INTRODUCTION

Gene mutations are common (>90%) in chronic myelomonocytic leukemia (CMLM) and involve epigenetic regulators (TET2 ~ 60% and ASXL1 ~ 40%), spliceosome components (SRSF2 ~ 50%) and cell signaling (RAS ~ 30% and CBL ~ 15%). Mutations involving ASXL1, TET2, RUNX1, CBL, SRSF2, RAS and IDH2 have demonstrated prognostic relevance on univariate survival analyses. However, on multivariable analyses that have included additional CMLM relevant factors, only ASXL1 mutations (frameshift and nonsense) have been shown to be prognostically detrimental. This has led to the incorporation of ASXL1 mutations into molecular prognostic models such as the Molecular Mayo Model and the Groupe Francais des Myelodysplasies model. TET2 mutations (chromosome 4q24) are frequent and are thought to be the driver mutations in CMML. TET2 catalyzes the conversion of 5-methyl-cytosine to 5-hydroxymethyl-cytosine, regulating methylation and transcription. The prognostic relevance of TET2 mutations remains unclear with some studies demonstrating favorable, unfavorable and no impact on overall survival (OS). 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 toward myeloid development. However, the mechanisms behind this effect and the prognostic interplay between TET2 and ASXL1 mutations remain unknown.

In the current study, we used a 27-gene panel assay to: (i) identify additional prognostically-relevant mutations in CMLM, (ii) to determine if the number of mutations carries prognostic relevance and (iii) to study the prognostic interplay between TET2 and ASXL1 mutations.
Table 1. Clinical and laboratory features and subsequent events in 175 patients with World Health Organization defined chronic myelomonocytic leukemia, stratified by ASXL1 and TET2 mutational status

| Variable | All patients with CMML (n = 175) | CMML patients with ASXL1 mutations (n = 82) | CMML patients with TET2 mutations (n = 80) |
|----------|----------------------------------|-------------------------------------------|------------------------------------------|
| Age in years; median (range) | 70 (18–90) | 69 (27–86) | 70 (40–90) |
| Males; n (%) | 116 (66) | 59 (72.0) | 56 (70) |
| Hemoglobin g/dL; median (range) | 11.5 (6.4–16.9) | 13.1 (1.8–264) | 9.3 (1.8–264) |
| WBC x 10^9/L; median (range) | 1.5 (0–151) | 2.6 (0.6–40) | 2 (0.3–40) |
| Platelets x 10^9/L; median (range) | 87 (10–585) | 82 (10–339) | 77 (10–585) |
| WHO morphological subtype; n (%) | | | |
| CMML-1 | 146 (83) | 67 (81.7) | 75 (93.8) |
| CMML-2 | 29 (17) | 15 (18.3) | 5 (6.1) |
| Mutational analysis | | | |
| IKZF | 0 (0) | 0 (0) | 0 (0) |
| PTPN11 | 8 (4.5) | 5 (6) | 0 (0) |
| SH2B3 | 8 (4.5) | 5 (6) | 6 (7.5) |
| SUZ12 | 2 (1.1) | 1 (1.2) | 1 (1.25) |
| ZRS22 | 9 (5.1) | 6 (7.3) | 7 (8.75) |
| CALR | 1 (0.57) | 0 (0) | 0 (0) |
| CBL | 25 (14.3) | 14 (17) | 12 (15) |
| CEBPA | 11 (6.3) | 6 (7.3) | 4 (5) |
| CSF3R | 3 (1.7) | 2 (2.4) | 1 (1.25) |
| DNM3A | 9 (5.1) | 3 (3.7) | 2 (2.5) |
| EZH2 | 2 (1.1) | 1 (1.2) | 1 (1.25) |
| FLT3 | 1 (0.57) | 1 (1.2) | 0 (0) |
| IDH1 | 3 (1.7) | 2 (2.4) | 0 (0) |
| IDH2 | 8 (4.5) | 5 (6) | 1 (1.25) |
| JAK2 | 7 (4) | 4 (4.9) | 1 (1.25) |
| KIT | 2 (1.1) | 1 (1.2) | 1 (1.25) |
| MPL | 0 (0) | 0 (0) | 0 (0) |
| NPM1 | 5 (2.9) | 0 (0) | 1 (1.25) |
| NRAS | 21 (12) | 12 (14.6) | 9 (11.25) |
| RUNX1 | 25 (14.3) | 13 (15.9) | 10 (12.5) |
| SETBP1 | 33 (18.9) | 23 (28) | 11 (13.75) |
| SF3B1 | 10 (5.7) | 1 (1.2) | 5 (6.25) |
| SRSF2 | 93 (53.1) | 39 (47.6) | 41 (51.25) |
| Tp53 | 9 (5.1) | 1 (1.2) | 1 (1.25) |
| U2AF1 | 14 (8) | 11 (13.4) | 2 (2.5) |
| ASXL1 | 82 (46.9) | N/A | 31 (38.75) |
| TET2 | 80 (45.7) | 31 (37.8) | N/A |
| Mayo-French cytogenetic risk stratification; n (%) | | | |
| Low | 118 (78) | 51 (70) | 66 (83) |
| Intermediate | 21 (10) | 11 (14) | 6 (8) |
| High | 18 (12) | 9 (16) | 1 (9) |
| MD Anderson prognostic risk categories; n (%) | | | |
| Low | 90 (51.4) | 35 (42.7) | 53 (66.25) |
| Intermediate-1 | 41 (23.4) | 22 (26.8) | 13 (16.25) |
| Intermediate-2 | 35 (20) | 21 (25.6) | 14 (17.5) |
| High | 9 (5.1) | 4 (4.9) | 0 (0) |
| Mayo model prognostic risk categories; n (%) | | | |
| Low | 76 (43.4) | 28 (34.1) | 40 (50) |
| Intermediate | 56 (32) | 32 (39) | 28 (35) |
| High | 43 (24.6) | 22 (26.8) | 12 (15) |
| Molecular Mayo Model risk categories; n (%) | | | |
| Low | 16 (9.1) | 3 (3.66) | 11 (13.75) |
| Intermediate-1 | 55 (31.4) | 12 (14.6) | 29 (36.25) |
| Intermediate-2 | 52 (29.7) | 30 (36.6) | 29 (36.25) |
| High | 52 (29.7) | 37 (45.1) | 11 (13.75) |
RESULTS

Among the 175 study patients, 115 (66%) were males with a median age of 70 years (range, 18–90). One hundred and forty-six (83%) patients were subclassified as CMMML-1 and the remainder had CMMML-2. At a median follow-up of 23 months, 146 (83%) deaths and 25 (14%) leukemic transformations were documented. Median survivals were 24 months for CMML-1 and 16 months for CMML-2 (P = 0.38). Cytogenetic risk stratification was carried out using the Mayo-French cytogenetic model,13 with the following distribution: 118 (78%) low, 21 (10%) intermediate and 18 (12%) high risk. Overall risk stratification was based on Mayo prognostic model:14 25% high, 32% intermediate and 43% low risk; and the Groupe Francais des Myelodysplasies model:19 high, 37% intermediate and 44% low risk. Baseline laboratory values and risk stratification are detailed in Table 1.

Mutational frequencies were as follows: TET2 46%, ASXL1 47%, SRSF2 45%, SETBP1 19%, CBL 14%, RUNX1 14%, NRAS 12%, U2AF1 8%, SF3B1 6%, ZRSR2 6%, Tp53 5%, DNMT3A 5%, IDH2 5%, PTPN11 5%, SH2B3 5%, JAK2V617F 4%, NPM1 3%, CSF3R 2%, IDH1 2%, EZH2 1%, SUZ12 1%, KIT 1%, FLT3 1% and CALR 1% (Figure 1 and Table 1). No mutations were detected in MPL or IKZF1. One hundred and seventy-two patients (98%) had at least one mutation, 21 (12%) had 2, 24 (14%) had 3, 20 (11%) had 4, 9 (5%) had 5; while one (1%) patient had 6 concurrent mutations (Figure 1).

In a univariate survival analysis that included the aforementioned mutations, only the presence of ASXL1 mutations (P = 0.01), absence of TET2 mutations (P = 0.005) and presence of DNMT3A mutations (P = 0.02) were associated with inferior survival. The number of concurrent mutations per patient did not affect outcome (P = 0.3). In a multivariable analysis, the presence of ASXL1 (P = 0.01) and the absence of TET2 (P = 0.03) mutations retained their negative prognostic impact. In order to determine the prognostic interaction between these two mutations, patients were stratified into four mutational categories: ASXL1wt/TET2wt (n = 56), ASXL1mut/TET2wt (n = 31), ASXL1mut/TET2mut (n = 50) and ASXL1wt/TET2mut (n = 38). Survival data in these four groups showed significant difference in favor of ASXL1wt/TET2wt (median survival 38 months; P = 0.016), compared with those with ASXL1wt/TET2wt (19 months), ASXL1mut/TET2wt (21 months) and ASXL1mut/TET2mut (16 months); there was no significant difference in survival among the latter three groups (P = 0.3). In multivariable analysis, presence of ASXL1 (P = 0.01) and absence of TET2 mutations (P = 0.003) remained significant when risk factors used in the Mayo prognostic model (hemoglobin < 10 g/dL, platelet count < 100x10^9/L, presence of circulating immature myeloid cells) were added to the model:14 the same was true for ASXL1wt/TET2wt (P = 0.036). In a separate multivariable analysis that included the Mayo prognostic model as a single variable along with presence of ASXL1 and absence of TET2 mutations or absence of ASXL1wt/TET2wt mutational status, the respective hazard ratios were 1.4 (95% CI 1.07–2.1; P = 0.012), 1.5 (95% CI 1.07–2.1; P = 0.03) and 1.8 (95% CI 1.2–2.7; P = 0.001). On a univariate analysis, LFS was worse in ZRSR2-mutated cases (P = 0.03). This relevance, however, was lost on a multivariable analysis that included circulating blasts (P = 0.01) and high risk karyotype (P = 0.03).

DISCUSSION

Clonal cytogenetic abnormalities are seen in ~30%,13,15 while gene mutations are seen in >90% of patients with CMMML.1,2,6 These mutations can broadly be classified into the following categories: (i) mutations involving epigenetic regulator genes: TET2 (~60%), DNMT3A, IDH1, and IDH2 (IDH mutations < 10%); (ii) mutations involving histone modification and chromatin regulation: ASXL1 (~40%) and EZH2 (~5%); (iii) mutations involving the splicing machinery: SF3B1, SRSF2 (~50%), U2AF1 and ZRSR2; (iv) mutations involving DNA damage response genes: Tp53 (~1%) and PHF6; (v) mutations in transcription factors and signal transduction pathways: JAK2, KRAS, NRAS (RAS ~30%),
**ASXL1** and **TET2** mutations in CMML

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**Figure 1.** Spectrum and frequency of gene mutations in 175 Mayo clinic patients with WHO defined chronic myelomonocytic leukemia.

**Figure 2.** Survival data for 175 patients with chronic myelomonocytic leukemia stratified by **ASXL1** and **TET2** mutational status.



CBL (−10−15%), FLT3, RUNX1 (~15%) and mutations such as SETBP1 (~15%),1,2,16−19 of these, mutations involving TET2 (~60%), SRSF2 (~50%), ASXL1 (~40%) and the RAS pathway (~30%) are most frequent, with only frameshift and nonsense **ASXL1** mutations independently impacting OS.1,2

The **ASXL1** (additional sex combs like 1) gene (chromosome 20q11) regulates chromatin by interacting with the polycomb-group repressive complex proteins (PRC1 and PRC2).20 Histone 2A lysine 119 (H2AK119Ub) and H3K27me3 play synergistic roles in PRC-mediated gene repression.11,21 Abdel-Wahab et al.21 demonstrated that **ASXL1** mutations resulted in loss of PRC2-mediated H3K27 tri-methylation, while Balasubramani et al.11 demonstrated that **ASXL1** truncations conferred enhanced activity on the **ASXL1**–BAP1 complex. This complex results in global erasure of H2AK119Ub and depletes H327Kme3, promoting dysregulated transcription. The current study once again demonstrates the frequent occurrence of **ASXL1** mutations (45%) in CMML and confirms the adverse prognostic impact imparted by frameshift and nonsense mutations on OS.

**TET2** (ten-eleven translocation (TET) oncogene family member 2) is a member of the TET family of proteins.22 Although **TET2** mutations are widely prevalent in CMML, thus far, they have not been shown to independently impact either OS or LFS.1 In the current study, **TET2** mutations were seen in 46% of CMML patients and the absence of **TET2** mutations negatively impacted OS. Additionally, the presence of clonal **TET2** mutations, in the absence of clonal **ASXL1** mutations (**ASXL1** wt/ **TET2** mut), had a favorable impact on OS. The mechanism behind this association is unclear. In MDS and younger patients with CMML (age <65 years), the presence of clonal **TET2** mutations, in the absence of clonal **ASXL1** mutations (**ASXL1** wt/ **TET2** mut), had a favorable impact on OS. The mechanism behind this association is unclear. In MDS and younger patients with CMML (age < 65 years), the presence of clonal **TET2** mutations, in the absence of clonal **ASXL1** mutations (**ASXL1** wt/ **TET2** mut), had a favorable impact on OS. The mechanism behind this association is unclear.

Approximately, 80% of patients with MDS have one or more oncogenic driver mutations (SF3B1 ~24%, **TET2** ~22%,
In a large study (n = 738), Papaemmanuil et al. demonstrated that driver mutations had an equivalent prognostic significance and LFS steadily declined as the number of driver mutations increased. 78% had at least one oncogenic mutation, while 43% had 2 or 3 and 10% had 4–8 mutations. Variants of unclear significance in oncogenic genes such as ASXL1 also adversely impacted outcomes. In the current study, 98% of the CMML patients had at least one mutation, 12% had 2, 14% had 3 and 17% had >3 mutations. The number of oncogenic mutations in CMML did not impact either the LFS or OS.

In summary, nearly all patients with CMML express one or more myeloid neoplasm-relevant mutations. Similar to prior studies, the LFS or OS on oncogenic mutations in CMML did not impact either the number of driver mutations increased. 78% had at least one mutation, 12% had 2, 14% had 3 and 17% had >3 mutations. These findings need validation in a larger data set.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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