**TET2** mutations were predictive of inferior prognosis in the presence of **ASXL1** mutations in patients with chronic myelomonocytic leukemia

Yajuan Cui¹²*, Hongyan Tong³*, Xin Du⁴*, Bing Li¹², Robert Peter Gale⁵, Tiejun Qin¹, Jinqin Liu², Zefeng Xu¹², Yue Zhang¹², Gang Huang⁶, Jie Jin³, Liwei Fang¹, Hongli Zhang⁷, Lijuan Pan¹, Naibo Hu¹, Shiqiang Qu¹, Zhijian Xiao¹²

¹MDS and MPN Center, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin 300020, China; ²State Key Laboratory of Experimental Hematology, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin 300020, China; ³Department of Hematology, The First Affiliated Hospital, Zhejiang University College of Medicine, Hangzhou 310000, China; ⁴Department of Hematology, Guangdong General Hospital, Guangzhou 510010, China; ⁵Hematology Research Center, Division of Experimental Medicine, Department of Medicine, Imperial College London, London, UK; ⁶Division of Experimental Hematology and Cancer Biology, Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio, USA

*These authors contributed equally to this work.

**Background:** Somatic mutations involving epigenetic regulators, histone modification and chromatin regulation, splicing components, transcription factors and signaling regulator genes are common in chronic myelomonocytic leukemia (CMML) patients. It has been consensus that **ASXL1** mutations have adversely impact on overall survival (OS), while the effect of **TET2** mutations remains controversial and undefined.

**Methods:** **ASXL1** and **TET2** mutations were analyzed in 141 patients with CMML using Sanger sequencing, with the aim to identify the interplay of **ASXL1** and **TET2** mutations in the prognosis of CMML.

**Results:** Sixty-five (46.1%) of the CMML patients harbored **ASXL1** mutations (frameshift and nonsense), and 46 (32.6%) had **TET2** mutations (frame shift, nonsense and missense). In a separate multivariable analysis that included the Mayo Prognostic Model as a single variable along with **ASXL1 wt/TET2 wt**, the respective hazard ratios of **ASXL1 mut/TET2 mut**, **ASXL1 mut/TET2 wt** and **ASXL1 wt/TET2 mut** were 4.7 (95% CI, 2.2–10.3; P<0.001), 2.2 (95% CI, 1.1–4.2; P=0.025) and 1.3 (95% CI, 0.6–2.5; P=0.521).

**Conclusions:** Our study showed that **ASXL1** mutations predict inferior OS, and additional **TET2** mutations were associated with poor survival in the presence of **ASXL1** mutations of CMML patients.

**Keywords:** **ASXL1** mutations; chronic myelomonocytic leukemia (CMML); prognosis; **TET2** mutations

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**Introduction**

Somatic mutations have been detected in about 90% of patients with chronic myelomonocytic leukemia (CMML) and the most frequent mutations occur in epigenetic regulators (**TET2**, 40–60%), histone modification and chromatin regulation (**ASXL1**, 30–50%), splicing components (**SRSF2**, 30–50%), transcription factors (**RUNXI**, 10–20%), and signaling regulator genes (**SETBP1**, 10–20%) (1-5). In proximately, over 40% CMML patients have at least two mutations (3,5,6). The diverse combinations of mutations detected in CMML suggest multi-step pathogenesis of the disease in some cases. For example, **ASXL1** and **TET2** mutations are the most frequent mutations and may be independent drivers of CMML in...
some patients (7), and the combination of TET2/SRSF2 mutations and ASXL1/SETBP1 mutations are consistent with a two-step ‘linear’ model of CMML development (8). In vitro studies have shown that ASXL1 mutations enhance the de-ubiquitinase activity of the ASXL1-BAP1 (BRCA associated protein 1) complex, which then cooperates with loss of TET2 to skew towards myeloid development (9). In a recent study, Patnaik et al. (6) demonstrated prognostic interaction of ASXL1 and TET2 mutations, which showed that TET2 mutations predict favorable survival in the absence of ASXL1 mutations. In this study, ASXL1 and TET2 mutations were detected in 141 patients with CMML and the interaction of ASXL1 and TET2 mutations in the prognostic stratification of CMML were conducted.

**Patients and methods**

From July 2007 to July 2015, 141 adult patients were reviewed and diagnosed as CMML according to the 2008 World Health Organization criteria (10). All patients received symptomatic treatment (blood transfusions, hydroxyurea, etc.). Risk stratifications of patients was based on Mayo Prognostic Model and Mayo Molecular Model (4,11). Overall survival (OS) was calculated from the date of first referral to the date of death (uncensored) or last contact (censored). This study was approved by the Ethical Committees of the Institute of Hematology, Chinese Academy of Medical Sciences (CAMS) and Peking Union Medical College (PUMC) following principles of the Declaration of Helsinki and all patients have signed informed consent.

Genomic DNA of bone marrow mononuclear cells collected at diagnosis was extracted using conventional methods. Mutation analysis was performed according to previously published methods (5). Sequencing was bi-directional. Sequence traces were analyzed with Mutation Surveyor Software (Applied Biosystems Genetic Analyzers). Single nucleotide polymorphisms previously annotated (www.hapmap.org) were discarded. Frameshift, nonsense and missense of TET2 mutations were considered pathogenic while only frameshift and nonsense of ASXL1 mutations were considered as pathogenic (only frameshift and nonsense mutations of ASXL1 independently impacting OS in previously published research).

Numerical variables are presented as medians and ranges. Categorical variables are described as counts and relative frequencies (%). Comparisons between categorical variables were performed using \( \chi^2 \) tests. Comparisons between continuous variables were performed using the Kruskal-Wallis U-test. Survival was analyzed by the Kaplan-Meier method and compared using the log-rank test. A Cox model was used to identify the prognostic variables. All the statistical analyses were conducted with SPSS version 18.0. All the P values are two-tailed, and statistical significance was set at P<0.05.

**Results**

The median age was 63 (range, 16–85) years, with a male 95 (67%) predilection. Following the WHO criteria, 80 (57%) of patients were identified as CMML-1 and the remainder as CMML-2, with median OS of 21 and 18 months, respectively. With a median follow-up of 15.4 months, 80 (57%) deaths were documented. Sixty-five (46.1%) of the CMML patients harbored ASXL1 mutations (frameshift and nonsense), and 46 (32.6%) had a TET2 mutation (frame shift, nonsense and missense). Following the Mayo Prognostic Model, 64.5% were classified as high, 31.2% as intermediate and 4.3% as low risk, while according to the Molecular Mayo Model, 45.4% corresponded to high, 34.8% to intermediate-2, 17.0% to intermediate-1 and 2.8% to low risk.

In univariate analysis, hemoglobin <100 g/L (P=0.004), presence of circulating immature myeloid cells (IMCs) (P=0.006), platelet <50×10⁹/L (P=0.022) and presence of ASXL1 mutations (P<0.001) predict inferior prognosis while the presence of TET2 mutations not (P=0.922). In multivariable analysis, hemoglobin <100 g/L (P=0.008), presence of IMCs (P=0.015) and ASXL1 (P=0.001) mutations remained significant.

We also stratified these 141 patients into four mutational categories to find out prognostic interaction between ASXL1 and TET2 mutations: ASXL1mut/TET2mut (n=17), ASXL1mut/TET2wt (n=48), ASXL1wt/TET2mut (n=29) and ASXL1wt/TET2wt (n=47). There was no statistical significance of hemoglobin, WBC, platelets, existence of IMCs except the ages among the four groups (details shown in Table 1). OS of the four groups from inferior to favorable was ASXL1mut/TET2mut (16.7 months), ASXL1mut/TET2wt (18.4 months) and ASXL1wt/TET2mut (36.0 months) ASXL1wt/TET2wt (36.0 months) (Figure 1). We did confirm the significant favorable impact of TET2 mutations in the absence of ASXL1 mutations (P=0.580).

In multivariable analysis that included the Mayo Prognostic Model as a single variable along with ASXL1wt/ TET2wt, the respective hazard ratios of ASXL1mut/
Table 1 Clinical and laboratory features and survival in 141 patients with WHO-defined chronic myelomonocytic leukemia, stratified by ASXL1 and TET2 mutational status

<table>
<thead>
<tr>
<th>Variable</th>
<th>ASXL1mut/TET2mut (n=17)</th>
<th>ASXL1mut/TET2wt (n=48)</th>
<th>ASXL1wt/TET2mut (n=29)</th>
<th>ASXL1wt/TET2wt (n=47)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males [%]</td>
<td>14 [82]</td>
<td>35 [73]</td>
<td>20 [69]</td>
<td>26 [55]</td>
<td>0.149</td>
</tr>
<tr>
<td>Hemoglobin, median (range) (g/L)</td>
<td>90.0 (43.0–146.0)</td>
<td>88.0 (43.0–158.0)</td>
<td>88.0 (56.0–166.0)</td>
<td>91.0 (44.0–157.0)</td>
<td>0.516</td>
</tr>
<tr>
<td>WBC, median (range) (×10^9/L)</td>
<td>25.6 (5.0–117.6)</td>
<td>22.8 (3.6–99.5)</td>
<td>20.0 (3.0–95.8)</td>
<td>21.4 (3.1–105.0)</td>
<td>0.377</td>
</tr>
<tr>
<td>Platelets, median (range) (×10^9/L)</td>
<td>85.0 (10.0–534.0)</td>
<td>100.5 (4.0–633.0)</td>
<td>88.0 (9.0–1,001.0)</td>
<td>60.0 (7.0–895.0)</td>
<td>0.305</td>
</tr>
<tr>
<td>WHO subtype [%]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.423</td>
</tr>
<tr>
<td>CMML-2</td>
<td>5 [29]</td>
<td>21 [44]</td>
<td>11 [38]</td>
<td>24 [51]</td>
<td></td>
</tr>
<tr>
<td>Mayo prognostic model [%]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.666</td>
</tr>
<tr>
<td>Mayo Molecular Model [%]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Survival (median) (months)</td>
<td>16.7</td>
<td>18.4</td>
<td>36.0</td>
<td>36.0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

CMML, chronic myelomonocytic leukemia.

TET2mut, ASXL1mut/TET2wt and ASXL1wt/TET2mut were 4.7 (95% CI, 2.2–10.3; P<0.001), 2.2 (95% CI, 1.1–4.2; P=0.025) and 1.3 (95% CI, 0.6–2.5; P=0.521).

Discussion

About 90% of patients with CMML had at least one somatic mutation, and over 20 relative genes were involved (6). The various combinations of genetic abnormalities in CMML did not only indicate a multi-step pathogenesis, but also likely contributed to the marked clinical heterogeneity of these disorders. In this study, we confirmed the negative prognostic impact on OS imparted by ASXL1 mutations, and also suggested an unfavorable prognostic
impact from TET2 mutations in the presence of ASXL1 mutations. ASXL1 and TET2 mutations were considered as independent drivers of CMML (7), and ASXL1mut/TET2mut suggested two ‘lineage’ clone development and might lead to poorer prognosis than other subgroups in patients receiving symptomatic treatment.

Although ASXL1 mutations negatively impact survival, there is not efficient evidence of correlation between overall response rate to hypomethylating agents (HMAs) and ASXL1 mutations (12). Total 5-methyl-cytosine levels in TET2mut cases were significantly higher than TET2wt cases in CMML (13). Patnaik et al. (6) demonstrated TET2 mutations predict favorable survival in the absence of ASXL1 mutations. Bejar et al. (14) also reported higher abundance TET2 mutations were associated with better response to HMAs in the absence of ASXL1 mutations in myelodysplastic syndrome patients. Thus, we speculated that TET2 mutations may make up inferior OS imparted by ASXL1 mutations and lead to better OS in ASXL1wt cases under treatment with HMAs in other studies, but could not show any advantage with symptomatic treatment in our study. At the same time, the combined mutation of TET2 and ASXL1 were considered as complicated clonal combination of CMML, and thus ASXL1mut/TET2mut indicated an aggressive disease evolution.

Conclusions

In summary, our study showed ASXL1 mutations predict inferior OS, and additional TET2 mutations present with an aggressive disease evolution in the presence of ASXL1 mutations in CMML patients received symptomatic treatment.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References


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