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Frequent *TET2* mutations in systemic mastocytosis: clinical, *KITD816V* and *FIP1L1-PDGFRA* correlates

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Abstract

TET2 (TET oncogene family member 2) is a candidate tumor suppressor gene located at chromosome 4q24, and was recently reported to be mutated in ~14% of patients with *JAK2* V617F-positive myeloproliferative neoplasms. We used high-throughput DNA sequence analysis to screen for *TET2* mutations in bone marrow-derived DNA from 48 patients with systemic mastocytosis (SM), including 42 who met the 2008 WHO (World Health Organization) diagnostic criteria for SM and 6 with *FIP1L1-PDGFRA*. Twelve (29%) SM, but no *FIP1L1-PDGFRA* patients, had *TET2* mutations. A total of 17 mutations (13 frameshift, 2 nonsense and 2 missense) were documented in 2 (15%) of 13 indolent SM patients, 2 (40%) of 5 aggressive SM, and 8 (35%) of 23 SM associated with a clonal non-mast cell-lineage hematopoietic disease ($P = 0.52$). *KITD816V* was detected by PCR sequencing in 50 or 20% of patients with or without *TET2* mutation ($P = 0.05$), respectively. Multivariable analysis showed a significant association between the presence of *TET2* mutation and monocytosis ($P = 0.0003$) or female sex ($P = 0.05$). The association with monocytosis was also observed in non-indolent SM ($n = 29$), in which the presence of mutant *TET2* did not affect survival ($P = 0.98$). We conclude that *TET2* mutations are frequent in SM, segregate with *KIT* D816V and influence phenotype without necessarily altering prognosis.

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Conflict of interest
The authors declare no conflict of interest.

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Keywords

KIT; PDGFRA; JAK2; MPL; myeloproliferative; eosinophilia

Introduction

Delhommeau *et al.*[1] recently reported an acquired loss of heterozygosity and somatic deletions at chromosome 4q24 in patients with myeloproliferative neoplasm (MPN). These findings are in consonance with a report by Viguie *et al.*[2] describing four female patients with acute myeloid leukemia or myelodysplastic syndrome and a 4q24 rearrangement; t(3;4)(q26;q24), t(4;5)(q24;p16), t(4;7)(q24;q21) and del(4)(q23;q24).[2] The specific cytogenetic abnormality was present in both myeloid and lymphoid lineage cells, as well as in lymphoma cells from one of the four patients who developed concomitant malignant lymphoma. Fluorescence *in situ* hybridization with bacterial artificial chromosome clones identified a 0.5-Mb commonly deleted region that accompanied the 4q24 breakpoint, and this microdeletion was subsequently observed in another patient with normal karyotype.[2] These observations were interpreted as indicating a stem cell-derived molecular event involving a putative tumor suppressor gene located at 4q24.[2]

Delhommeau *et al.*[1] showed that *TET2* (TET oncogene family member 2), contained in the minimal loss of heterozygosity region of 4q24, harbored loss-of-function mutations in patients with myeloid malignancy, including a stop codon mutation in exon 3 and a 9 nucleotide deletion in exon 6. Subsequently, the authors sequenced *TET2* in 181 *JAK2* V617F-positive patients with polycythemia vera, essential thrombocythemia and primary myelofibrosis, and identified a spectrum of putative loss-of-function mutations, with an overall mutational frequency of 14%.[1] Additional studies showed that these mutations often involved both copies of the *TET2* gene, consistent with a tumor suppressor function for the wild-type alleles, occurred in both multipotent and committed progenitors, and antedated acquisition of the *JAK2* V617F allele in a small cohort of informative patients.[1] Here we report that loss-of-function mutations in *TET2* occur at a high frequency in systemic mastocytosis (SM), and are associated with *KITD816V* mutations. Collectively, these observations indicate that acquired *TET2* mutations contribute to pathogenesis of a spectrum of myeloid malignancies that are associated with activating mutations in tyrosine kinases.[3]

Material and methods

After approval by the institutional review board, the Mayo Clinic database of adult SM patients (age 18 years) was utilized to select consecutive patients in whom stored bone marrow (BM) cells were available for DNA extraction and mutation analysis. All study patients met the 2001 WHO (World Health Organization) diagnostic criteria for SM[4] but data analysis was adjusted by considering *FIP1L1-PDGFRA* -positive patients separately, in line with the 2008 WHO criteria.[3] Patients were seen at the Mayo Clinic between January 1976 and October 2007 and were required to have BM pathology reviewed on site. Special stains, including tryptase and KIT, were used to identify mast cells in biopsy material and BM mast cell burden and other aspects of BM histology were recorded for each case.

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Cytogenetic analysis and mutation screening for *KIT* D816V (PCR sequencing) and *JAK2* V617F (reverse transcriptase-PCR) were performed using BM-derived cells, according to the earlier published methods.[5–7]

M13-appended gene-specific primers were designed to amplify and sequence all coding exons of all isoforms of *TET2*. In all, 1 μg of genomic DNA was used to amplify all exons of *TET2*, followed by bidirectional sequencing (Agencourt Bioscience, Beverly, MA, USA). Mutations were detected in bidirectional sequence traces using Mutation Surveyor (Soft-genetics, Inc., State College, PA, USA), and all traces were manually reviewed for the presence of frameshift mutations. Frameshift and nonsense mutations were annotated according to the predicted effects on the TET2 coding sequence (Table 2). Non-synonymous mutations were annotated as somatic mutations on the basis of sequence analysis of paired MPN/myelodysplastic syndrome samples that showed that these mutations were present in tumor and not in matched normal DNA.

Statistical procedures utilized were conventional and all data were analyzed by using StatView (SAS Institute, Cary, NC, USA). All *P*-values were two-tailed and statistical significance was set at the level of $P<0.05$. Clinical and laboratory correlative studies considered values obtained at the time of diagnosis or referral to the Mayo Clinic. Continuous variables were summarized as medians and ranges. Categorical variables were described as count and relative frequency (%). Comparison between categorical variables was performed by the χ^2 statistics. Comparison between categorical and continuous variables was performed by either the Mann–Whitney *U* test or Kruskal–Wallis test. The association of variables selected from univariate analysis was explored using logistic regression models. Survival curves for the patients with and without *TET2* mutation were constructed by Kaplan–Meier method, taking the interval from the date of diagnosis to death or last contact, and compared using the log-rank test.

Results

Forty-eight patients were included in the current study, including 42 who fulfilled the 2008 WHO diagnostic criteria for SM (Table 1) and the six with *FIP1L1-PDGFRA* fusion gene that contributes to eosinophilia associated with mastocytosis. SM disease subclassification included 13 patients with indolent SM (ISM), 5 with aggressive SM (ASM), 23 with SM associated with a clonal non-mast cell-lineage hematopoietic disease (SM-AHNMD) and one with mast cell leukemia (MCL). Presenting clinical and laboratory features for each of these SM subcategories are outlined in Table 1. Overall, 12 (29%) of the 42 patients with WHO-defined SM, but none of the 6 with *FIP1L1-PDGFRA*, displayed a *TET2* mutation. Furthermore, 5 of the 12 *TET2* -mutated patients had additional *TET2* mutations (all involving different exons) bringing the total to 17 mutations, including 13 frameshift, 2 nonsense and 2 missense; the location of these mutations (i.e. exon number), the specific nucleotide changes and their consequences are detailed in Table 2. Exons 4 and 12 were the most frequently affected regions.

TET2 mutational frequencies for ISM, ASM and SM-AHNMD were 15% (2 of 13), 40% (2 of 5) and 35% (8 of 23) ($P = 0.52$). Among the 23 patients with SM-AHNMD, the non-mast

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cell-lineage hematopoietic disease was chronic myelomonocytic leukemia in 8, myelodysplastic syndrome in 6, MPN in 6 and lymphoid neoplasm in 3; the corresponding *TET2* mutational frequencies were 50, 33, 33 and 0% ($P = 0.5$). Six (50%) of the 12 *TET2*-mutated patients also displayed *KIT* D816V and one *JAK2* V617F (low allele burden). Only one other patient (also with SM-AHNMD) expressed *JAK2* V617F. The single patient with MCL was negative for all the three mutations; *TET2*, *KIT* and *JAK2*. *KIT* D816V was not detected in the 6 patients with *FIP1L1-PDGFRA*.

Correlations of *TET2* mutation presence with several clinical and laboratory parameters were performed in the 42 patients with WHO-defined SM, as well as in the subgroup of 29 patients with non-indolent SM (Table 3). Multivariable analysis of all the 42 patients showed that monocytosis and female sex remained significant in their association with the presence of *TET2* mutation. A similar analysis restricted to the 29 patients with non-indolent SM (i.e. SM-AHNMD, ASM and MCL) validated the significant association between monocytosis and the presence of *TET2* mutation. The presence of mutant *TET2* did not affect the survival of patients with non-indolent SM (Figure 1). Median follow-up in this latter group of patients was 14 months (range 0.1–69). ISM patients were excluded from the survival analysis because of their well-known longevity; regardless, the two *TET2* mutated ISM patients in the current study were both female and remain alive and well after a follow-up period of 15–22 months.

Discussion

Primarily on the basis of its stem cell-derived clonal heritage,[8] SM is currently classified with polycythemia vera, essential thrombocythemia and primary myelofibrosis, under the MPN category of myeloid neoplasms.[3] Phenotypic diversity and similarity among chronic myeloid neoplasms is currently attributed to differences in their underlying molecular pathogenesis. Some molecular markers, such as *JAK2* V617F, are widely shared among MPN and, albeit infrequently, are also present in SM.[9,10] Others, such as *BCR-ABL1* in CML, are more specific. The current study suggests that *TET2* mutations transcend clinicopathological class and are not unique to *JAK2* V617F-positive MPN.[1] The high prevalence of *TET2* mutations in SM is of particular interest because, although present in the majority of affected patients,[11] *KIT* D816V might require the presence of a collaborating mutation that contributes to known phenotypic heterogeneity.[12] The striking association between the presence of a *TET2* mutation and monocytosis in the current study lends further support to this contention. Furthermore, the existence of *KIT* -independent recurrent mutations in SM might explain the limited clinical activity of KIT-targeted therapy in this disease.[13,14]

World Health Organization-defined SM is further subclassified into ISM, ASM, SM-AHNMD and MCL.[15] The presence of organ dysfunction (e.g. ascites, cytopenia and lytic bone lesions) distinguishes indolent from ASM. Both myeloid and lymphoid neoplasms qualify as the ‘second’ non-mast cell-lineage neoplasm in SM-AHNMD whereas a diagnosis of MCL requires the presence of 10% circulating mast cells. In the current study, there was only one patient with MCL and he was negative for both *TET2* mutation and *KIT* D816V. Obviously, additional comments regarding *TET2* mutations in MCL, based on this single

case, are not warranted but *KIT* D816V-negative MCL has been reported earlier by others as well.[11] The demonstration of *TET2* mutations in ISM, with a mutational frequency that was not significantly different than that seen with the more aggressive disease variants, undermines but does not exclude their role as markers of tumor progression. Similarly, the statistically non-significant but apparently lower associated mutational frequency might be a function of tumor burden rather than tumor aggression. This is also consistent with the lack of significant association between *TET2* mutation and survival in patients with non-indolent SM. However, a larger study with longer follow-up is necessary before refuting the prognostic relevance of *TET2* mutations in SM.

It has been suggested that all patients with SM carry a *KIT* mutation, but this may be mitigated in part by cell source and assay sensitivity.[11,16] For example, among patients carrying a *KIT* mutation in purified mast cell preparations, the mutation is detected in less than half of the patients when other nucleated BM cells were studied.[11] It may therefore not be surprising that *KIT* D816V was detected in less than a third of the patients in this study, as we used a direct sequencing technique of whole BM specimens that were not enriched for mast cells. As such, *KIT* D816V detection in the current study will reflect the degree of BM infiltration by clonal mast cells, but will lack sensitivity for mutation in the mast cell lineage. Additional studies will be needed to explore clonal distribution in terms of both *TET2* and *KIT* mutations in early progenitor cells and specific cell compartments, including mast cells, monocytes, neutrophils and eosinophils.

The association between *TET2* mutations and the female gender is of interest. Of note, the four patients with 4q24-rearranged acute myeloid leukemia/myelodysplastic syndrome, described in the original report by Viguie *et al.*,[2] were also all females. Such gender predilection has also been observed in *PDGFR*-rearranged MPN, in which virtually all affected patients are male. There are no mechanistic insights into this gender predilection.[17] In contrast and unlike the case with *JAK2* V617F in essential thrombocythemia[18] and primary myelofibrosis,[19] the association between age and the detection of *TET2* mutations in SM has not been verified by multivariable analysis. Whether or not these observations hold true in the setting of other myeloid neoplasms is expected to be clarified soon.

Because clonal BM mastocytosis also occurs in association with another WHO category of myeloid malignancies, *FIP1L1-PDGFRA* -positive myeloid neoplasm,[20,21] we included six such patients in the current study, none of whom displayed a *TET2* mutation. Although the number of patients studied is too small to allow one to make definitive conclusions, the particular observation reinforces the distinction between WHO-defined and *FIP1L1-PDGFRA*-associated SM, and is consistent with the contention that FIP1L1-PDGFRA is oncogenically more robust than KITD816V.[12,22] Of note, patients with *FIP1L1-PDGFRA*-associated MPN are also not known to co-express *JAK2* V617F.[23] Taken together, and as has been pointed out earlier in the context of acute myeloid leukemia,[24] the observations from the current study underscore the complexity of molecular pathogenesis in myeloid neoplasms and, in this regard, suggest a cooperative but not necessarily a primary role for *TET2* mutations.

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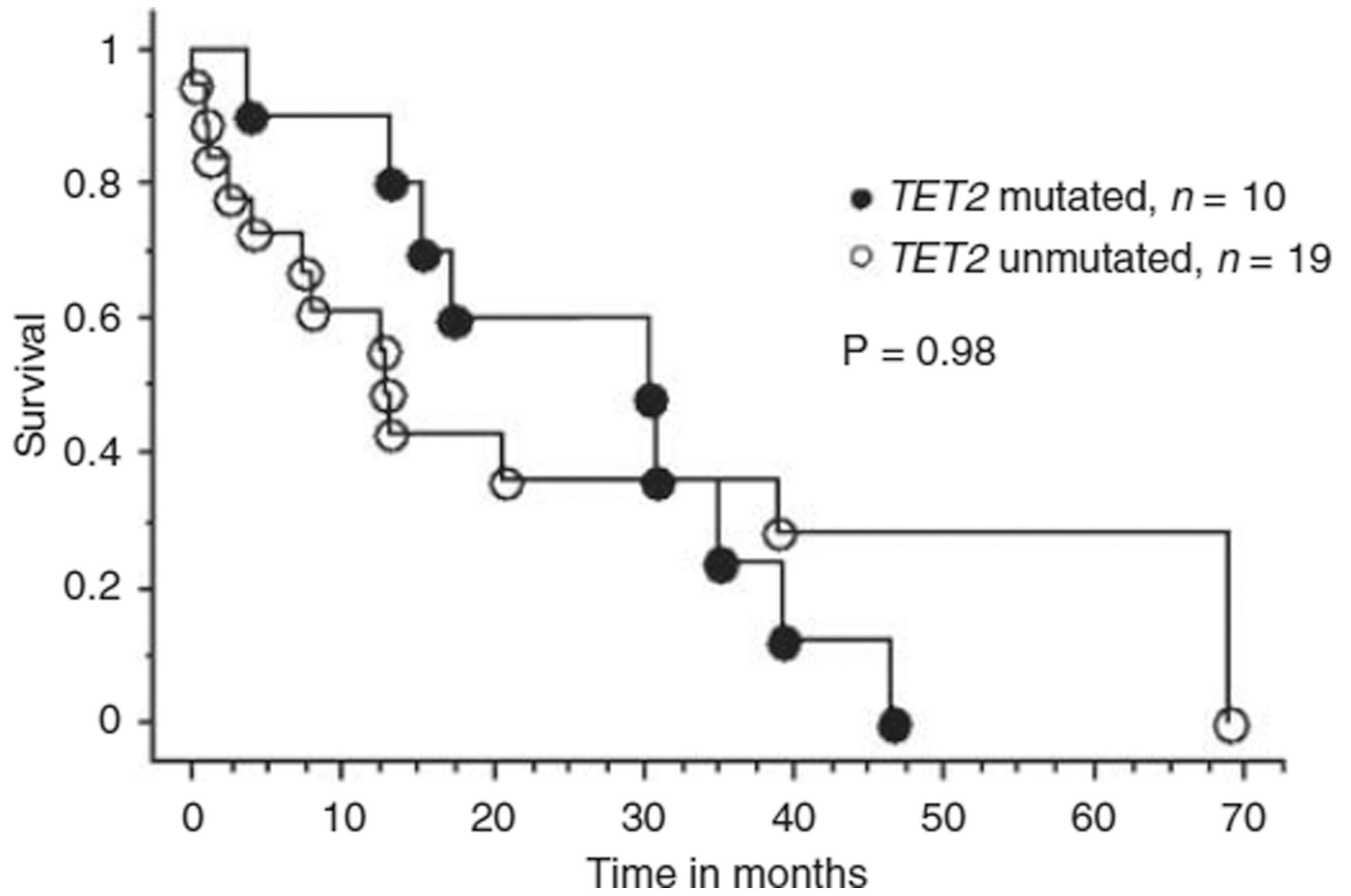

Figure 1.

Survival curves of *TET2* mutated and unmutated patients with non-indolent systemic mastocytosis ($n = 29$).

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Table 1

Clinical and laboratory features of 42 patients with World Health Organization-defined systemic mastocytosis

	All patients	TET2 mutated	TET2 unmutated	ISM	ASM	SM-AHNMD
N (%)	42 (100)	12 (29)	30 (71)	13 (31)	5 (12)	23 (55)
Males, *n* (%)	27 (64)	5 (42)	22 (73)	8 (62)	3 (60)	15 (65)
Age in years, median (range)	64.5 (28–87)	69.5 (50–83)	62 (28–87)	57 (28–78)	69 (43–80)	67 (34–87)
UP, *n* (%)	12 (29)	2 (17)	10 (33)	8 (62)	0	4 (17)
MCMRS[a], *n* (%)	19 (45)	2 (17)	17 (57)	9 (69)	2 (40)	7 (30)
Splenomegaly, *n* (%)	14 (33)	7 (58)	7 (23)	0	1 (20)	12 (52)
AML transformation, *n* (%)	4 (10)	1 (8)	3 (10)	0	0	4 (17)
Hemoglobin (g/dl), median (range)	11.9 (7.3–16)	10.7 (7.9–15)	12.3 (7.3–16)	14.3 (9.9–15.2)	10.8 (10.5–16)	9.7 (7.3–15.9)
Leukocyte count ($\times 10^9$/l), median (range)	8.3 (1.2–73.4)	13.7 (3.2–73.4)	8.3 (1.2–21.3)	6.6 (1.6–9.9)	11.1(6.5–21.5)	9.1 (1.2–73.4)
Monocyte count ($\times 10^9$/l), median (range)	0.6 (0–11.2)	1.4 (0.4–11.2)	0.4 (0–4)	0.4 (0–0.7)	0.6 (0.4–2.8)	0.7 (0–11.2)
Eosinophil count ($\times 10^9$/l), median (range)	0.3 (0–9.2)	0.1 (0–8.8)	0.3 (0–9.2)	0.2 (0–0.5)	0.4 (0–9.2)	0.3 (0–8.8)
Platelet count ($\times 10^9$/l), median (range)	179 (12–431)	106 (12–238)	204.5 (14–431)	233 (39–431)	149 (20–345)	115 (12–392)
Serum tryptase (ng/ml), median (range) *n* evaluated = 26	55.8 (13.1–1450)	179 (13.1–674)	47.2 (13.2–1450)	38 (13.1–440)	66.5 (29–1450)	73.7 (13.2–674)
BM mast cell %, median (range)	10 (5–70)	20 (5–30)	10 (5–70)	10 (5–25)	17.5 (10–30)	10 (5–30)
BM blast % 5, *n* (%)	6 (14)	2 (17)	4 (13)	0	0	6 (26)
Abnormal karyotype, *n* (%), *n* evaluated = 39	11 (28)	3 (25)	8 (30)	1 (9)	1 (20)	9 (41)
KITD816V, *n* (%)	12 (29)	6 (50)	6 (20)	2 (15)	2 (40)	8 (35)
JAK2V617F, *n* (%)	2 (5)	1 (8)	1 (3)	0	0	2 (9)

Abbreviations: AHNMD, associated hematologic non-mast cell lineage disease; AML, acute myeloid leukemia; ASM, aggressive SM; BM, bone marrow; ISM, indolent SM; MCMRS, mast cell mediator release symptoms; SM, systemic mastocytosis; UP, urticaria pigmentosa.

[a]Included headache, dizziness/light-headedness, syncope/pre-syncope, hypotension, anaphylaxis, palpitation/tachycardia, bronchoconstriction/wheezing, and peptic ulcer disease. One of the 42 study patients had mast cell leukemia.

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Table 2

Clinical and laboratory details of 12 systemic mastocytosis patients with *TET2* mutation

SM type	Exon	Nucleotide change	Consequence	Age	Sex	Hgb g/ 100 ml	WBC $\times 10^9$/L	Plt. $\times 10^9$/L	AMC $\times 10^9$/L	JAK2 V617F	KIT D816V	Cyto.
ISM	4	1777_1778insG	Frameshift	78	F	9.9	3.2	238	0.4	Neg.	Pos.	Normal
ISM	12	723_724delAG	Frameshift	64	F	12.7	5.0	202	0.6	Neg.	Neg.	Normal
ASM	4	3248_delT	Frameshift	69	M	12.6	6.5	149	1.6	Neg.	Neg.	-Y
	8	278_279insA	Frameshift									
ASM	4	1554_delG	Frameshift	80	F	10.6	21.5	64	2.8	Neg.	Pos.	Normal
SM-CMML-1	12	1305A>G	H1881R	83	M	15.0	26.3	202	1.3	Pos.	Neg.	13q-
SM-CMML-1	4	2628C>T	Nonsense	70	F	12.4	5.1	153	1.4	Neg.	Pos.	Normal
SM-	10	231 C>T	R1358C	69	F	8.9	21.5	72	8.3	Neg.	Neg.	Normal
CMML-1	11	287_delA	Frameshift									
SM-CMML-1	12	695_696insT	Frameshift	75	M	10.8	51.1	12	11.2	Neg.	Pos.	Normal
SM-	4	3070_3071insA	Frameshift	73	F	7.9	11.0	115	1.3	Neg.	Neg.	Normal
RAEB-1	12	955_956insT	Frameshift									
SM-	4	2812_2813insT	Frameshift	50	M	8.7	8.2	97	NA	Neg.	Pos.	−7
RCMD	12	252_262delCTCTACAGAAG	Frameshift									
SM-MPN	8	236_delC	Frameshift	53	M	8.8	73.4	54	2.9	Neg.	Neg.	Normal
SM-MPN	4	2468_2469insA	Frameshift	61	F	14.5	16.4	33	0.7	Neg.	Pos.	Normal
	6	208 C>T	Nonsense									

Abbreviations: AMC, absolute monocyte count; ASM, aggressive SM; CMML, chronic myelomonocytic leukemia; Cyto., bone marrow cytogenetic results; Hgb, hemoglobin level; ISM, indolent SM; MPN, myeloproliferative neoplasm; NA, not assessed; neg., negative; Plt., platelet count; pos., positive; RAEB, refractory anemia with excess blasts; RCMD, refractory cytopenia with multilineage dysplasia; SM, systemic mastocytosis; WBC, white blood cell count.

Table 3

P-values during univariate analysis correlating the presence of *TET2* mutations with clinical and laboratory features at presentation

Variables (continuous)	Variables (categorical)	Patients with WHO-defined SM (N = 42)	Patients with non-indolent SM (SM-AHNMD/ASM/MCL) (N = 29)
Age		*0.02*	0.19
	Female gender	***0.05***	0.20
	SM subgroup	0.52	0.74
	Palpable spleen	0.03	0.09
Hemoglobin level		0.23	0.99
	Hemoglobin <10 g/100 ml	0.47	0.70
Leukocyte count		0.09	***0.03***
	Leukocyte >10 × 10^9/l	***0.02***	0.05
Platelet count		***0.05***	0.15
	Platelet <100 × 10^9/l	0.09	0.14
Monocyte count		***0.002***	***0.005***
	Monocyte >1 × 10^9/l	***0.0003***	***0.001***
Eosinophil count		0.68	0.64
Serum tryptase level (*n* = 27)		0.18	0.10
BM mast cell content		0.07	0.06
	BM blasts 5%	0.78	0.95
	KITD816V	***0.05***	0.20
	Abnormal karyotype	0.50	0.68

Abbreviations: AHNMD, associated hematologic non-mast cell lineage disease; ASM, aggressive SM; BM, bone marrow; MCL, mast cell leukemia; SM, systemic mastocytosis; WHO, World Health Organization.

Bold, italic and underline entries are statistically significant.

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