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Research Paper

Prognostic Role of Gene Mutations in Chronic Myelomonocytic Leukemia Patients Treated With Hypomethylating Agents

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ABSTRACT

Somatic mutations contribute to the heterogeneous prognosis of chronic myelomonocytic leukemia (CMML). Hypomethylating agents (HMAs) are active in CMML, but analyses of small series failed to identify mutations predicting response or survival. We analyzed a retrospective multi-center cohort of 174 CMML patients treated with a median of 7 cycles of azacitidine ($n = 68$) or decitabine ($n = 106$). Sequencing data before treatment initiation were available for all patients, from Sanger ($n = 68$) or next generation ($n = 106$) sequencing. Overall response rate (ORR) was 52%, including complete response (CR) in 28 patients (17%). In multivariate analysis, *ASXL1* mutations predicted a lower ORR (Odds Ratio [OR] = 0.85, $p = 0.037$), whereas $TET2^{mut}/ASXL1^{wt}$ genotype predicted a higher CR rate (OR = 1.18, $p = 0.011$) independently of clinical parameters. With a median follow-up of 36.7 months, overall survival (OS) was 23.0 months. In multivariate analysis, $RUNX1^{mut}$ (Hazard Ratio [HR] = 2.00, $p = .011$), CBL^{mut} (HR = 1.90, $p = 0.03$) genotypes and higher WBC ($\log_{10}$(WBC) HR = 2.30, $p = .005$) independently predicted worse OS while the $TET2^{mut}/ASXL1^{wt}$ predicted better OS (HR = 0.60, $p = 0.05$). CMML-specific scores CPSS and GFM had limited predictive power. Our results stress the need for robust biomarkers of HMA activity in CMML and for novel treatment strategies in patients with myeloproliferative features and *RUNX1* mutations.

1. Introduction

Chronic myelomonocytic leukemia (CMML) is a clonal bone marrow disorder, classified by WHO as a myelodysplastic/myeloproliferative neoplasm (MDS/MPN) [18]. It is characterized by persistent monocytosis associated with a variable degree of bone marrow blast excess, cytopenias and myeloproliferation. Its prognosis is variable but overall poor, with a median survival of 20–32 months and a risk of acute myeloid leukemia (AML) transformation of 14–29% [8,11].

Recurrent somatic mutations found in CMML affect genes encoding epigenetic regulators, signaling, splicing and transcription regulator genes [11,14,16,19]. The high frequency of *TET2*, *ASXL1*, *SRSF2*, and RAS pathway (*NRAS*, *KRAS*, and *CBL*) mutations may represent a mutational fingerprint of the disease [11,19]. Frameshift and nonsense *ASXL1* mutations have invariably been shown to confer poor prognosis [8,11,19],

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while the poor prognostic impact of *TET2* and *SRSF2* mutations [11,13,21] is more controversial, and could depend on specific mutation combinations. Mutations in *EZH2* [9], *SETBP1 [8]* and *DNMT3A [22]* could also be detrimental, but their impact is more difficult to assess because of their lower incidence. Recently, several prognostic scoring systems accounting for gene mutations have been developed in CMML, but most of them were developed in cohorts of untreated patients, or with heterogeneous treatments [8,11]. The prognostic value of gene mutations is highly dependent on the therapeutic context, as exemplified by the specific poor prognosis of *RAS* mutations in CMML in the context of allogeneic stem cell transplantation (ASCT) [30].

Retrospective studies [1,5,23] and limited prospective data [3,6,25], mostly non-randomized, have reported activity of hypomethylating agents (HMA) in CMML. In these studies, azacitidine (AZA) and decitabine (DAC) provided an overall response rate (ORR) of 40–70%, translating in median overall survivals (OS) of 12–22 months. Response to HMA is difficult to predict and is loosely correlated to survival in CMML [7]. In MDS patients treated with HMA, mutations in the epigenetic regulators *TET2* [2,10,28] and *ASXL1* [2] affect response rates but not overall survival (OS). In retrospective studies of CMML treated with HMA, older age, higher bone-marrow (BM) and peripheral blood (PB) blast count, higher white blood cell counts (WBC), splenomegaly and cytogenetic risk have been found to impair survival [1].

The impact of gene mutations in this setting has so far been addressed in small series, precluding the identification of mutations predicting response or survival in CMML treated with HMA [3,15,25]. Here we report the largest retrospective cohort of CMML patients treated with DAC or AZA to date with available molecular data for the most frequently mutated genes.

2. Patients and Methods

2.1. Patients

We updated clinical data from 174 patients with CMML treated with AZA or DAC between February 2007 and December 2016, in Groupe Francophone des Myélodysplasies (GFM) centers ($n = 61$, including Dresden), Firenze ($n = 37$), Mayo clinic ($n = 41$), Memorial Sloan Kettering (MSKCC) and Moffitt (MCC) Cancer Centers ($n = 35$). Patients provided written informed consent and the study was approved by each institution's IRB (GFM: PHRC MAD-06 and clinical trial EudraCT #2008-000470-21; Mayo clinic: 15-003786 and 11-005599; Firenze NCT01251627; MCC/MSKCC: 00014416). Patients with previous intensive treatment (intensive chemotherapy or ASCT) or with AML transformation prior to HMA were excluded.

CMML diagnosis and stratification was made according to WHO 2008 criteria [18]. Splenomegaly was defined as a clinical or radiological spleen enlargement. Bone marrow blasts included agranular blasts, myeloblasts and promonocytes as recommended. Cytogenetic risk was assessed according to CMML-specific cytogenetic risk classification [27]. Prognosis at initiation of treatment was evaluated according to CMML-specific prognostic scoring system (CPSS) [27] and GFM score [11]. Information on RBC-transfusion dependency was not available and was substituted by hemoglobin level (Hb < 10 g/dL) to calculate CPSS as proposed by the authors [27]. CPSS-mol [8] was assessed in patients with either available *SETBP1* information, or for whom the risk was unchanged whatever the *SETBP1* mutational status. Patients received HMA according to standard schedules (AZA: 75 mg/m^2/d subcutaneously d1-7/28d cycles; DAC: 20 mg/m^2/d intravenous d1-5/28d cycles). Responses were assessed according to MDS IWG-2006 criteria [4].

2.2. Gene Mutation Analyses

DNA extracted from peripheral blood (PB) CD14$^+$ monocytes or bone marrow (BM) mononucleated cells (BMNCs) for GFM centers, BMNCs for Mayo Clinic and Firenze, BMNCs or PB mononucleated cells (PBMCs) for MSKCC and MCC. Analysis of somatic mutations was done by Sanger sequencing (GFM) or Next-Generation Sequencing (NGS), using custom target capture with Agilent SureSelect (Mayo Clinic), Agilent HaloPlex (Firenze), Fluidigm Access Array multiplex PCR technologies (MSKCC and MCC) followed by sequencing on Illumina platforms. The overlapping genomic regions interrogated by all platforms included exon 12 of *ASXL1*, and all coding exons in the *SRSF2*, *TET2*, *NRAS*, *RUNX1*, *CBL*, *U2AF1*, *DNMT3A*, *IDH2*, *KRAS*, *SF3B1*, *JAK2*, *EZH2*, *IDH1* and *TP53* genes. Details on mutational analysis pipelines have previously been published [11,15,20,24].

2.3. Statistical Analyses

Variables are reported as medians and interquartile ranges (IQR) and numbers and proportions for continuous and categorical variables respectively. Group comparisons for dichotomic, ordinal and continuous variables were carried by Fisher's exact tests, Kendall's correlation tests, and Mann-Whitney's tests respectively. Univariate analyses of variables influencing response rates were stratified on HMA and tested with linear regressions. All significant variables with significant impact ($p < 0.05$) in univariate analyses were then included in multivariate linear regressions adjusted on HMA.

OS was defined as time between initiation of HMA and date of death from any cause or date of last follow-up. AML free survival (AMLFS) was defined as the time between initiation of HMA and date of AML transformation, death or last follow-up. OS and AMLFS were obtained according to the Kaplan-Meier method and univariate analyses stratified on HMA were done with the Cox regression model. Follow-up duration was calculated with the inverse method. The prognostic impact of WBC was assessed with the $\log_{10}$-transformed variable (logWBC), and age, hemoglobin level, platelets count were analyzed as continuous variables. Multivariate survival analyses were performed by Cox regression followed by backward stepwise selection. The proportional hazard assumption was validated by visual inspection of Schöenfeld residuals. Interactions were studied by comparing through a likelihood ratio test Cox models including the two studied variables with or without an interaction term. The goodness-of-fit of a given model was assessed with Harrell's C concordance index (C-index), a value ranging from 0.5 (no relevance) to 1 (perfect prediction).

There was no sample size calculation prior to this retrospective study. In a post hoc power analysis using two-sided log-rank tests with an alpha risk of 0.05, a study population of 174 patients provided a power of 0.70 to detect a hazard ratio (HR) of 2.5 or higher for mutations present in only 10% of patients or to detect a milder effect (HR ≥ 1.5) for more frequent mutations (40% of patients). Mutations present in <10% of patients were thus not analyzed. Thus, only complete cases were analyzed, with imputation of missing data.

Propensity Score Matching (PSM) was performed by logistic regression using indicated variables, and patients were matched one to one by nearest neighbour according to HMA received. Quality of matching was checked by inspecting the reduction of bias for each variable and testing for differences in matched samples for each variable. All statistical analyses were stratified on HMA and two-sided, retaining $p < 0.05$ as statistically significant. Analyses were performed with R 3.3.2 (cran.r-project.org) or STATA 12 (Stata Corp).

3. Results

3.1. Patients Characteristics

We included 174 patients in this study, 118 men (68%) and 56 women (32%) with a median age of 72 years (Inter-quartile range [IQR] 66–78). Characteristics of patients at initiation of HMA are summarized in Table 1. Diagnosis at HMA onset was CMML-1 and CMML-2 in 64% and 36% respectively. Cytogenetic risk was low, intermediate,

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