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# *SETBP1* mutations in 415 patients with primary myelofibrosis or chronic myelomonocytic leukemia: independent prognostic impact in CMML

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*SETBP1* encodes SET-binding protein 1, a binding partner for the multi-function SET protein. This protein is encoded by the SET nuclear oncogene and is involved in apoptosis, transcription and nucleosome assembly.[1] The proposed functional outcome of this interaction is based on *in vitro* studies that demonstrate a protection of SET protein from protease cleavage that results in inhibition of protein phosphatase 2A activity, leading to higher rates of cell proliferation.[1] Initial identification of germline *SETBP1* alterations affecting amino-acid residues between 858 and 871 have been described in patients with Schinzel–Giedion syndrome, associated with a congenital phenotype including mental retardation and facial deformities.[2]

Recently, analysis of exome sequencing data from eight cases of atypical chronic myelogenous leukemia (aCML) led to the identification of recurrent somatic mutations involving *SETBP1*.[3] Mutational frequency was 24% among 70 patients with aCML, and 4% among 82 patients with chronic myelomonocytic leukemia (CMML). The investigators were not able to detect similar mutations among 106 patients with acute myeloid leukemia (AML), 100 with myelodysplastic syndromes (MDS), 42 with chronic myeloid leukemia, 33 with primary myelofibrosis (PMF), 42 with polycythemia vera and 36 with essential thrombocythemia.[3] A more recent study identified *SETBP1* mutations with an overall prevalence of 3.2% in a total of 658 cases

**Table 1.** SETBP1 mutational frequency and distribution in PMF and CMML

| *SETBP1 mutations* | *PMF* n = *236* | *CMML* n = *179* | |
|---|---|---|---|
| *SETBP1* mutated | 6/236 (2.5%) | 8/179 (4.5%) | |
| D868N | 3/236 | 5/179 | |
| D868Y | 0/236 | 1/179 | |
| G870S | 2/236 | 1/179 | |
| I871T | 1/236 | 1/179 | |
| *SETBP1 with concomitant mutations* | *PMF* | *P*-value | *CMML* |
| *JAK2*V617F mutated | 3/136 | 0.68 | —[a] |
| *JAK2*V617F unmutated | 3/98 | — | |
| *MPL* | 0/6 | — | —[a] |
| *ASXL1* | 2/6 | 0.7 | 6/8 |
| *EZH2* | 0/6 | 0.52 | —[a] |
| *SRSF2* | 1/6 | 0.65 | 3/8 |
| *IDH* | 0/6 | 0.6 | —[a] |
| *SF3B1* | 0/3 | 0.61 | 0/8 |
| *U2AF35* | —[a] | —[a] | 2/8 |

Abbreviations: CMML, chronic myelomonocytic leukemia; PMF, primary myelofibrosis. [a]Not tested in this patient group.

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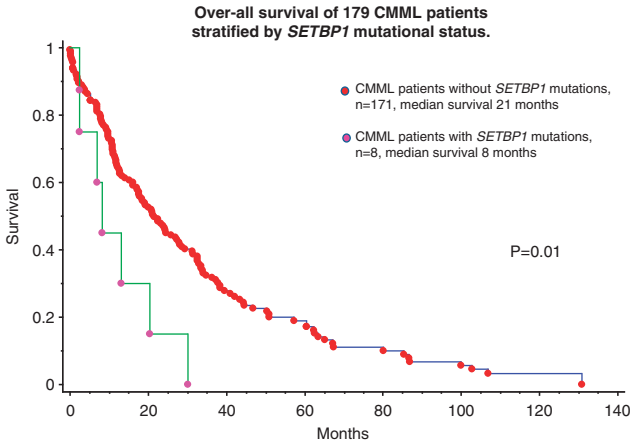


**Figure 1.** Overall survival of 179 CMML patients stratified by SETBP1 mutational status.

consisting of 195 patients with CMML, 222 with MDS and 241 with secondary acute myeloid leukemia (sAML). *SETBP1* mutations were identified in 6.2% of CMML patients, 2.2% of MDS patients and 1.7% of patients with sAML.[4]

In an effort to further investigate the prevalence and prognostic value of *SETBP1* mutations in PMF and CMML, we studied a total of 415 patients with either PMF ($n=236$) or CMML ($n=179$). PCR and Sanger sequencing was used for mutation screening in PMF patients (forward primer 5′-ATGCACCCACTTTCAACACA-3′ and Reverse primer 5′-AAAAGGCACCTTTGTCATGG-3′ to generate sequence for amino-acid region 825–1013). For the CMML cohort, we used the ViiA7 quantitative RT-PCR platform (qPCR) and MeltDoctor high-resolution melting assay (Life Technologies, Grand Island, NY, USA) using forward primer 5′-GCGA GATTGGCTCCCTAAAG-3′ and reverse primer 5′-CCAGGGAGCA GAAATCAAAA-3′ to generate sequence for amino-acid region 860–1000. Targeted cases in the CMML cohort were validated using Sanger sequencing to confirm the presence of a mutation.

Among the 236 patients with PMF (median age 63 years; 63% males), Dynamic International Prognostic Scoring System (DIPSS)-plus[5] risk distributions were high in 30%, intermediate-2 in 37%, intermediate-1 in 20% and low in 13%. Only six (2.5%) patients displayed *SETBP1* mutations including three with D868N, two with G870S and one with I871T (Table 1). These mutations have all been previously described in other myeloid malignancies but not in PMF.[3] We found no significant correlations between the presence of *SETBP1* mutations and age ($P=0.74$), sex ($P=0.5$), DIPSS-plus risk category ($P=0.38$), red cell transfusion need ($P=0.3$), hemoglobin $<10$ g/dl ($P=0.34$) or karyotype ($P=0.48$; three normal and three abnormal karyotype). *SETBP1* mutations significantly correlated with higher leukocyte count ($P=0.047$), and borderline significance was seen with lower platelet count ($P=0.08$). Among 234 patients with concomitant *JAK2*V617F analysis, *SETBP1* mutations were seen in 3 of 136 *JAK2*V617F-mutated and 3 of 98 unmutated cases ($P=0.68$). Table 1 outlines the patterns of concomitant mutations in other genes, including *MPL*, *ASXL1*, *EZH2*, *SRSF2* and *IDH*, for all six *SETBP1*-mutated cases. Three of the six *SETBP1*-mutated patients were also screened for *SF3B1* mutations and were all negative ($P=0.61$). At a median follow-up of 47 months, 129 (55%) deaths and 22 (9%) leukemic transformations were documented. Although the number of informative cases were too small to be definitive, the differences in either overall (hazard ratio (HR) 1.9; 95% confidence interval (CI) 0.7–5.2) or leukemia-free survival (HR 2.6; 95% CI 0.34–19.4) did not reach statistical significance.

Among the 179 study patients with CMML, median age was 70 years and 122 (68%) were males. Distribution of patients based on the Mayo CMML prognostic model were: 93 (52%) low risk, 45 (25%) intermediate risk and 41 (23%) high risk.[6] Eight (4.5%) patients with CMML displayed *SETBP1* mutations. These included previously described mutations in seven patients (five with D868N, one with G870S and one with I871T) and a previously undescribed variant affecting amino-acid 868 (D868Y; Table 1). We found no significant correlations between the presence of *SETBP1* mutations and age ($P=0.4$), sex ($P=0.6$), absolute monocyte count ($P=0.77$), hemoglobin ($P=0.4$), platelet count ($P=0.34$), bone marrow blasts ($P=0.8$), distribution across the Spanish cytogenetic risk stratification system ($P=0.17$),[7] MD Anderson prognostic scoring system (MDAPS) ($P=0.19$),[8] Mayo prognostic scoring system ($P=0.65$) and the global MDAPS ($P=0.56$).[9] *SETBP1* mutations significantly correlated with higher circulating immature myeloid cells ($P=0.03$) and circulating blasts ($P=0.032$), and a borderline significance was noted for leukocyte count ($P=0.08$). *SETBP1*-mutated patients with CMML coexpressed mutations involving *ASXL1* in six cases (75%), *SRSF2* in three (38%), *U2AF35* in two (25%) and *SF3B1* in none; there was no statistically significant difference between *SETBP1*-mutated and unmutated cases in their coexpression frequencies. At a median follow-up of 17 months, 134 (75%) deaths and 24 (13%) leukemic transformations were documented. In univariate analysis, *SETBP1* mutations were found to have a negative impact on overall survival ($P=0.01$, HR; 95% CI) (Figure 1). In multivariable analysis, *SETBP1* mutations retained their negative prognostic impact against other parameters of prognostic importance that are listed in conventional prognostic models for CMML. Low number of events did not allow accurate statistical evaluation for leukemia-free survival.

Increased expression of *SETBP1* has been reported occurring in 27% of patients with AML.[1] Similarly, increased expression of *SETBP1* has been associated with decreased expression of *SETBP1*-embedded regulatory micro-RNA miR_4319 in a patient with PMF progressing to AML.[10] Accordingly, using published primer sets[11] and *GAPDH* controls, we measured levels of gene expression using qPCR and the SYBR green mastermix (Life Technologies) in 20 PMF patients who were studied for the presence of *SETBP1* mutations, including 4 who harbored the mutation. *SETBP1* expression levels in 19 of the 20 PMF patients were similar to normal controls ($n=4$) and the single patient with $>5$-fold increased expression of *SETBP1* was wild-type for *SETBP1*. The role of *SETBP1* in disease progression, including leukemic transformation, is currently poorly understood, although it was recently reported that constitutive expression of *SETBP1* in an *in vivo* murine system may be involved in conferring self-renewal properties to leukemic stem cells.[12] Regardless, the strong prognostic value of the particular mutation in CMML, as suggested by the current study as well as that of Damm *et al.*, raises the possibility of its incorporation into current prognostic models.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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# Baseline differential blood count and prognosis in CD20-positive post-transplant lymphoproliferative disorder in the prospective PTLD-1 trial

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Post-transplant lymphoproliferative disorder (PTLD) is a spectrum of lymphatic diseases associated with the use of potent immunosuppressive drugs after transplantation and ranges from polyclonal early lesions associated with primary Epstein-Barr virus (EBV) infection to monomorphic lymphoma.[1] The PTLD-1 trial, the largest prospective phase II trial in the field so far, has demonstrated the efficacy and safety of sequential therapy (rituximab followed by cyclophosphamide, doxorubicin, vincristin, prednisolone (CHOP) chemotherapy) with an overall response rate (ORR) of 90% and 6.6 years median overall survival (OS) in CD20-positive PTLD unresponsive to reduction of immunosuppression.[2] Because of the risk of treatment-related complications, such as infections in immunosuppressed transplant recipients, tailoring treatment to the individual patient is of particular importance in PTLD.[3] The European study groups on PTLD have already implemented risk stratification according to the response to rituximab (NCT00590447), with encouraging interim results.[4] However, stratification according to baseline parameters could potentially improve therapy even further.

With this in mind, we have noted with interest that a number of publications have demonstrated a significant prognostic effect of the differential blood count at initial diagnosis on OS in immunocompetent patients with diffuse large B-cell lymphoma (DLBCL): these included a poorer outcome in patients with an absolute lymphocyte count (ALC) $\leqslant$1000/μl in DLBCL treated with rituximab-cyclophosphamide, doxorubicin, vincristin, prednisolone (R-CHOP) immunochemotherapy,[5–8] inferior OS and progression-free survival (PFS) for patients with a baseline neutrophil/lymphocyte ratio (NLR) $\geqslant$3.5 in a cohort of 255 consecutive patients with DLBCL treated with R-CHOP at a single centre,[9] and significantly poorer treatment response, OS as well as PFS, for patients with a lymphocyte-to-monocyte ratio (LMR) $\leqslant$2.6 in 438 patients with DLBCL treated with R-CHOP.[10] In addition, Wilcox *et al.*[8] demonstrated a poor OS outcome in a cohort of 366 patients with DLBCL treated from 1993 to 2007 with CHOP or R-CHOP at a single institution not only for a low ALC $\leqslant$1000/μl but also for a high absolute monocyte count (AMC) $\geqslant$630/μl, and combined both in a model, the absolute monocyte and lymphocyte prognostic score (AMLPI), assigning one point each for either low lymphocytes or high monocytes. This model demonstrated a highly significant effect on OS and PFS—confirmed in a subgroup analysis of those patients receiving R-CHOP. The cohort of 70 patients treated in the PTLD-1 trial is the largest prospectively treated trial cohort in this disease entity so far. Because of uniform diagnostic criteria and treatment, it is ideally suited to examine the prognostic value of the baseline differential blood count in PTLD under sequential immunochemotherapy.

The current analysis is based on the published data set of the international, prospective, multicentre phase II PTLD-1 trial (NCT01458548, $n = 70$, data cut-off 1 June 2011):[2] solid organ transplant recipients with CD20-positive PTLD unresponsive to immunosuppression reduction received four weekly courses of 375 mg/m$^2$ rituximab followed by 4 weeks without treatment and four cycles of CHOP chemotherapy (cyclophosphamide 750 mg/m$^2$ IV day (d) 1, doxorubicin 50 mg/m$^2$ IV d1, vincristine 1.4 mg/m$^2$ IV d1 and prednisone 50 mg/m$^2$ Per OS (PO) d1–5) at 3-week intervals starting at day 50. In case of disease progression under rituximab treatment, patients proceeded to chemotherapy immediately (therefore, starting before day 50). Supportive treatment included granulocyte colony-stimulating factor support (mandatory) as well as antibiotic prophylaxis (cotrimoxazole and ciprofloxacin, recommended). Key exclusion criteria were central

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