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ORIGINAL ARTICLE

# Genetic alterations and their clinical implications in older patients with acute myeloid leukemia

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A number of patient-specific and leukemia-associated factors are related to the poor outcome in older patients with acute myeloid leukemia (AML). However, comprehensive studies regarding the impact of genetic alterations in this group of patients are limited. In this study, we compared relevant mutations in 21 genes between AML patients aged 60 years or older and those younger and exposed their prognostic implications. Compared with the younger patients, the elderly had significantly higher incidences of *PTPN11*, *NPM1*, *RUNX1*, *ASXL1*, *TET2*, *DNMT3A* and *TP53* mutations but a lower frequency of *WT1* mutations. The older patients more frequently harbored one or more adverse genetic alterations. Multivariate analysis showed that *DNMT3A* and *TP53* mutations were independent poor prognostic factors among the elderly, while *NPM1* mutation in the absence of *FLT3*/ITD was an independent favorable prognostic factor. Furthermore, the status of mutations could well stratify older patients with intermediate-risk cytogenetics into three risk groups. In conclusion, older AML patients showed distinct genetic alterations from the younger group. Integration of cytogenetics and molecular mutations can better risk-stratify older AML patients. Development of novel therapies is needed to improve the outcome of older patients with poor prognosis under current treatment modalities.

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## INTRODUCTION

Acute myeloid leukemia (AML) is a clinically and biologically heterogeneous hematologic malignancy characterized by uncontrolled proliferation of hematopoietic precursors and loss of the ability to differentiate. Although the clinical outcome improves steadily in younger patients in the past 40 years, the survival for older patients remains very poor.[1,2]

In addition to patient-specific factors, such as concomitant comorbidity, poor performance status and intolerance to intensive chemotherapy,[3,4] a number of leukemia-associated factors are related to the poor outcome in older AML patients.[5,6] Traditionally, cytogenetic findings establish the backbone for prognostic and therapeutical strategies in AML and are long used to risk-stratify AML patients and guide the treatment plan.[7,8] Appelbaum *et al.*[5] demonstrated that older patients had less frequently favorable-risk but more commonly unfavorable-risk cytogenetics, particularly abnormalities in chromosomes 5, 7 and 17.

Many acquired gene mutations have been detected in AML patients, especially those with intermediate-risk cytogenetics, and some of them, such as mutations in *NPM1*, *CEBPA*, *RUNX1*, *WT1*, *DNMT3A*, *ASXL1*, *IDH2* and *FLT3* genes, have been shown to have prognostic significance.[9–22] However, less is known about the clinical implications of gene mutations in older patients with AML. In this study, we aimed to comprehensively investigate the clinico-biological features and molecular genetic alterations and their clinical relevance in older AML patients. The findings from this study may pave ways for future targeted therapies in this group of patients with poor clinical outcome under the current treatment modality.

## MATERIALS AND METHODS

### Subjects

Totally, 462 adult patients who were newly diagnosed as having *de novo* non-M3 AML according to the FAB Cooperative Group Criteria[23] at the National Taiwan University Hospital (NTUH) had cryopreserved cells for mutational analyses, and had complete clinical, cytogenetic and laboratory data were recruited for this study. Among them, 177 patients were 60 years or older. Patients with antecedent hematological diseases, history of cytopenia, family history of myeloid neoplasms or therapy-related AML were all excluded. Diagnosis and classification of AML were made according to the FAB Cooperative Group Criteria.[23] This study was approved by the Research Ethics Committee of the NTUH and written informed consents were obtained from all participants in accordance with the Declaration of Helsinki. Among these patients, 329 (71.2%) received standard induction chemotherapy (Idarubicin 12 mg/m$^2$ per day on days 1–3 and Cytarabine 100 mg/m$^2$ per day on days 1–7) and then consolidation chemotherapy with two to four courses of high-dose Cytarabine (2000 mg/m$^2$ q12h, total eight doses), with or without an anthracycline (Idarubicin or Mitoxantrone), after achieving complete remission (CR).[20,22] Because hypomethylating agents have not been reimbursed for the treatment of AML by the Taiwan government, only few patients received hypomethylating agents in this cohort; analysis of prognostic impact of hypomethylating agents was not carried out. The remaining patients received palliative therapy with supportive care and/or

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low-dose chemotherapy owing to underlying comorbidities or based on the decision of the physicians and patients.

### Cytogenetics

Bone marrow cells were harvested directly or after 1–3 days of unstimulated culture as described previously.[24] Metaphase chromosomes were banded by trypsin-Giemsa technique and karyotyped according to the International System for Human Cytogenetic Nomenclature.

### Mutation analysis

Analyses of relevant mutations in 21 genes, including Class I mutations, such as *FLT3*/ITD,[25] *FLT3*/TKD,[26] *NRAS*,[27] *KRAS*,[27] *JAK2*,[27] *KIT*[28] and *PTPN11*[ref. 28] mutations, and Class II mutations, such as *CEBPA*[29] and *RUNX1*[ref. 17] mutations, as well as mutations in *NPM1*,[12] *WT1*,[30] *TP53*,[31] *Cohesin* complex genes (including *STAG1/2*, *SMC1A*, *SMC3*, and *RAD21*),[32] and those genes related to epigenetic modification, such as *MLL*/PTD,[33] *ASXL1*,[34] *IDH1*,[35] *IDH2*,[36] *TET2*[ref. 37] and *DNMT3A*[20] were performed as previously described. Abnormal sequencing results were confirmed by at least two repeated analyses.

### Statistical analysis

The discrete variables of patients with and without specific molecular alteration were compared using the Fisher exact test. If the continuous data were not normally distributed, Mann–Whitney *U* tests were used to compare continuous variables and medians of distributions. To evaluate the impact of age, molecular alterations and other variables on clinical outcome, only the patients who received conventional standard chemotherapy were included in analyses.[20,22] Overall survival (OS) was measured from the date of first diagnosis to the date of last follow-up or death from any cause, whereas relapse was defined as a reappearance of at least 5% leukemic blasts in bone marrow aspiration smears or new extramedullary leukemia in patients with a previously documented CR.[38] Disease-free survival (DFS) was applied to patients receiving standard intensive chemotherapy and was measured from the date of CR until relapse from CR or death from any cause, whichever occurred first. Multivariate Cox proportional hazards regression analysis was used to investigate independent prognostic factors for OS and DFS. The proportional hazards assumption (constant hazards assumption) was examined by using time-dependent covariate Cox regression before conducting multivariate Cox proportional hazards regression. The variables including age, white blood cell counts at diagnosis, karyotype, *NPM1/FLT3*-ITD, *WT1*, *CEBPA*, *RUNX1*, *MLL*/PTD, *ASXL1*, *TET2*, *IDH2*, *DNMT3A* and *TP53* mutations that showed prognostic implication with *P* value less than 0.1 in univariate analysis were used as covariates. Those patients who received hematopoietic stem cell transplantation (HSCT) were censored at the time of HSCT in survival analysis to ameliorate the influence of the treatment. A $P$ value $< 0.05$ was considered statistically significant. All statistical analyses were performed with the SPSS 20 (SPSS Inc., Chicago, IL, USA) and Statsdirect (Cheshire, England, UK).

## RESULTS

### Comparison of clinical and laboratory features between older and younger patients

Among the 462 AML patients recruited, 261 were males and 201 were females (Table 1). One hundred and seventy-seven patients were 60 years or older with a median age of 71 years (range 60–90 years). There was no difference in gender, hemogram and lactate dehydrogenase level between younger patients and older patients. Older age was negatively associated with the expression of CD19 ($P = 0.022$), CD15 ($P = 0.007$) and CD34 ($P = 0.002$) on the leukemic cells (Supplementary Table 1). There was no difference in the expression of other antigens.

### Comparison of cytogenetic abnormalities and molecular gene mutations between older and younger patients

Chromosome data were available in 444 patients at diagnosis, including 166 older and 278 younger patients (Table 2). Compared with younger patients, the elderly had more frequently unfavorable-risk cytogenetic changes (21.1 vs 10.8%, $P = 0.004$), but less commonly favorable-risk cytogenetics (4.2 vs 19.4%, $P < 0.001$),

**Table 1.** Clinical manifestations of AML patients stratified by age

| *Variables* | *Total* (n = *462*) | *Older patient* (n = *177, 38.3%*) | *Younger patient* (n = *285, 61.7%*) | P *value* |
|---|---|---|---|---|
| *Age* | 51.5 (15–90) | 71 (60–90) | 40 (15–59) | |
| *Sex*[a] | | | | |
| Male | 261 | 107 (60.5%) | 154 (54.0%) | 0.179 |
| Female | 201 | 70 (39.5%) | 131 (46.0%) | |
| *Lab data*[b] | | | | |
| WBC (/μl) | 21 850 (120–627 800) | 20 810 (650–627 800) | 21 950 (120–423 000) | 0.656 |
| Hb (g/dl) | 8.0 (3.0–16.0) | 8.1 (3.0–16.0) | 7.9 (3.0–14.0) | 0.956 |
| Platelet (×1000 /μl) | 44.0 (3.0–802.0) | 41.5 (3.0–455.0) | 45.0 (3.0–802.0) | 0.221 |
| Blast (/μl) | 9863 (0–456 725) | 6968 (0–456 725) | 11 149 (0–369 070) | 0.189 |
| LDH (U/l) | 858 (206–15 000) | 811 (274.0–15 000) | 907 (206–13 130) | 0.424 |
| *FAB*[a] | | | | |
| M0 | 10 | 5 (2.8%) | 5 (1.8%) | 0.517 |
| M1 | 112 | 35 (19.8%) | 77 (27.0%) | 0.094 |
| M2 | 171 | 64 (36.2%) | 107 (37.5%) | 0.843 |
| M4 | 124 | 53 (29.9%) | 71 (24.9%) | 0.237 |
| M5 | 24 | 12 (6.8%) | 12 (4.2%) | 0.281 |
| M6 | 12 | 2 (1.1%) | 10 (3.5%) | 0.142 |
| Undetermined | 9 | 6 (3.4%) | 3 (1.1%) | 0.092 |
| *Induction response*[a,c] | | | | |
| CR | 252 | 39 (56.5%) | 213 (81.9%) | < 0.001 |
| PR/refractory | 54 | 17 (24.6%) | 37 (14.2%) | 0.300 |
| Induction death | 23 | 13 (18.9%) | 10 (3.9%) | 0.079 |
| *Relapse*[a,c] | 137 | 25 (64.1%) | 112 (52.6%) | 0.222 |

Abbreviations: AML, acute myeloid leukemia; CR, complete remission; FAB, French-American-British classification; Hb, hemoglobin; LDH, lactate dehydrogenase; PR, partial remission; WBC, white blood cell. [a]Number of patients (%) among older (≥60 years old) or younger group (< 60 years old). [b]Median (range). [c]Total 329 patients received conventional chemotherapy, including 69 older patients and 260 younger ones.

**Table 2.** Association of age with cytogenetic abnormalities[a]

| *Variables* | *Total* | *Older patient* | *Younger patient* | P *value* |
|---|---|---|---|---|
| *Karyotype*[b] | | | | |
| Favorable | 61 (13.8%) | 7 (4.2%) | 54 (19.4%) | < 0.001 |
| Intermediate | 318 (71.6%) | 124 (74.7%) | 194 (69.8%) | 0.680 |
| Unfavorable | 65 (14.6%) | 35 (21.1%) | 30 (10.8%) | 0.004 |
| Normal | 223 (50.2%) | 90 (54.2%) | 133 (47.9%) | 0.203 |
| Simple | 170 (38.3%) | 49 (29.5%) | 121 (43.5%) | 0.004 |
| Complex | 51 (11.5%) | 27 (16.3%) | 24 (8.6%) | 0.032 |
| t(8;21) | 42 (9.5%) | 4 (2.4%) | 38 (13.7%) | < 0.001 |
| inv (16) | 19 (4.3%) | 3 (1.8%) | 16 (5.8%) | 0.053 |
| t(11q23) | 16 (3.6%) | 5 (3.0%) | 11 (4.0%) | 0.612 |
| t(7;11) | 10 (2.3%) | 0 (0%) | 10 (3.6%) | 0.016 |
| − 5/5q −[c] | 19 (4.3%) | 14 (8.4%) | 5 (1.8%) | 0.001 |
| − 7/7q −[c] | 22 (5.0%) | 14 (8.4%) | 8 (2.9%) | 0.012 |
| +8[c] | 29 (6.6%) | 14 (8.5%) | 15 (5.5%) | 0.236 |
| +11[c] | 3 (0.7%) | 0 (0%) | 3 (1.1%) | 0.289 |
| +13[c] | 1 (0.2%) | 1 (0.6%) | 0 (0%) | 0.383 |
| +21[c] | 9 (2.0%) | 1 (0.6%) | 8 (2.9%) | 0.163 |

[a]444 patients, including 166 older patients and 278 younger ones, had chromosome data at diagnosis. [b]Favorable, t(8;21), inv(16); unfavorable, − 7, del(7q), − 5, del(5q), 3q abnormality, complex abnormalities; intermediate, normal karyotype and other abnormalities. [c]Only including simple chromosomal abnormalities with two or less changes, but not those with complex abnormalities with three or more aberrations.

such as t(8;21) (2.4 vs 13.7%, $P < 0.001$) based on the Medical Research Council (MRC) classification.[39] Specifically, the older patients had higher frequencies of complex chromosomal abnormalities (16.3 vs 8.6%, $P = 0.032$), monosomy 5/5q deletion (8.4 vs 1.8%, $P = 0.001$) and monosomy 7/7q deletion (8.4 vs 2.9%, $P = 0.012$) but a lower incidence of t(7;11) (0 vs 3.6%, $P = 0.016$). The distribution of simple chromosomal abnormalities with two or less changes involving chromosomes 8, 11, 13 and 21 was not different between the two groups.

To investigate the difference of gene mutations in the pathogenesis of leukemia between older and younger AML patients, a complete mutational screening of 21 genes was performed. The most common molecular event in total cohort was *FLT3*/ITD (22.5%), followed by *NPM1* (22.3%), *DNMT3A* (15.2%), *TET2* (14.3%) and *CEBPA* mutations (14.3%). Among the elderly, the most prevalent molecular event was *NPM1* (28.2%), followed by *TET2* (24.3%), *FLT3*/ITD (22.6%), *DNMT3A* (20.9%) and *RUNX1* mutations (19.8%) (Table 3). The median number of molecular gene mutations at diagnosis was higher in the older patients than the younger ones (2.0, range 0–5 vs 1.0, range 0–5, $P < 0.001$). Older patients had significantly higher incidences of *PTPN11*, *NPM1*, *RUNX1*, *ASXL1*, *TET2*, *DNMT3A* and *TP53* mutations than younger patients (6.2% vs 2.5%, $P = 0.050$; 28.2% vs 18.6%, $P = 0.021$; 19.8% vs 9.5%, $P = 0.002$; 17.6% vs 6.7%, $P < 0.001$; 24.3% vs 8.1%, $P < 0.001$; 20.9% vs 11.6%; $P = 0.008$; and 13.0% vs 4.2%, $P = 0.001$, respectively). On the contrary, *WT1* mutations were rarely seen in patients aged 60 years or older (3.4 vs 9.1%, $P = 0.023$). Other genetic alterations were not significantly different between these two age groups. The distributions of molecular gene mutations in these two groups are distinct (Figure 1 and Supplementary Figure 1). Older patients had a higher frequency to harbor one or more adverse genetic alterations (including *FLT3*/ITD, *WT1*, *RUNX1*, *ASXL1*, *DNMT3A* and *TP53* mutations)[22,31] than younger ones (69.5 vs 49.5%, $P < 0.001$), and the difference remained similar between the two groups when two or more such gene mutations were counted (26.6 vs 13.3%, $P = 0.001$). We further showed that 85 pairwise associations were significant with $P < 0.1$ in the elderly cohort (Supplementary Figure 2).

## Prognostic impact of gene mutations in older patients

Fewer older patients received standard chemotherapy than younger patients (69/177, 40.0% vs 260/285, 91.2%, $P < 0.001$); however, standard chemotherapy lead to a longer OS than palliative care only in this group of patients (median, 10.0 vs 3.0 months, $P < 0.001$).

**Table 3.** Distribution of molecular genetic alterations by age

| *Variables* | *Examined No.* | *Patients with alteration (%)* | | | P *value* |
|---|---|---|---|---|---|
| | | *Whole cohort* | *Older patients* | *Younger patients* | |
| *FLT3*/ITD | 462 | 22.5 | 22.6 | 22.5 | > 0.999 |
| *FLT3*/TKD | 462 | 6.5 | 6.8 | 6.3 | 0.848 |
| *NRAS* | 462 | 12.1 | 13.0 | 11.6 | 0.662 |
| *KRAS* | 462 | 3.2 | 2.3 | 3.9 | 0.426 |
| *PTPN11* | 462 | 3.9 | 6.2 | 2.5 | 0.050 |
| *KIT* | 462 | 3.2 | 2.3 | 3.9 | 0.426 |
| *JAK2* | 462 | 0.6 | 0.6 | 0.7 | > 0.999 |
| *WTI* | 462 | 6.9 | 3.4 | 9.1 | 0.023 |
| *NPM1* | 462 | 22.3 | 28.2 | 18.6 | 0.021 |
| *CEBPA* | 462 | 14.3 | 10.2 | 16.8 | 0.055 |
| *RUNX1* | 462 | 13.4 | 19.8 | 9.5 | 0.002 |
| *MLL*/PTD | 462 | 5.8 | 6.8 | 5.3 | 0.543 |
| *ASXL1* | 462 | 10.9 | 17.6 | 6.7 | < 0.001 |
| *IDH1* | 462 | 5.8 | 6.8 | 5.3 | 0.543 |
| *IDH2* | 462 | 11.9 | 14.7 | 10.2 | 0.183 |
| *TET2* | 462 | 14.3 | 24.3 | 8.1 | < 0.001 |
| *DNMT3A* | 462 | 15.2 | 20.9 | 11.6 | 0.008 |
| *TP53* | 462 | 7.6 | 13.0 | 4.2 | 0.001 |
| *Cohesin* | 411 | 10.0 | 9.6 | 10.2 | > 0.999 |

Among the total cohort of 329 AML patients undergoing conventional intensive induction chemotherapy, 252 (76.6%) patients achieved CR. Older patients had a lower CR rate than younger population (56.5 vs 81.9%, $P < 0.001$, Table 1). With a median follow-up of 69 months (ranges, 0.1–160), the elderly had significantly poorer OS and DFS than those aged below 60 years (median, 10.0 vs 61.0 months, $P < 0.001$, Figure 2a, and median, 3.0 vs 9.0 months, $P = 0.001$, Figure 2b, respectively). In multivariate Cox proportional hazards regression analysis for total cohort (Table 4), the independent poor risk factors for OS and DFS were older age, high white blood cell count > 50 000/μl, and *WT1*, *DNMT3A* and *TP53* mutations. On the other hand, *NPM1*$^+$/*FLT3*-ITD$^-$ and *CEBPA*$^{\text{double-mutation}}$ were independent favorable prognostic factors. We also found that unfavorable-risk cytogenetics and *RUNX1* mutations independently conferred poorer DFS and *IDH2* predicted better OS.

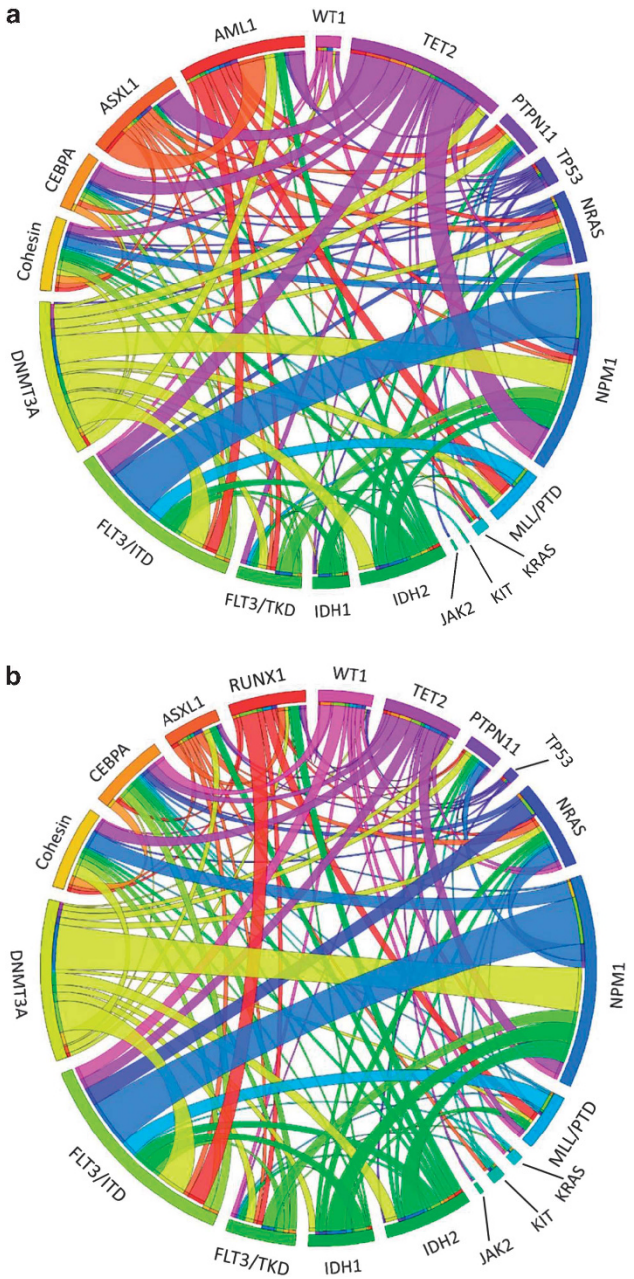


**Figure 1.** The Circos plots depicted the relative frequency and pairwise co-occurrence of genetic alterations in the older (**a**) and younger AML patients (**b**). The length of the arc corresponds to the frequency of the first gene mutation, and the width of the ribbon corresponds to the proportion of the second gene mutation.

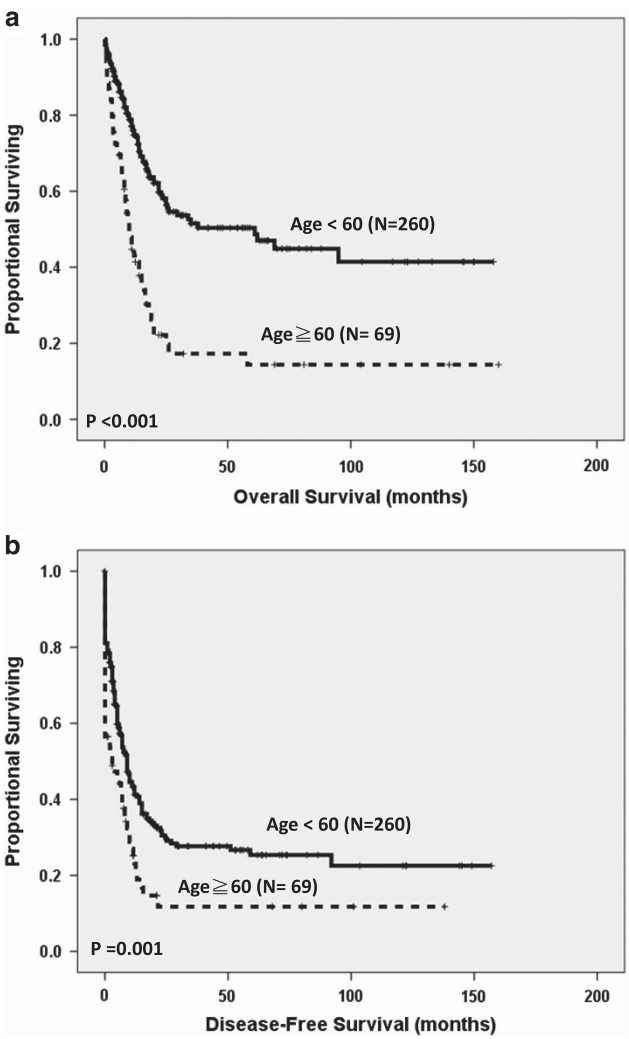


**Figure 2.** The Kaplan–Meier survival curves for OS (**a**) and DFS (**b**) in 329 AML patients who received standard intensive chemotherapy. The older patients have significantly poorer OS and DFS than those aged below 60 years (median, 10.0 vs 61.0 months, $P < 0.001$, and median, 3.0 vs 9.0 months, $P = 0.001$, respectively).

In the multivariate Cox proportional hazards regression analysis for OS in the elderly, *DNMT3A* and *TP53* mutations were independent poor prognostic factors, while *NPM1*$^{+}$/*FLT3*-ITD$^{-}$ remained to have good prognostic impact. Intriguingly, the older patients harboring any unfavorable genetic alteration, including *FLT3*/ITD, *DNMT3A* or *TP53* mutation, had more dismal survival compared with those not (median OS, 7.0 vs 14.0 months, $P = 0.042$, Figure 3).

The poor prognostic impacts of some mutations, such as *FLT3*/ITD, *RUNX1* and *DNMT3A* mutations were lost when the patients receiving HSCT were not censored on the date of transplantation, implying allogeneic HSCT might overcome the poor risk of the patients with these mutations. Unfortunately, none of the 69 elder patients underwent allogeneic HSCT.

Further, the older patients with intermediate-risk cytogenetics could be further separated into three risk groups according to the molecular genotype:[22] mutations of *NPM1* or *IDH2* or *CEBPA*$^{\text{double-mutation}}$ in the absence of *FLT3*/ITD as a favorable genotype,[9] mutations of *RUNX1*, *WT1*, *ASXL1*, *DNMT3A* or *TP53* as an unfavorable genotype,[9,27] and the remaining mutation patterns as an intermediate-risk genotype. Among the older patients with intermediate-risk cytogenetics, those with a favorable genotype had a higher CR rate and a trend of lower relapse rate than those with intermediate-risk and unfavorable genotypes (CR, 81.8 vs 64.7 vs 30.3%, $P = 0.002$ and relapse, 44.4 vs 72.7 vs 87.5%, $P = 0.150$). These three groups also had distinct OS (median, 26.0 vs 15.0 vs 8.0 months, $P < 0.001$, Figure 4a) and DFS (median, 12.0 vs 7.0 vs 0 months, $P = 0.002$, Figure 4b). Patients with intermediate-risk cytogenetics but favorable genotype had OS and DFS similar to those with favorable-risk cytogenetics ($P = 0.349$), while patients with intermediate-risk cytogenetics but unfavorable genotype had OS and DFS similar to those with unfavorable-risk cytogenetics ($P = 0.420$).

**Table 4.** Multivariate analysis (Cox regression) on the disease-free survival and overall survival

| *Variables* | *Disease-free survival* | | | | *Overall survival* | | | |
|---|---|---|---|---|---|---|---|---|
| | *RR* | *95% CI* | | P *value* | *RR* | *95% CI* | | P *value* |
| | | *Lower* | *Upper* | | | *Lower* | *Upper* | |
| *Total cohort* (n = *329*) | | | | | | | | |
| Age[a] | 1.556 | 1.112 | 2.178 | 0.010 | 2.494 | 1.707 | 3.644 | < 0.001 |
| WBC[b] | 1.440 | 1.058 | 1.960 | 0.020 | 1.767 | 1.208 | 2.585 | 0.003 |
| Karyotype[c] | 1.686 | 1.033 | 2.751 | 0.037 | 1.621 | 0.844 | 3.116 | 0.147 |
| *NPM1/FLT3*-ITD[d] | 0.296 | 0.159 | 0.550 | < 0.001 | 0.324 | 0.156 | 0.673 | 0.003 |
| *CEBPA*[double-mutation] | 0.541 | 0.331 | 0.883 | 0.014 | 0.434 | 0.215 | 0.876 | 0.020 |
| *RUNX1* | 1.656 | 1.060 | 2.585 | 0.027 | 1.691 | 0.976 | 2.932 | 0.061 |
| *WT1* | 1.893 | 1.221 | 2.935 | 0.004 | 1.816 | 1.037 | 3.181 | 0.037 |
| *ASXL1* | 0.958 | 0.553 | 1.660 | 0.878 | 1.298 | 0.727 | 2.317 | 0.377 |
| *TET2* | 0.991 | 0.623 | 1.577 | 0.969 | 1.017 | 0.594 | 1.743 | 0.950 |
| *IDH2* | 0.772 | 0.486 | 1.226 | 0.273 | 0.459 | 0.235 | 0.899 | 0.023 |
| *MLL*/PTD | 1.316 | 0.725 | 2.391 | 0.367 | 1.471 | 0.673 | 3.211 | 0.333 |
| *DNMT3A* | 1.932 | 1.273 | 2.932 | 0.002 | 2.167 | 1.316 | 3.567 | 0.002 |
| *TP53* | 2.441 | 1.194 | 4.992 | 0.015 | 5.184 | 2.276 | 11.808 | < 0.001 |
| *Older cohort* (n = *69*) | | | | | | | | |
| WBC[b] | 1.491 | 0.750 | 2.964 | 0.254 | 1.833 | 0.808 | 4.158 | 0.147 |
| Karyotype[c] | 0.958 | 0.304 | 3.022 | 0.941 | 1.828 | 0.635 | 5.266 | 0.264 |
| *NPM1/FLT3*-ITD[d] | 0.371 | 0.126 | 1.087 | 0.071 | 0.228 | 0.070 | 0.738 | 0.014 |
| *CEBPA*[double-mutation] | 0.931 | 0.349 | 2.482 | 0.886 | 0.570 | 0.130 | 2.489 | 0.454 |
| *RUNX1* | 1.426 | 0.571 | 3.560 | 0.447 | 1.615 | 0.656 | 3.977 | 0.297 |
| *ASXL1* | 1.147 | 0.421 | 3.123 | 0.788 | 1.290 | 0.480 | 3.471 | 0.614 |
| *TET2* | 0.856 | 0.385 | 1.900 | 0.702 | 1.042 | 0.427 | 2.543 | 0.928 |
| *IDH2* | 0.438 | 0.172 | 1.117 | 0.084 | 0.425 | 0.145 | 1.246 | 0.119 |
| *MLL*/PTD | 1.542 | 0.520 | 4.571 | 0.435 | 2.669 | 0.825 | 8.630 | 0.101 |
| *DNMT3A* | 1.638 | 0.786 | 3.410 | 0.188 | 3.396 | 1.471 | 7.840 | 0.004 |
| *TP53* | 2.222 | 0.547 | 9.027 | 0.264 | 4.306 | 1.069 | 17.347 | 0.040 |

Abbreviation: CI, confidence interval; RR, relative risk; WBC, white blood cell. [a]Age ⩾ 60 relative to Age < 60 (the reference). [b]WBC greater than 50 000/μl vs less than 50 000/μl. [c]Unfavorable cytogenetics vs others. [d]*NPM1*$^{+}$*/FLT3*-ITD$^{-}$ vs other subtypes.

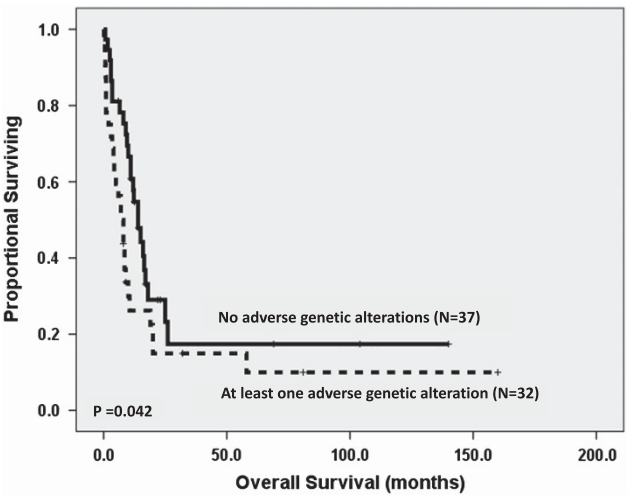


**Figure 3.** The Kaplan–Meier survival curve for OS in older AML patients stratified by having adverse genetic alterations or not. The survival of older patients who harbor any unfavorable genetic alterations is more dismal compared with those who do not (OS, 7.0 vs 14.0 months, $P = 0.042$).

Genetic ontogeny was first proposed by Lindsley *et al.*[40] Three types of mutations were defined: secondary-type mutations, *TP53* mutations and *de novo*/pan AML mutations. Presence of secondary-type mutations predicted characteristic phenotype and poor outcome. We validated the impact of genetic ontogeny in our cohort. Five secondary-type genes, including three splicing factor genes (*SRSF2*, *SF3B1* and *U2AF1*),[41] *ASXL1* and *STAG2*, were analyzed. In the 177 *de novo* AML patients aged 60 years or older, 34.5% had secondary-type mutations, 13.0% had *TP53* mutation, 49.2% had *de novo*/pan AML mutations and 3.3% were undetermined. Among the 69 patients receiving standard chemotherapy, patients with secondary-type or *TP53* mutations had a lower CR rate and a poor OS than those with *de novo*/pan AML mutations (43.5% vs 25.0% vs 66.7%, $P = 0.006$, and median, 10.0 vs 3.0 vs 14.0 months, $P = 0.004$, respectively).

## DISCUSSION

Most studies concerning prognostic factors in AML patients were focused on younger patients with less comorbidities and better performance status, and thus might not be representative of the general AML population in the real world.[42] In this study, we recruited consecutively all *de novo* AML patients who had adequate samples for mutation analyses without restriction of age, so that we could compare the genetic alterations between older and younger patients and explored their clinical implications. We found that older AML patients had distinct clinicobiological features and genetic alterations from younger patients, and the status of mutations could predict the prognosis in this group of patients.

Traditionally, karyotype is one of the strongest prognostic factors in AML patients.[43,44] We showed that older patients had more frequently poor risk cytogenetics, such as complex chromosomal abnormalities, or aberrations involving chromosomes 5 and 7, but less frequently the core binding factor abnormalities. To better stratify AML patients into different risk groups, the European LeukemiaNet (ELN) panel first proposed a

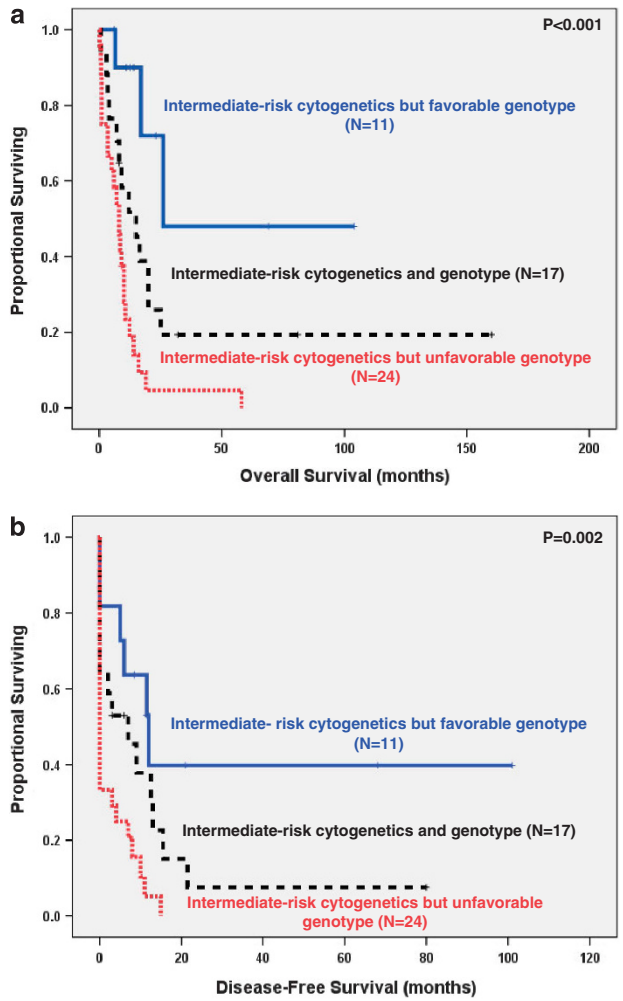


**Figure 4.** The Kaplan–Meier survival curve for OS (**a**) and DFS (**b**) in older AML patients stratified by molecular genotypes. Older patients could be risk-stratified into groups with distinct outcomes.

standardized classification according to both cytogenetics and molecular mutations in three genes, including *FLT3*/ITD, *NPM1* and *CEBPA* mutations.[7] Recently, several other genetic alterations were also found to have prognostic significance and were incorporated into risk stratification of AML patients.[22,31,45,46] However, there were only few reports in literature regarding the clinical impact of molecular alterations on older AML patients. In the studies from Cancer and Leukemia Group B (CALGB), *RUNX1* and *ASXL1* mutations were found to be more prevalent in the elder population with cytogenetically normal AML and were poor prognostic factors.[14,15] Older patients with *WT1*[(ref. 47)] or *TP53*[(ref. 48)] mutations had a shorter OS, while those with *NPM1*[49] mutations had a better CR rate and OS. Ostronoff *et al.*[50] further depicted that *NPM1* mutations in the absence of *FLT3*/ITD had a survival benefit among patients aged 55–65 years, but not in those older than 65 years. To our knowledge, this study is the first to comprehensively investigate the molecular genetic alterations of 21 genes among older patients with non-M3 AML. First, we showed that the distribution of genetic alterations and the burdens of gene mutations differed across the age groups. Second, older patients had higher incidences of *PTPN11*, *NPM1*, *RUNX1*, *ASXL1*, *TET2*, *DNMT3A* and *TP53* mutations but less *WT1* mutations. With the exception of *NPM1* mutation, most of the other mutations that were more prevalent in older patients had unfavorable prognostic impact. Furthermore, older patients had a higher frequency to harbor one or more adverse genetic alterations (including *FLT3*/ITD, *WT1*, *RUNX1*, *ASXL1*, *DNMT3A* and *TP53*) than younger ones. Taken together, in addition to a higher incidence of adverse cytogenetics, the higher frequency and burdens of molecular mutations that are associated with poor prognosis in the elderly might explain the dismal outcome in this group of patients. Another possible cause to explain the dismal clinical outcome in older patients is that they are more vulnerable to the toxicity of chemotherapy agents, so may have higher treatment-related mortality.[5,51] However, our study demonstrated that the early mortality rate were comparable between the two age groups, similar to the national registration data of the United States[52] and Swedish Acute Leukemia Registry.[53,54]

Cytogenetic changes could well separate older AML patients into three risk groups in this study, similar to previous reports.[55–58] However, about 60–70% of the patients were in the intermediate-risk cytogenetic group which would hinder risk stratification of these patients for proper treatment. With the incorporation of nine gene mutations, including *FLT3*/ITD and mutations of *CEBPA*, *NPM1*, *RUNX1*, *WT1*, *IDH2*, *ASXL1*, *DNMT3A* and *TP53*, that are associated with prognosis,[9,27] we showed that older AML patients with intermediate-risk cytogenetics could be further stratified into three groups with different outcomes. The patients with favorable genotype (*NPM1*, *IDH2* and *CEBPA*$^{\text{double-mutation}}$ in the absence of *FLT3*/ITD) had the longest survival, whereas those with unfavorable genotype (*DNMT3A*, *ASXL1*, *WT1*, *RUNX1* or *TP53*) had the poorest outcome. The incorporation of the mutation status of these genes is helpful to stratify this highly heterogeneous population with intermediate-risk cytogenetics into distinct risk groups.

Genetic ontogeny, first proposed by Lindsley *et al.*,[40] can help risk-stratify AML patients irrespective of their clinical assignment. In the 42 *de novo* AML patients aged 60 years or older in their study, 33.3% had secondary-type mutations, 21.4% had *TP53* mutations and 45.2% had *de novo*/pan AML mutations. The frequencies of these three types of mutations in our cohort were not much different from those reported by Lindsley *et al.* The poor prognostic implication of secondary-type mutations in elderly patients with *de novo* AML was shown in this study as that of Lindsley *et al.*[40] Similar to the high incidence of secondary-type mutations, elderly AML patients aged 60 years or older had higher incidence of dysplastic morphological features than those younger than 60 years (23.7 vs 14.4%; $P=0.013$); even none of them had a history of myelodysplastic syndrome, myeloproliferative neoplasm or other hematologic diseases.

In summary, we showed that older AML patients had distinct clinico-biological features, more frequently high-risk cytogenetics and gene mutations, and poorer prognosis. Integration of both cytogenetics and molecular alterations can better stratify older patients into different risk groups with distinct outcomes. It is warranted to develop novel therapies to improve the outcome of older patients with poor prognosis under current treatment modalities.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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## AUTHOR CONTRIBUTIONS

C-HT was responsible for data management and interpretation, statistical analysis and manuscript writing; H-AH was responsible for study design and plan, literature collection, data management and interpretation, statistical analysis and manuscript writing; C-YL was responsible for statistical analysis and interpretation of the statistical findings; Y-YK, L-IL was responsible for mutation analysis and interpretation; C-YC, W-CC, M-Y, S-YH, J-LT, B-SK, S-CH, C-TL, C-CL, S-JW, S-CC, WT and Y-CC contributed patient samples and clinical data; M-HT, C-FH, Y-CC, C-YL, F-YL and M-CL performed the gene mutation and chromosomal studies and H-FT designed and coordinated the study over the entire period and wrote the manuscript.

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Supplementary Information accompanies this paper on the Leukemia website (http://www.nature.com/leu)