, *BCOR*, *BCORL1*, *EZH2* or *SMARCA2* is mutated. Cohesin complex is mutated if *RAD21*, *SMC1A*, *SMC3* or *STAG2* is mutated. Kinases is mutated if *AXL*, *FLT3*-ITD, *FLT3*-TKD, *KIT* or *TYK2* is mutated. Methylation-related is mutated if *DNMT3A*, *IDH1*, *IDH2*, or *TET2* is mutated. *NPM1* is mutated if *NPM1* is mutated. *RAS* pathway is mutated if *CBL*, *KRAS*, *NRAS* or *PTPN11* is mutated. Spliceosome is mutated if *SF3B1*, *SRSF2*, *U2AF1* or *ZRSR2* is mutated. Transcription factors is mutated if *CEBPA*, *ETV6*, *IKZF1*, *GATA2*, *NOTCH1* or *RUNX1* is mutated. Tumor suppressors is mutated if *PHF6*, *TP53* or *WT1* is mutated. [b]*P*-values are from Fisher's exact test and compare the five major cytogenetic groups.

NPM1 (57%), and kinases (46%) dominated the mutation pattern, which is consistent with recent findings of Metzeler *et al.*[4]

In concordance with previous reports,[8,9] *TP53* mutations were the most common ones in patients with CK-AML (red color; subgroups #2 and 3), being found in 38% of the patients (Figures 1 and 2; Supplementary Table S2). The second most common mutation, *TET2*, was detected only half as often (17%). We found differences in the mutation patterns associated with typical vs atypical complex karyotypes (subgroup #2 vs #3; Figures 1 and 2). The *TP53* mutations were present in 52% of patients with typical CK-AML compared with only 5% of patients with atypical CK-AML ($P < 0.001$). Conversely, patients with atypical complex karyotype harbored more often mutations in another tumor suppressor gene, *PHF6* (15% vs 2%, $P = 0.03$). Furthermore, patients with atypical CK-AML had a broader spectrum of recurrent mutations, with nine mutations, namely *FLT3*-TKD and mutations in the *DNMT3A, IDH2*, *NPM1*, *NRAS*, *PHF6*, *RUNX1*, *TET2* and *ZRSR2* genes, occurring in $\geqslant 10\%$ of patients as compared with mutations in only three genes, *DNMT3A*, *TP53* and *TET2*, detected in $\geqslant 10\%$ of patients with typical CK-AML (Figures 1 and 2), suggesting the existence of important biological differences between typical and atypical CK-AML.

Patients with CBF-AML (gray color; subgroups #4 and #5) had very few detectable mutations in addition to their respective disease-defining *RUNX1-RUNX1T1* or *CBFB-MYH11* gene fusions, with a median of one mutation (range, 0–5) vs two (range, 0–7) or three mutations (range, 0–9) detected in AML patients belonging to the remaining major cytogenetic groups ($P < 0.001$; Supplementary Table S2). Specifically, CBF-AML was characterized by a complete absence of *NPM1* and biallelic *CEBPA* mutations (Supplementary Table S2), and a paucity of mutations in methylation-related genes, which were found in only 2% of CBF-AML patients compared with 51% of the remaining AML cohort ($P < 0.001$, Table 1). As previously reported,[10,11,36] the most frequently mutated genes in patients with CBF-AML were *NRAS* (found in 23% of CBF-AML patients) and *KIT* (20%). Mutations in *CCND2*, detected in 12% of t(8;21) patients, have just recently been discovered as a novel mutational feature of AML with t(8;21).[37,38] We also noted an enrichment of mutations in cohesin complex genes in patients with t(8;21), 15% of whom carried these mutations, as opposed to patients with inv(16), none of whom did. This is in line with the report from Duployez *et al.*,[36] who detected cohesin complex mutations in 18% of their t(8;21) patients and in none of those with inv(16). They also reported an enrichment of

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Table 2. Frequencies of gene mutations assigned to the functional groups detected in patients with *de novo* acute myeloid leukemia aged < 60 years and patients ⩾ 60 years of age

Functional group[a]	*Patients aged < 60 years* n = *1080*	*Patients aged ⩾ 60 years* n = *523*	*P*[b]
Chromatin remodeling, n *(%)*			< 0.001
Mutated	147 (14)	118 (23)	
Wild-type	933 (86)	405 (77)	
Cohesin complex, n *(%)*			0.68
Mutated	126 (12)	65 (12)	
Wild-type	954 (88)	458 (88)	
Kinases, n *(%)*			< 0.001
Mutated	392 (39)	141 (29)	
Wild-type	623 (61)	345 (71)	
Methylation-related, n *(%)*			< 0.001
Mutated	394 (36)	320 (61)	
Wild-type	686 (64)	203 (39)	
NPM1, n *(%)*			0.52
Mutated	337 (32)	155 (30)	
Wild-type	726 (68)	360 (70)	
RAS pathway, n *(%)*			0.004
Mutated	285 (26)	104 (20)	
Wild-type	795 (74)	419 (80)	
Spliceosome, n *(%)*			< 0.001
Mutated	130 (12)	191 (37)	
Wild-type	944 (88)	327 (63)	
Transcription factors, n *(%)*			< 0.001
Mutated	171 (18)	130 (29)	
Wild-type	797 (82)	325 (71)	
Tumor suppressors, n *(%)*			0.28
Mutated	143 (13)	80 (15)	
Wild-type	937 (87)	443 (85)	
Total number of mutations			< 0.001
Median	2	3	
Range	0–9	0–8	

Abbreviation: *n*, number. [a]Chromatin remodeling is mutated if *ASXL1*, *BCOR*, *BCORL1*, *EZH2* or *SMARCA2* is mutated. Cohesin complex is mutated if *RAD21*, *SMC1A*, *SMC3* or *STAG2* is mutated. Kinases is mutated if *AXL*, *FLT3*-ITD, *FLT3*-TKD, *KIT* or *TYK2* is mutated. Methylation-related is mutated if *DNMT3A*, *IDH1*, *IDH2*, or *TET2* is mutated. *NPM1* is mutated if *NPM1* is mutated. *RAS* pathway is mutated if *CBL*, *KRAS*, *NRAS* or *PTPN11* is mutated. Spliceosome is mutated if *SF3B1*, *SRSF2*, *U2AF1* or *ZRSR2* is mutated. Transcription factors is mutated if *CEBPA*, *ETV6*, *IKZF1*, *GATA2*, *NOTCH1* or *RUNX1* is mutated. Tumor suppressors is mutated if *PHF6*, *TP53* or *WT1* is mutated. Only genes whose mutation frequencies in all patients were ⩾ 2% were included in the aforementioned functional groups. A given functional group was considered to be involved if at least one of the genes assigned to this functional group was found mutated. [b]*P*-values for discrete variables are from Fisher's exact test and for continuous variables are from the Wilcoxon rank sum test and compare patients aged < 60 years with those aged ⩾ 60 years.

mutations in chromatin remodeling genes in t(8;21) AML. Although those mutations were also more frequent in our t(8;21) cohort (13 vs 6% in patients with inv(16)), the difference did not reach statistical significance ($P = 0.18$). We suspect that the difference between our results and those of Duployez *et al.*[36] may be related in part to the fact that we did not test for *ASXL2* mutations in our study.

Patients with other, non-CBF-AML-associated balanced rearrangements (yellow color; subgroups #6-18; Figures 1 and 2; Table 1) shared some mutational features with CBF-AML.[6,36] Specifically, there was a paucity of biallelic *CEBPA* mutations (1%) and *NPM1* mutations (5%), whereas mutations in the RAS pathway (29%) and kinases (22%) functional groups were relatively frequent. As previously described,[39,40] AML patients with 11q23/*MLL*-rearrangements often had mutations in RAS pathway genes, whereas they rarely harbored mutations in other functional groups compared with patients with non-*MLL*-rearranged balanced rearrangements. For example, only 9% of *MLL*-rearranged patients (subgroups #6–12) harbored mutations in chromatin remodeling genes vs 26% of patients with non-*MLL*-rearranged balanced rearrangements (subgroups #13–18), and similar differences were observed for tumor suppressor (3% vs 18%), methylation-related (12% vs 23%) and spliceosome (12% vs 24%) functional groups.

Some of the recurrent cytogenetic subgroups presented with particular molecular features. For example, 47% of patients with t(6;11)(q27;q23) (subgroup #7) harbored *KRAS* mutations, as compared with only 3% of all AML patients without t(6;11), and 6% of patients with balanced translocations other than t(6;11) (Figures 1 and 2). We detected *FLT3*-ITD in 5 of 7 (71%) patients with sole t(6;9)(p23;q34) (subgroup #15), which is consistent with the literature.[41] However, remarkably, only three other single gene mutations were found in this patient cohort (*NRAS* mutation in a patient without *FLT3*-ITD, and *TET2* and *ZRSR2* accompanying *FLT3*-ITD in one patient each). Of the AML patients with the t(9;22)(q34;q11.2)/*BCR-ABL1*[42] (subgroup #16), 47% had mutations in *RUNX1*, and 27% harbored mutations in *ZRSR2*. This observation is of special interest, because AML with t(9;22) has been added as a new entity to the 2016 revision of the WHO classification of myeloid neoplasms and acute leukemia,[31] and specific molecular features of this rare subset are largely unknown.[42] In agreement with a previous report,[43] patients with inv(3)(q21q26)/t(3;3)(q21;q26) (subgroup #13) had frequent mutations in *SF3B1* and *BCOR* (both found in 38% of patients). Notably, patients with rare recurrent balanced rearrangements (subgroup #17) and patients with unique balanced rearrangements (subgroup #18) had *TP53* mutations detectable in 20% and 15% of the patients, respectively. This is in sharp contrast to patients with all other, more frequent recurrent balanced rearrangements, who totally lack *TP53* mutations. However, on closer inspection, the karyotypes of 75% of *TP53* mutation-positive patients in both subgroup #17 and subgroup #18 were complex as opposed to, respectively, only 22 and 38% of *TP53* mutation-negative patients in these subgroups having a complex karyotype. This suggests that, in contrast to well-established balanced rearrangements, the presence of a unique or even a rare recurrent balanced rearrangement within a complex karyotype should not be the reason for excluding such patients from the CK-AML category. Furthermore, 18% of patients with unique balanced translocations (subgroup #18) harbored mutations in *NPM1*, which are only rarely seen in patients with other balanced rearrangements (subgroups #13–17).

The last major cytogenetic group, comprised of patients with gains or losses of chromosome material in non-complex karyotypes (blue color; subgroups #19–34; Figures 1 and 2; Table 1), was characterized by high frequencies of mutations in methylation-related genes, found in 54% of patients, transcription factor genes (33%) and spliceosome genes (32%). Interestingly, spliceosome mutations were predominantly enriched in patients with gains of chromosomal material, with almost half of these patients harboring one or more such mutations. With the exception of patients with sole trisomy 11 (subgroup #21), who most frequently harbored *U2AF1* mutations (43%),[44] *SRSF2* was the most often mutated spliceosome gene in patients with unbalanced chromosomal abnormalities. It was mutated in 17% of patients with unbalanced abnormalities, and in 18–50% of patients with sole gain of specific chromosomes, being especially

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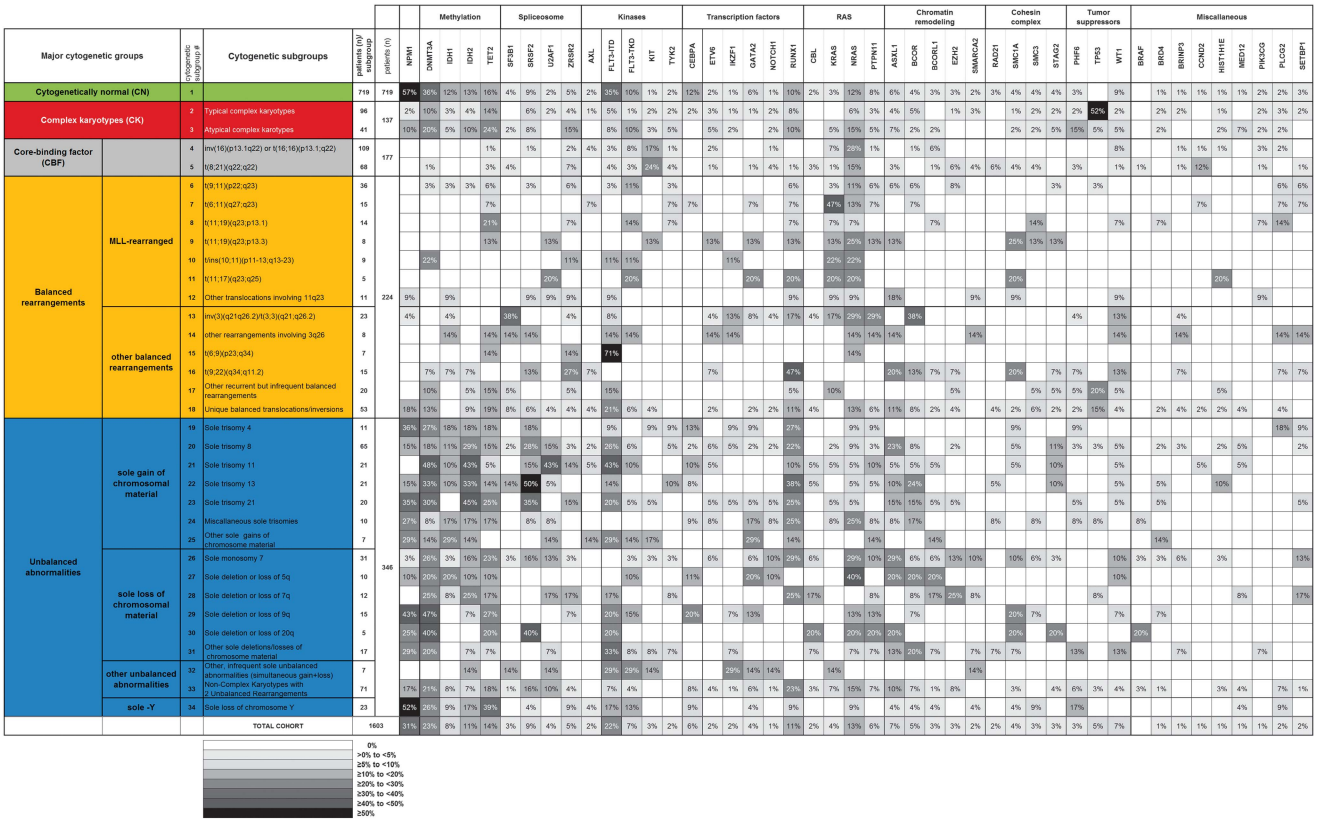

Figure 1. Oncoprint of mutations found in patients with *de novo* acute myeloid leukemia who had specific cytogenetic findings. The color coding of rows indicate the assignment of recurrent cytogenetic abnormalities to five major cytogenetic groups. Each individual line corresponds to one of 34 specific cytogenetic subgroups. Columns represent single gene mutations that are clustered into the previously described functional groups.[2] The frequency of each mutation detected within a given cytogenetic subset is indicated by a black-to-white color gradient, with black indicating a mutation frequency $\geqslant 50\%$, and white indicating a mutation frequency $< 1\%$ of patients. In addition, the specific frequencies (in percent) are indicated in each cell.

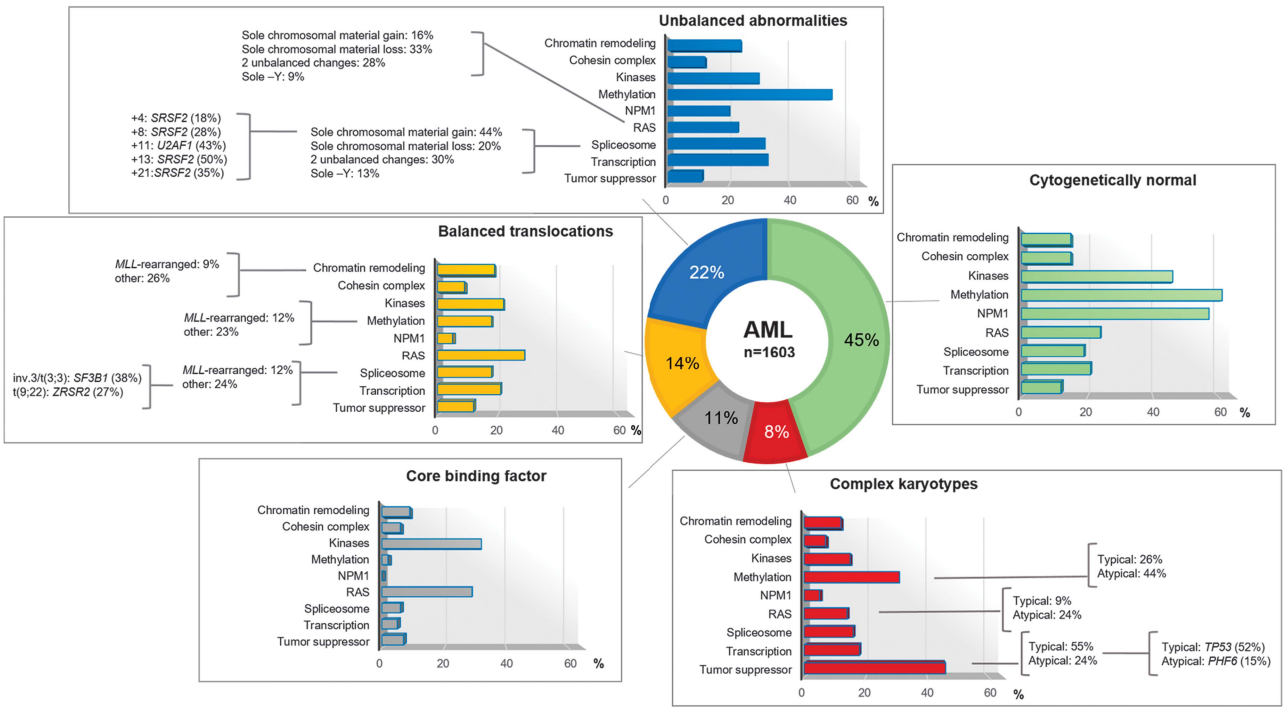

Figure 2. Circle plot illustrating proportions of patients belonging to the five major cytogenetic groups, indicated by the identical color code as in Figure 1, and bar graphs indicating frequencies of mutations belonging to specific functional groups detected in cytogenetic subsets analyzed.

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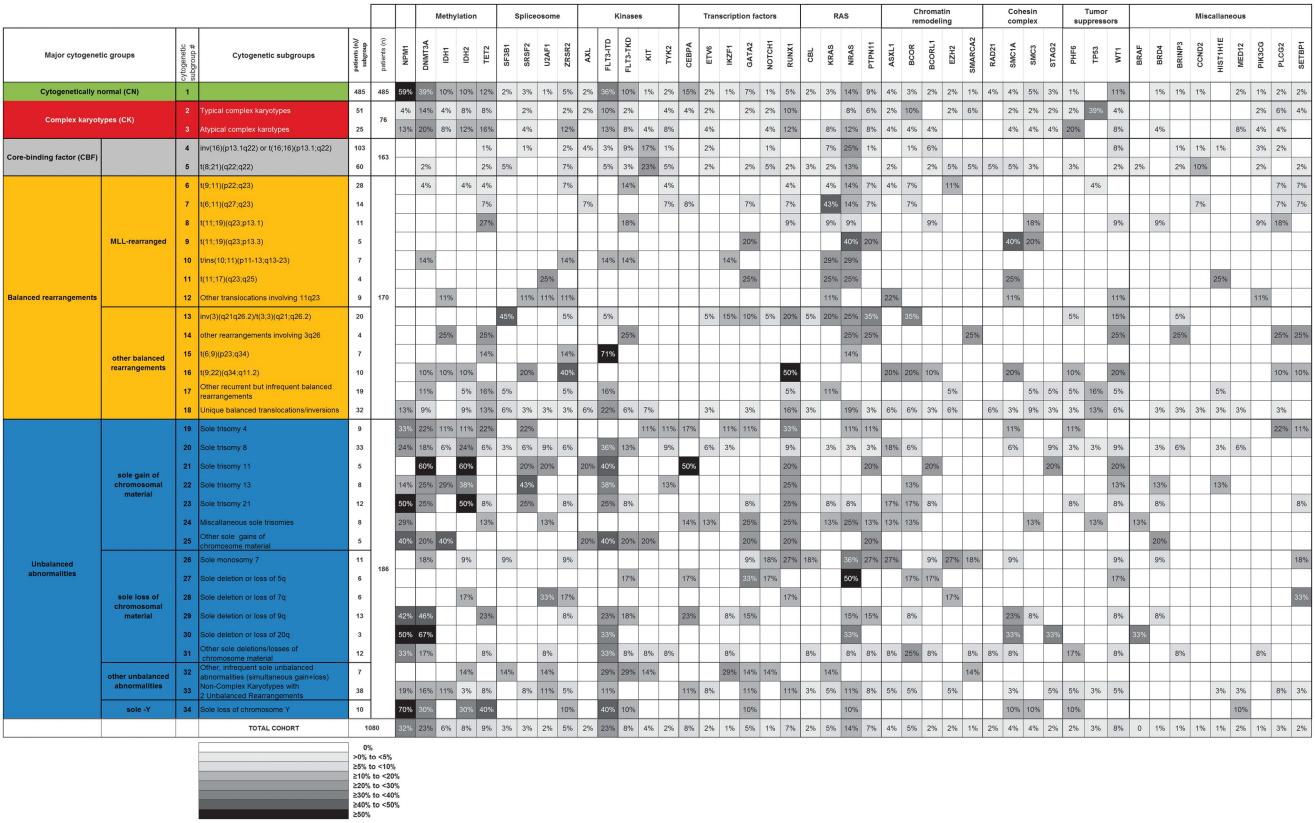

Figure 3. Oncoprint of mutations found in patients < 60 years of age, with respect to their cytogenetic findings.

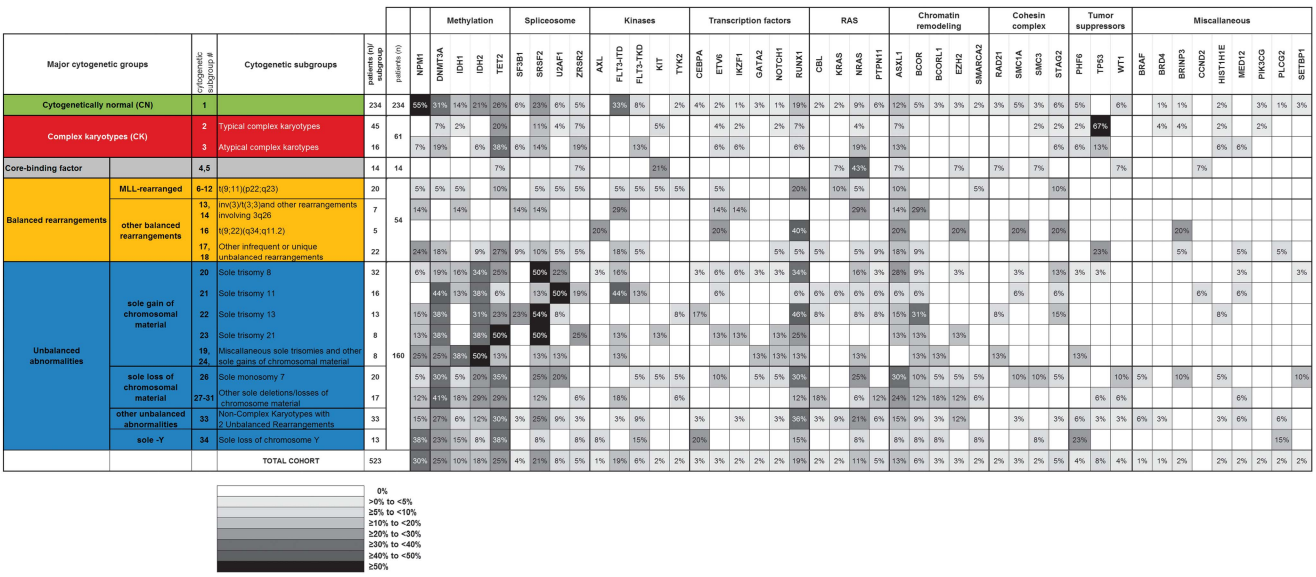

Figure 4. Oncoprint of mutations found in patients $\geqslant 60$ years of age with respect to their cytogenetic findings.

frequent in patients with sole trisomy 13 (50%; Figures 1 and 2). Collectively, this suggests that splicing defects and acquisition of trisomies may cooperate during leukemogenesis. As with CBF-AML and *KIT* mutations, this provides a rationale for the potential use of therapies that include agents pharmacologically targeting the splicing machinery in AML patients with sole trisomies and mutated spliceosome genes.[45]

In addition to 34 cytogenetic groups, we also analyzed the mutational spectrum of patients with monosomal karyotype. The monosomal karyotype is defined as the presence of two or more autosomal monosomies or one autosomal monosomy and at least one structural aberration (other than those denoting CBF-AML),[46] and has been associated with an extremely poor prognosis in AML.[46,47] The most frequent mutation in patients with monosomal karyotype was that of *TP53*, which was detected in 39% of the patients (Supplementary Figure 3). Only two other mutations were detected in at least 10% of patients, namely *NRAS* (10%) and *TET2* (10%), which resembles the findings in patients with typical complex karyotype.

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Lastly, we prepared mutational oncoprints separately for patients younger than 60 years and those aged 60 years or older (Figures 3 and 4). This revealed important differences in both their cytogenetic and mutational backgrounds. Whereas the proportions of CN-AML patients in both age groups were virtually identical (44.9% among younger vs 44.7% among older patients, $P = 0.96$), as previously reported[48,49] CBF-AML was more than five times more frequent in younger than older patients (15.1% vs 2.7%, $P < 0.001$), and balanced rearrangements other than those associated with CBF-AML were also more frequent in younger patients (15.7% vs 10.3%, $P = 0.003$). In contrast, older patients harbored more frequently CK-AML (17.2% vs 7%, $P = 0.003$) and unbalanced rearrangements present in a non-complex karyotype (30.6% vs 11.7%, $P < 0.001$) than younger patients under 60 years of age (Supplementary Table S3). Our data support previous reports showing age-related differences in the distribution of recurring cytogenetic abnormalities.[48–50] The enrichment of CK-AML and unbalanced rearrangements in patients aged $\geqslant 60$ years is also consistent with a higher incidence of AML with myelodysplasia-related changes in older patients.[31]

With respect to their mutational features, in general, younger AML patients harbored fewer mutations than older patients (median, 2 vs 3 mutations; $P < 0.001$; Table 2). Mutations in kinase genes (39% of younger vs 29% of older patients, $P < 0.001$) and RAS pathway mutations (26% vs 20%, $P = 0.004$) were more frequent in younger AML patients, whereas mutations involving methylation-related genes (61% of older vs 36% of younger patients, $P < 0.001$), spliceosome (37% vs 12%, $P < 0.001$), transcription factor (29% vs 18%, $P < 0.001$), and chromatin remodeling genes (23% vs 14%, $P < 0.001$) were more often found in older patients. Examination of age-related mutation distributions within five major cytogenetic groups has shown that the aforementioned overall differences in the mutational features were largely driven by patients with CN-AML (Supplementary Table S4) and patients with unbalanced rearrangements (Supplementary Table S8). Among patients with CK-AML only mutations in genes encoding kinases were more frequent in younger patients (22% vs 7%, $P = 0.02$) and spliceosome mutations in older patients (25% vs 8%, $P = 0.008$; Supplementary Table S5). In patients with balanced rearrangements other than those associated with CBF-AML, only mutations in the RAS pathway genes were more common in younger patients (33% vs 17%, $P = 0.02$), whereas mutations in the *NPM1* gene (13% vs 2%, $P = 0.005$) and genes belonging to the methylation-related functional group (30% vs 15%, $P = 0.02$) were more frequent in patients aged 60 years or older (Supplementary Table S7). Taken together, the above findings are consistent with differences in the biology of AML between the younger and older patients.

In summary, our mutational oncoprint data represent a comprehensive depiction of single gene mutations and functional groups in recurrent cytogenetic subsets to date. The presence and absence of particular gene mutations in specific cytogenetic subgroups contribute to better understanding of both the pathogenesis of AML and differences in outcomes of patients belonging to the same specific cytogenetic subgroup who differ with regard to the presence or absence of specific mutations. Furthermore, if these data are confirmed, the mutational oncoprint may be used as a guide for mutation testing in personalized characterization of the leukemia in individual AML patients (for example, spliceosome mutations in patients with sole trisomies, *PHF6* mutations in atypical CK), and may ultimately lead to a more focused application of targeted therapy in AML.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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DISCLAIMER

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

AUTHOR CONTRIBUTIONS

A-KE, KM, JCB and CDB contributed to the study design; A-KE, KM, AdlC, JCB and CDB contributed to the data interpretation, A-KE, KM, JK, JCB and CDB wrote the manuscript; A-KE and SO performed laboratory-based research; JSB and KWK performed the data processing; JK and DN performed statistical analysis; RMS, AJC, KM, JEK, BLP, ESW 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.

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