2014

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Published in final edited form as:
*Leukemia*. 2014 August ; 28(8): 1754–1758. doi:10.1038/leu.2014.114.

# Prognostic gene mutations and distinct gene- and microRNA-expression signatures in acute myeloid leukemia with a sole trisomy 8

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Presented in part at the 52nd Annual Meeting of the American Society of Hematology, Orlando, FL, December 6, 2010 and published in abstract form in *Blood* 2010; **116**: 255 (abstract 577).

**AUTHOR CONTRIBUTIONS**

HB, K Maharry, K Mrózek, SV, MDR, GM and CDB contributed to the design and analysis of this study and the writing of this manuscript, and all authors agreed on the final version; HB, A-KE, KHM, SS, SPW, JHM, Y-ZW and PP carried out laboratory-based research; K Maharry, SV, MDR, JK and DN performed statistical analyses; and BLP, THC, MW, JEK, AJC, MRB, MAC, RMS, GM and CDB were involved directly or indirectly in the care of patients and/or sample procurement.

**CONFLICT OF INTEREST**

The authors declare no competing financial interests in relation to the work.

Trisomy 8 (+8) is the most frequent numerical chromosome aberration in acute myeloid leukemia (AML), occurring in approximately 9% of adult patients.[1] In one-third of such patients, +8 is the sole cytogenetic abnormality.[1] These patients are mostly classified as having an intermediate prognosis.[1,2] The few available studies suggest that sole +8 AML is molecularly heterogeneous,[3-5] but the clinical impact of mutations remains to be established. Moreover, although the biologic features of sole +8 AML have been investigated using genome-wide gene-[6,7] or microRNA-expression[8] analyses, these studies included small numbers of patients.

We report herein the molecular and clinical characterization of 80 adults with *de novo* AML and sole +8 enrolled on Cancer and Leukemia Group B/Alliance clinical trials. Methodological details are described in the Supplementary Information.

Ninety-four percent of the sole +8 AML patients harbored at least one mutation (Supplementary Figure S1). The most frequently mutated genes were *RUNX1* (32%), *ASXL1* (29%), *FLT3* (specifically *FLT3*-ITD: 29%), *IDH2* (26%), *DNMT3A* (25%) and *NPM1* (22.5%) (Table 1). Younger (<60 years) patients less often harbored mutations in *RUNX1* (*P*=0.008), *ASXL1* (*P*=0.002), *IDH2* (*P*=0.04) and *TET2* (*P*=0.001) than older (≥60 years) patients.

We compared the mutational features of the +8 AML cohort with CN-AML patients, the largest and molecularly best characterized cytogenetic subset of AML.[1,2] Among younger and older patients, those with sole +8 more often had mutations in *ASXL1* (younger, *P*=0.04; older, *P*<0.001) and *RUNX1* (younger, *P*=0.08; older, *P*<0.001), and less often in *NPM1* (younger and older, *P*<0.001) (Supplementary Table S1). Younger sole +8 patients also less frequently had *TET2* (*P*=0.002) and *CEBPA* (*P*=0.005) mutations than CN-AML patients. Hence, particularly mutations in *ASXL1* and *RUNX1* associate with sole +8 AML. However, no single mutation was as tightly associated with +8 AML as reported for AML with other numeric aberrations, e.g., +11 and *MLL*-PTD,[9] +13 and *RUNX1* mutations.[10] Future studies may determine whether +8 favors acquisition of *RUNX1* and *ASXL1* mutations or whether CN-AML with such mutations is prone to the gain of +8.

Patients included in the outcome analyses received cytarabine/daunorubicin-based induction and consolidation, and no allogeneic hematopoietic stem-cell transplantation in first complete remission (CR) (Supplementary Table S2). As in previous reports,[1] the outcomes of sole +8 AML patients were relatively poor; 64% achieved a CR and 5-year rates were 9% for disease-free survival (DFS) and 15% for overall survival (OS) (Table 1). Notably, there were no significant differences in CR rates, DFS or OS between younger and older patients (Table 1), despite differences in treatment intensity. This is in contrast with the better outcomes of younger patients previously observed in CN-AML[2] and could be related to differences in the mutation or gene-expression patterns (described below) between the cytogenetic subsets.

To further characterize the outcome of sole +8 AML, we evaluated it in comparison with CN-AML and in the context of the European LeukemiaNet (ELN) classification.[2] Among younger adults, sole +8 AML associated with worse CR rates (*P*=0.003), and shorter DFS

(*P*=0.01) and OS (*P*=0.003) than CN-AML; among older individuals, sole +8 patients had only a trend for shorter DFS (*P*=0.09) (Supplementary Table S3). In the ELN recommendations,[2] the Intermediate-II Genetic Group consists of two subsets: patients with t(9;11)(p22;q23) and patients with cytogenetic aberrations not classified in the Favorable or Adverse Genetic Groups, which also include sole +8. Thus, we compared the outcome of sole +8 patients with that of t(9;11) patients and the remaining Intermediate-II patients (Supplementary Table S3). Among younger adults, sole +8 patients had shorter DFS (*P*=0.02) and OS (*P*=0.02) than t(9;11) patients, and worse CR rates (*P*=0.04) and OS (*P*=0.05) but no significant differences in DFS compared with the remaining Intermediate-II patients. Among older patients, there were no significant outcome differences between sole +8, t(9;11) and the remaining Intermediate-II patients.

It is currently unknown whether molecular markers allow risk stratification in sole +8 AML. Thus, we tested the prognostic significance of the various markers in multivariable models (Table 2).

In younger patients, only *BAALC* expression impacted on CR attainment, with high *BAALC* expressers having lower odds of achieving a CR (*P*=0.04). *FLT3*-ITD status was the only variable associated with DFS; patients with *FLT3*-ITD had worse DFS than those without (*P*=0.054). Both harboring *FLT3*-ITD (*P*=0.02) and being non-white (*P*=0.01) associated with worse OS. Patients with *FLT3*-ITD had almost three times higher risk of death than those without *FLT3*-ITD (Table 2). At 5 years, *FLT3*-ITD-positive patients had a DFS rate of 0% and OS rate of 7% compared with the respective 22% and 31% for patients without *FLT3*-ITD (DFS: *P*=0.054, OS: *P*=0.02; unadjusted rates; Supplementary Figure S2a, Supplementary Table S4).

Among older patients, *TET2* mutation status was the only significant marker for CR and DFS (Table 2). Only 38% of the *TET2*-mutated patients achieved a CR compared with 80% of wild-type *TET2* patients (*P*=0.04), and the former had shorter DFS (*P*=0.048; Supplementary Table S4). Both *TET2* mutations (*P*=0.003) and wild-type *RAS* (*P*=0.03) were associated with shorter OS (Table 2). *TET2*-mutated patients had almost fourfold higher risk of death than those with wild-type *TET2*; the respective 3-year OS rates were 0% and 25% (*P*=0.004; unadjusted rates; Supplementary Figure S2b, Supplementary Table S4). The patient numbers were too small to formally investigate the significance of the combined *TET2* and *RAS* mutation status. However, three of the four older +8 patients with both wild-type *TET2* and *RAS* mutation were alive 3 years after diagnosis, whereas no patient with mutated *TET2* (and mostly wild-type *RAS*) was.

We previously reported that sole +8 AML associates with the overexpression of chromosome 8-located genes due to their higher genomic dosage,[6] and subsequent, small studies made similar observations.[7] The present study of a relatively large patient cohort further characterizes this finding. We compared the gene-expression profiles between sole +8 and CN-AML patients, and derived a signature comprising 452 genes significantly upregulated and 329 downregulated in +8 AML (Supplementary Table S5). Consistent with a gene dosage effect, 189 (42%) of the genes significantly upregulated in +8 AML were located on chromosome 8 (Supplementary Figure S3a). Accordingly, in gene set enrichment

analyses of the chromosome locations of all genes studied, chromosome 8 exhibited marked upregulation in +8 AML compared with CN-AML (familywise error rate $P<0.001$; normalized enrichment score 16.15). In a leave-one-out cross-validated analysis, 399 (92%) of 434 patients were correctly classified as sole +8 or CN-AML based on the expression pattern of the chromosome 8-located genes of the signature (80.6% sensitivity to correctly predict the presence of +8, 94% specificity to correctly predict its absence). In line with our earlier analysis of 41 genes in ten +8 AML patients,[6] the upregulation of genes mapped to chromosome 8 was evenly distributed along the entire chromosome (Supplementary Figure S3b).

Among the most upregulated genes on chromosome 8 was *BAALC*. We originally identified *BAALC* because of its high expression in +8 AML and subsequently described its high expression as a prognostically adverse marker in CN-AML.[11] As detailed above, higher *BAALC* expression also associated with lower odds for CR attainment among younger sole +8 patients, despite its overall high expression in sole +8 AML. A genomic neighbor of *BAALC*, i.e., *FZD6* (receptor of the Wnt signaling cascade) was also highly expressed in +8 AML. Strongly upregulated in +8 AML, but located on chromosome 21, was *APP* (overexpressed in *IDH2* R172-mutated CN-AML[12] and AML with amplification of 21q-material[13]). In gene ontology analyses of genes expressed ≥1.5-fold in sole +8 AML, significantly overrepresented terms were response to chemical stimulus and extracellular matrix organization (Supplementary Figure S4). Among the downregulated genes were *CD33* (target of gemtuzumab ozogamicin) and histone genes.

From the comparison of 354 mature microRNAs between sole +8 and CN-AML patients, we derived a signature of 7 microRNAs – 5 upregulated and 2 downregulated in +8 AML (Supplementary Table S6). In contrast to the protein-coding genes, none of the chromosome 8-located microRNAs that we studied was significantly upregulated in +8 AML (Supplementary Table S7). Hence, it is currently uncertain whether microRNA expression is affected by genomic dosage in the same manner as gene expression. MicroRNAs overexpressed in +8 AML were *miR-34b* and *miR-107* (activated by TP53),[14,15] *miR-370* (downregulates NF1, the deficiency of which causes hyperactive RAS signaling in myeloid neoplasms)[16] and *miR-342* (upregulated by all-*trans*-retinoic acid in acute promyelocytic leukemia).[17]

In summary, sole +8 AML is molecularly heterogeneous, with mutations in *RUNX1*, *ASXL1*, *IDH2*, *DNMT3A* and *NPM1*, and *FLT3*-ITD being most frequent. Mutation frequencies differ between +8 AML and CN-AML, and between younger and older sole +8 patients, whereas the outcomes of younger and older sole +8 AML patients are only slightly different. High *BAALC* expression and *FLT3*-ITD associated with worse outcomes among younger and *TET2* mutations and wild-type *RAS* among older sole +8 AML patients. Moreover, sole +8 AML is characterized by distinct gene- and microRNA-expression patterns. The increased dosage of chromosome 8-located genes leads to their overexpression, an effect not observed for microRNAs. Our findings should be useful for the guidance of treatment decisions and the development of new therapies that improve the poor outcome of patients with sole +8 AML.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

The Cancer and Leukemia Group B (CALGB) institutions, principal investigators, and cytogeneticists participating in this study are provided in the Supplementary Information. The research was supported in part by National Cancer Institute, Bethesda, MD grants CA101140, CA140158, CA114725, CA129657, CA16058, CA77658, The Coleman Leukemia Research Foundation, the Deutsche Krebshilfe - Dr. Mildred Scheel Foundation (to Heiko Becker) and the Research Committee of the University Freiburg, Germany (to Heiko Becker), the Pelotonia Fellowship Program (to Ann-Kathrin Eisfeld), and the Conquer Cancer Foundation (to Jason H. Mendler). The research for CALGB 8461, 20202 and 9665 (Alliance) was supported, in part, by grants from the National Cancer Institute (CA31946) to the Alliance for Clinical Trials in Oncology (Monica M. Bertagnolli, Chair) and to the Alliance Statistics and Data Center (Daniel J. Sargent, CA33601). The content of this manuscript is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute.

We thank Donna Bucci and the CALGB Leukemia Tissue Bank at The Ohio State University Comprehensive Cancer Center, Columbus, OH, for sample processing and storage services, Colin G. Edwards, PhD, Lisa J. Sterling and Christine Finks for data management, and Dean Margeson and Kelsi B. Holland for assistance in the statistical analyses. Moreover, we thank Stephan M. Tanner, PhD and Albert de la Chapelle, MD, PhD for scientific discussions.

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## Table 1

Pretreatment clinical and molecular characteristics and outcome of patients with *de novo* acute myeloid leukemia and sole +8 and comparison by age group (<60 years vs ≥60 years)

| *Characteristic* | *Sole +8 AML (n=80)* | *Sole +8 AML <60 years (n=40)* | *Sole +8 AML ≥60 years (n=40)* | *P* [f] |
|---|---|---|---|---|
| *Age, years* | | | | - |
| Median | 59 | 43 | 71 | |
| Range | 18-84 | 18-59 | 60-84 | |
| *Male sex, no. (%)* | 50 (63) | 26 (65) | 24 (60) | 0.82 |
| *Race, no. (%)* | | | | 0.85 |
| White | 69 (87) | 35 (90) | 34 (85) | |
| Black or African American | 8 (10) | 3 (8) | 5 (13) | |
| Other | 2 (3) | 1 (2) | 1(2) | |
| *Hemoglobin, g/dL* | | | | 0.64 |
| Median | 9.2 | 9.2 | 9.1 | |
| Range | 5.0-15.8 | 5.0-15.8 | 5.3-14.1 | |
| *Platelet count, × $10^9$/L* | | | | 0.63 |
| Median | 46 | 49 | 41 | |
| Range | 5-233 | 11-148 | 5-233 | |
| *WBC, × $10^9$/L* | | | | 0.003 |
| Median | 8.9 | 20.7 | 4.2 | |
| Range | 0.6-302.3 | 0.6-302.3 | 0.8-187.0 | |
| *Blood blasts, %* | | | | 0.02 |
| Median | 38 | 49 | 23 | |
| Range | 0-97 | 2-97 | 0-91 | |
| *Bone marrow blasts, %* | | | | 0.14 |
| Median | 70 | 77 | 56 | |
| Range | 18-94 | 22-90 | 18-94 | |
| *FAB, no. (%)* [a] | | | | <0.001 (M1/M2 vs M4/M5) |
| M0 | 3 (5) | 1 (3) | 2 (7) | |
| M1 | 9 (15) | 2 (6) | 7 (26) | |
| M2 | 17 (29) | 6 (19) | 11 (41) | |
| M4 | 9 (15) | 5 (16) | 4 (15) | |
| M5 | 19 (32) | 17 (53) | 2 (7) | |
| M6 | 2 (3) | 1 (3) | 1 (4) | |
| *Extramedullary involvement, no. (%)* | 15 (20) | 9 (24) | 6 (16) | 0.57 |
| CNS | 0 (0) | 0 (0) | 0 (0) | NA |

| *Characteristic* | *Sole +8 AML (n=80)* | *Sole +8 AML <60 years (n=40)* | *Sole +8 AML ≥60 years (n=40)* | *P* [f] |
|---|---|---|---|---|
| Hepatomegaly | 2 (3) | 2 (5) | 0 (0) | 0.24 |
| Splenomegaly | 3 (4) | 3 (8) | 0 (0) | 0.24 |
| Lymphadenopathy | 5 (7) | 4 (11) | 1 (3) | 0.19 |
| Skin infiltrates | 6 (8) | 1 (3) | 5 (14) | 0.11 |
| Gum hypertrophy | 6 (8) | 4 (11) | 2 (5) | 0.67 |
| Mediastinal mass | 1 (1) | 0 (0) | 1 (3) | 0.49 |
| *+8 metaphases in BM, no. (%)* ≥ 80% | 50 (63) | 26 (65) | 24 (60) | 0.82 |
| *RUNX1, no. (%)* | | | | 0.008 |
| Mutated | 25 (32) | 7 (18) | 18 (46) | |
| Wild-type | 54 (68) | 33 (82) | 21 (54) | |
| *ASXL1, no. (%)* | | | | 0.002 (mutated vs wild-type) |
| Mutated | 22 (29) | 5 (13) | 17 (46) | |
| c.1934dupG | 10 | 1 | 9 | |
| Other | 12 | 4 | 8 | |
| Wild-type | 54 (71) | 34 (87) | 20 (54) | |
| *FLT3-ITD, no. (%)* | | | | 0.14 |
| Positive | 23 (29) | 15 (38) | 8 (20) | |
| Negative | 57 (71) | 25 (62) | 32 (80) | |
| *IDH2, no. (%)* | | | | 0.04 (mutated vs wild-type) |
| Mutated | 21 (26) | 6 (15) | 15 (38) | |
| R140 mutated | 13 | 4 | 9 | |
| R172 mutated | 8 | 2 | 6 | |
| Wild-type | 59 (74) | 34 (85) | 25 (62) | |
| *DNMT3A, no. (%)* | | | | 0.58 (mutated vs wild-type) |
| Mutated | 17 (25) | 8 (22) | 9 (29) | |
| R882 | 12 | 7 | 5 | |
| Non-R882 | 5 | 1 | 4 | |
| Wild-type | 51 (75) | 29 (78) | 22 (71) | |
| *NPM1, no. (%)* | | | | 0.18 |
| Mutated | 18 (22.5) | 12 (30) | 6 (15) | |
| Wild-type | 62 (77.5) | 28 (70) | 34 (85) | |
| *FLT3-TKD, no. (%)* | | | | 0.54 |
| Positive | 13 (17) | 5 (13) | 8 (21) | |
| Negative | 65 (83) | 34 (87) | 31 (79) | |
| *IDH1, no. (%)* | | | | 0.19 |

| *Characteristic* | *Sole +8 AML (n=80)* | *Sole +8 AML <60 years (n=40)* | *Sole +8 AML ≥60 years (n=40)* | *P* [f] |
|---|---|---|---|---|
| Mutated | 11 (14) | 3 (8) | 8 (20) | |
| Wild-type | 69 (86) | 37 (92) | 32 (80) | |
| *RAS, no. (%)* | | | | 0.31 (mutated vs wild-type) |
| Mutated | 10 (13) | 3 (8) | 7 (18) | |
| *NRAS* mutated | 9 | 3 | 6 | |
| *KRAS* mutated | 1 | 0 | 1 | |
| Wild-type | 70 (87) | 37 (92) | 33 (82) | |
| *TET2, no. (%)* | | | | 0.001 |
| Mutated | 8 (11) | 0 (0) | 8 (24) | |
| Wild-type | 64 (89) | 39 (100) | 25 (76) | |
| *CEBPA, no. (%)* | | | | 0.20 (mutated vs wild-type) |
| Mutated | 6 (8) | 1 (3) | 5 (13) | |
| Single mutated | 4 | 1 | 3 | |
| Double mutated | 2 | 0 | 2 | |
| Wild-type | 74 (92) | 39 (97) | 35 (87) | |
| *WT1, no. (%)* | | | | 1.00 |
| Mutated | 3 (4) | 2 (5) | 1 (3) | |
| Wild-type | 77 (96) | 38 (95) | 39 (97) | |
| *BAALC expression, no. (%)* [b] | | | | 0.62 |
| High | 33 (50) | 16 (55) | 17 (46) | |
| Low | 33 (50) | 13 (45) | 20 (54) | |
| *miR-155 expression, no. (%)* [c] | | | | 1.00 |
| High | 31 (48) | 14 (48) | 17 (49) | |
| Low | 33 (52) | 15 (52) | 18 (51) | |
| *miR-3151 expression, no. (%)* [b] | | | | 1.00 |
| High | 21 (50) | 8 (50) | 13 (50) | |
| Low | 21 (50) | 8 (50) | 13 (50) | |

| *Endpoint* | *Sole +8 AML (n=59)* [d] | *Sole +8 AML <60 years (n=30)* | *Sole +8 AML ≥60 years (n=29)* | *OR/HR* [e] *(95% CI)* | *P* [f] |
|---|---|---|---|---|---|
| Complete remission | | | | 0.68 (0.23-1.98) | 0.47 |
| No. in complete remission (%) | 38 (64) | 18 (60) | 20 (69) | | |
| *Disease-free survival* | | | | 0.59 (0.30-1.16) | 0.13 |
| No. of events | 36 | 16 | 20 | | |

| *Endpoint* | ***Sole +8 AML (n=59)*** [d] | ***Sole +8 AML <60 years (n=30)*** | ***Sole +8 AML ≥60 years (n=29)*** | ***OR/HR*** [e] ***(95% CI)*** | ***P*** [f] |
|---|---|---|---|---|---|
| Median, years | 0.7 | 1.1 | 0.6 | | |
| % Disease-free at 3 years (95% CI) | 12 (4-25) | 21 (6-42) | 5 (0-21) | | |
| % Disease-free at 5 years (95% CI) | 9 (2-21) | 14 (3-35) | 5 (0-21) | | |
| *Overall survival* | | | | 0.67 (0.38-1.16) | 0.15 |
| No. of events | 52 | 24 | 28 | | |
| Median, years | 1.3 | 1.5 | 1.2 | | |
| % Alive at 3 years (95% CI) | 23 (13-35) | 29 (14-46) | 17 (6-33) | | |
| % Alive at 5 years (95% CI) | 15 (7-26) | 19 (7-36) | 10 (3-24) | | |

Abbreviations: WBC, white blood count; FAB, French-American-British classification; NA, not applicable; *FLT3-ITD*, internal tandem duplication of the *FLT3* gene; *FLT3*-TKD, tyrosine kinase domain mutations of the *FLT3* gene; OR, odds ratio; HR, hazard ratio; CI, confidence interval.

[a] FAB morphology was centrally reviewed.

[b] The median expression value was used as a cut point. It was calculated based on the expression levels assessed by RT-PCR.

[c] The median expression value was used as a cut point. It was calculated based on the expression levels on the Affymetrix array.

[d] Of the 80 patients, 59 were evaluable for outcome. Pretreatment clinical and molecular characteristics of the patients included in outcome analyses are provided in Supplementary Table S2.

[e] Ratios are comparing outcome of patients <60 years vs ≥60 years.

[f] P-values compare patients who are <60 years vs ≥60 years. For baseline continuous variables the Wilcoxon rank sum test was used, for baseline categorical variables the Fisher's exact test was used. For CR, the Wald test was used from the logistic regression model. For overall and disease-free survival, the Wald test was used from the Cox regression models.

**Table 2**

Multivariable outcome analyses in patients with de novo acute myeloid leukemia and sole +8

| *Variables in final models* | *Complete remission* | | *Disease-free survival* | | *Overall survival* | |
|---|---|---|---|---|---|---|
| | ***P*** | ***OR (95% CI)*** | ***P*** | ***HR (95% CI)*** | ***P*** | ***HR (95% CI)*** |
| **Sole +8 AML <60 years** | | | | | | |
| *BAALC* expression (high vs low) | 0.04 | 0.13 (0.02-0.91) | - | - | - | - |
| *FLT3*-ITD (positive vs negative) | - | - | 0.054 | 2.87 (0.98-8.40) | 0.02 | 2.77 (1.19-6.45) |
| Race (white vs non-white) | - | - | - | - | 0.01 | 0.20 (0.06-0.69) |
| **Sole +8 AML ≥60 years** | | | | | | |
| *TET2* (mutated vs wild-type) | 0.04 | 0.15 (0.03-0.91) | 0.048 | 3.87 (1.01-14.78) | 0.003 | 3.94 (1.61-9.68) |
| *RAS* (mutated vs wild-type) | - | - | - | - | 0.03 | 0.26 (0.08-0.91) |

Abbreviations: *FLT3*-ITD, internal tandem duplication of the FLT3 gene; OR, odds ratio; HR, hazard ratio; CI, confidence interval.

Notes: We considered 30 patients aged <60 years and 29 patients aged ≥60 years for complete remission (CR) and overall survival (OS) and 18 patients aged <60 years and 20 patients aged ≥60 years for disease-free survival (DFS). An odds ratio less than 1.0 means lower CR rate for the first category listed for the variable. Hazard ratios greater than (less than) 1.0 indicate higher (lower) risk for relapse or death (DFS), or death (OS) for the first category listed for the variables.

Variables considered were those significant at $\alpha$=0.20 in univariable models, i.e., in patients aged <60 years: for CR, *NPM1* (mutated vs wild-type), *FLT3*-ITD (positive vs negative), *BAALC* expression (high vs low) and age (10 year increase); for DFS, *FLT3*-ITD (positive vs negative), hemoglobin (continuous), platelets ($50x10^9$/L increase), WBC ($50\times10^9$/L increase), race (white vs non-white), sex (male vs female) and extramedullary involvement (present vs absent); for OS, *FLT3*-ITD (positive vs negative), platelets ($50\times10^9$/L increase), WBC ($50\times10^9$/L increase), race (white vs non-white), sex (male vs female) and extramedullary involvement (present vs absent). In patients aged ≥60 years: for CR, *TET2* (mutated vs wild-type), *RUNX1* (mutated vs wild-type), *ASXL1* (mutated vs wild-type), *miR-155* expression (high vs low), hemoglobin (continuous) and +8 metaphases in bone marrow (≥80% vs <80%); for DFS, *NPM1* (mutated vs wild-type), *FLT3*-ITD (positive vs negative), *TET2* (mutated vs wild-type), and *RAS* (mutated vs wild-type); for OS, *TET2* (mutated vs wild-type), *IDH2* (mutated vs wild-type), *RAS* (mutated vs wild-type), *miR-3151* expression (high vs low) and sex (male vs female).