Modern Pathology (2020) 33:1135–1145
https://doi.org/10.1038/s41379-019-0447-x

ARTICLE

Detection of the *KIT*^{D816V} mutation in myelodysplastic and/or myeloproliferative neoplasms and acute myeloid leukemia with myelodysplasia-related changes predicts concurrent systemic mastocytosis

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Received: 30 October 2019 / Revised: 16 December 2019 / Accepted: 16 December 2019 / Published online: 2 January 2020

Abstract

Greater than 90% of cases of systemic mastocytosis (SM) harbor pathogenic *KIT* mutations, particularly *KIT*^{D816V}. Prognostically-significant pathogenic *KIT* mutations also occur in 30–40% of core binding factor-associated acute myeloid leukemia (CBF-AML), but are uncommonly associated with concurrent SM. By comparison, the occurrence of SM in other myeloid neoplasms bearing pathogenic *KIT* mutations, particularly those with a chronic course, is poorly understood. Review of clinical next-generation sequencing (NGS) performed at our institutions in patients with known or suspected hematologic malignancies over an 8-year period revealed 64 patients with both a pathogenic *KIT* mutation detected at one or more timepoints and available bone marrow biopsy materials. Patients with *KIT*^{D816V}-mutated myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN), or overlap MDS/MPN ($n = 22$) accounted for approximately one-third of our cohort (34%). Comprehensive morphologic and immunophenotypic characterization revealed that nearly all cases ($n = 20$, 91%) exhibited concurrent SM. In contrast, of the 18 patients (28%) with AML and *KIT*^{D816V}, only eight (44%) showed evidence of SM at any point in their disease course ($p = 0.0021$); of these eight, the AML component was characterized as AML with myelodysplasia-related changes (AML-MRC) in all but one instance ($n = 7$, 87%). Twelve patients (19%) had pathogenic *KIT* mutations other than p.D816V, all in the setting of AML (CFB-AML, $n = 7$; AML, not otherwise specified, $n = 2$; AML-MRC, $n = 1$; acute promyelocytic leukemia, $n = 1$); only two of these patients (17%), both with CBF-AML, exhibited concurrent SM. The remaining 12 patients (19%) had SM without evidence of an associated hematological neoplasm (AHN). For nearly one-third of the 30 SM-AHN patients in our cohort ($n = 9$, 30%), the SM component of their disease was not initially clinicopathologically recognized. We propose that identification of the *KIT*^{D816V} mutation in patients diagnosed with MDS, MPN, MDS/MPN, or AML-MRC should trigger reflex testing for SM.

Introduction

The KIT receptor tyrosine kinase proto-oncogene (*KIT*) encodes a type 3 transmembrane receptor (KIT, CD117) found on the surface of multiple cell types, including hematopoietic stem cells, mast cells, germ cells,

Supplementary information The online version of this article (https://doi.org/10.1038/s41379-019-0447-x) contains supplementary material, which is available to authorized users.

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melanocytes, and interstitial cells of Cajal [1]. Activation of KIT through the binding of its ligand stem cell factor (SCF) drives diverse signaling pathways involved in cell survival, growth, proliferation, differentiation, and migration. Various point mutations and in-frame insertions/deletions in *KIT* result in a constitutively active and SCF-independent oncogenic tyrosine kinase that can induce neoplastic transformation in KIT-expressing cell types [2]. Among hematopoietic neoplasms, *KIT* mutations are found at highest frequency in mast cell disorders, particularly systemic mastocytosis (SM) (>90% of cases), and the core-binding factor acute myeloid leukemias [CBF-AML: AML with t(8;21) and AML with inv(16) or t(16;16)] (30–40% of cases) [3–5]. By comparison, *KIT* mutations are infrequent in non-CBF AML, myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN), or myelodysplastic/myeloproliferative neoplasms (MDS/MPN) such as chronic myelomonocytic leukemia (CMML) [6–9].

One particular subtype of SM, SM with associated hematological neoplasm (SM-AHN), is distinct in requiring diagnostic evidence of both SM and a separate clonal hematologic non-mast cell lineage disease, which are often detected simultaneously in diagnostic bone marrow (BM) biopsy specimens [10]. Like other subtypes of SM, acquired *KIT* mutations are common in SM-AHN, particularly the hallmark KIT^{D816V} variant, which is found in the majority of cases [11–13]. Establishing a diagnosis of SM-AHN can be challenging, especially in situations where the AHN component predominates and obscures the presence of SM. In fact, therapy that targets the non-mast cell neoplasm can reveal the presence of previously unsuspected underlying SM [14, 15].

The hematologic neoplasms most frequently associated with SM-AHN are myeloid diseases, including non-CBF-AML, MDS/MPN (especially CMML), and to a lesser extent MDS and MPN [16, 17]. Despite the high frequency of KIT^{D816V} mutations in CBF-AML, it is less often associated with concurrent SM [18, 19]. In contrast, recent work suggests a strong correlation between non-CBF-AML and concurrent SM when KIT^{D816V} is detected [20]. However, the diagnostic significance of detecting a pathogenic *KIT* mutation (p.D816V or otherwise) in other myeloid neoplasms, particularly those with a chronic component (i.e., chronic myeloid neoplasms), is not yet well understood. To address this question, we systematically evaluated a series of BM biopsies from patients with known or suspected myeloid neoplasms associated with pathogenic *KIT* mutations. Our results demonstrate that most myeloid neoplasms with a chronic course (MDS/MPN, MDS, and MPN) harboring the KIT^{D816V} mutation are associated with SM, which may be unrecognized both clinically as well as by pathologists conducting routine histologic evaluation of biopsy specimens. We also found a strong correlation between SM and AML with myelodysplasia-related changes (AML-MRC) bearing the KIT^{D816V} mutation, whereas other non-CBF, KIT^{D816V}-mutated AML subtypes were only rarely associated with SM in our cohort.

Materials and methods

Cohort selection

Institutional Review Board approval was obtained at all participating institutions. The cohort ($n = 64$) included all patients seen at Brigham and Women's Hospital/Dana-Farber Cancer Institute (BWH/DFCI) or Massachusetts General Hospital (MGH) during the time period of July 2011 to February 2019 with each of the following: (a) a pathogenic *KIT* mutation detected at any point during the disease course in peripheral blood (PB), BM, or cerebrospinal fluid by clinical next-generation sequencing (NGS); and (b) BM biopsy materials available for histologic review and further evaluation as described below. *KIT* mutations were detected on at least 1 of 4 custom NGS panels as follows: Rapid Heme Panel (*KIT* exons 8–9, 11, and 17; BWH/DFCI) [21]; OncoMap (*KIT* hotspots including p.D816V; BWH/DFCI) [22]; OncoPanel (*KIT*, all exons; BWH/DFCI) [23, 24]; or SNaPshot (*KIT* exons 1–2, 5, 8–15, 17, and 18; MGH) (see also Supplementary Materials and Methods). Single-gene *KIT* mutation testing performed as part of the workup for SM in patients without suspicion for another hematologic malignancy was not included. Details of one patient in the cohort have been published previously [25, 26].

Clinicopathologic evaluation

Clinical data, including pathologic diagnoses, patient symptoms, physical exam findings, laboratory results, and disease course and treatment, were obtained from the electronic medical record. When possible, each respective NGS sample was paired with histologic findings from the most proximate BM biopsy. Original pathology reports and slides were reviewed, and case materials including blocks were recovered from institutional archives when required for further immunohistochemistry (IHC). For patients with established SM, pathologic diagnoses were confirmed by re-review of original histologic sections and relevant IHC. For patients without a documented history of SM, available BM biopsy specimens, including when possible those obtained within 6 months of detection of any pathogenic *KIT* mutation, were reassessed for SM using an IHC panel composed of CD25, KIT (CD117), and/or mast cell tryptase (MCT) if such studies were not performed as part of the original diagnostic workup (see "Immunohistochemistry" section below). Materials from the latter group were

independently reviewed by two pathologists with expertise in hematopathology and mast cell neoplasia who were blinded to the diagnosis (JCA; JLH). When appropriate, pathologic diagnoses were revised according to the 2017 WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues (revised 4th edition) [27]. As a control cohort, thirty BM biopsies from patients diagnosed with either CMML ($n = 15$) or MDS ($n = 15$) without pathogenic *KIT* mutations, randomly selected from the same study period, were included in the blinded review.

Immunohistochemistry

IHC for MCT [mouse monoclonal clone AA1 (DAKO Corp, Carpenteria, CA), titer 1:4000, retrieval 10 min with Enzyme 1 (Leica Microsystems, Buffalo Grove, IL)], and CD25 [mouse monoclonal clone 4C9 (Leica), titer 1:50, retrieval 30 min with Bond Epitope Retrieval Solution 2 (Leica)] were performed on the Leica immunostainer (BOND III) using a 60 min primary antibody incubation period and the BOND Polymer Refine DAB Detection system (Leica). IHC for KIT [rabbit polyclonal (DAKO), titer 1:300, no retrieval] was performed manually using a 50 min primary antibody incubation period with detection performed using Rabbit Power-Vision (Leica) for 30 min; staining was developed with the DAB+ kit (DAKO). For all studies, slides were counterstained manually with either hematoxylin or methyl green, dehydrated through solvents, and cover slipped.

Statistical analysis

When appropriate, Fisher's exact test (two-tailed) was used to assess conditional distribution probabilities associated with the presence or absence of SM (https://www.graphpad.com/quickcalcs/). P values < 0.05 were considered statistically significant.

Results

Pathogenic *KIT* mutations

Our final cohort ($n = 64$) included all patients in whom a pathogenic *KIT* mutation was detected at one or more timepoints by clinical NGS sequencing and for whom BM biopsy materials were available to evaluate for involvement by SM. The canonical p.D816V *KIT* variant was the most common *KIT* mutation detected within our patient cohort ($n = 52$, 81%), including one patient with an additional p.D816Y *KIT* mutation. Samples from the remaining 12 patients exhibited one or more pathogenic non-D816V *KIT* mutations without a concurrent KIT^{D816V} mutation.

KIT^{D816V} mutation in myeloid neoplasms

Forty of the 52 patients with a KIT^{D816V} mutation were diagnosed with a myeloid neoplasm other than isolated SM (Fig. 1a, Supplementary Table 1). This included 22 patients with chronic myeloid neoplasms (MDS, MPN, or MDS/MPN) and 18 patients with AML. Based on comprehensive IHC testing for CD25, KIT, and/or MCT and expert evaluation, nearly all ($n = 20$, 91%) chronic myeloid neoplasms with a KIT^{D816V} mutation fulfilled criteria for SM-AHN, whereas only 8 of 18 (44%) AML cases fulfilled criteria for SM-AHN ($p = 0.0021$), as described below.

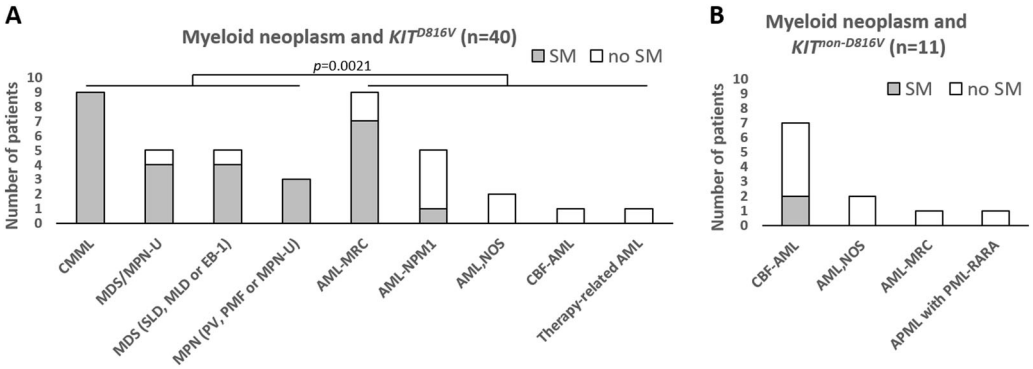

Fig. 1 Systemic mastocytosis in patients with myeloid neoplasms and *KIT* mutations. a Forty patients in the cohort had a KIT^{D816V} mutation detected by next-generation sequencing in the setting of an acute or chronic myeloid neoplasm. Twenty of twenty-two (91%) patients with a chronic myeloid neoplasm (chronic myelomonocytic leukemia (CMML), myelodysplastic/myeloproliferative neoplasm, unclassifiable (MDS/MPN-U), myelodysplastic syndrome (MDS) with single lineage dysplasia (SLD), multilineage dysplasia (MLD), or excess blasts-1 (EB-1), myeloproliferative neoplasm (MPN) including polycythemia vera (PV), primary myelofibrosis (PMF), and MPN unclassifiable (MPN-U)) also had systemic mastocytosis (SM), while only 8 of 18 (44%) patients with acute myeloid leukemia (AML; AML with myelodysplasia-related changes, AML-MRC; AML with mutated *NPM1*, AML-*NPM1*; AML, not otherwise specified, AML, NOS; core binding factor-associated AML, CBF-AML) also had SM ($p = 0.0021$). **b** Eleven patients in the cohort had a pathogenic *KIT* mutation other than the p.D816V variant in the setting of a myeloid neoplasm, all of which were AML; two patients exhibited evidence of SM, both CBF-AML.

KIT^{D816V} mutations in chronic myeloid neoplasms

The 20 patients with SM-AHN where the AHN component was a chronic myeloid neoplasm (MDS-single lineage dysplasia, $n = 2$; MDS-multilineage dysplasia, $n = 2$; CMML, $n = 9$; MDS/MPN, unclassifiable (MDS/MPN-U), $n = 4$; polycythemia vera, $n = 1$; primary myelofibrosis, $n = 1$; MPN, unclassifiable, $n = 1$) included three patients with a preceding (>1 year prior) diagnosis of SM [aggressive SM (ASM), $n = 2$; indolent SM (ISM), $n = 1$], two patients with a preceding (>1 year prior) diagnosis of the myeloid neoplasm, and 15 patients with concurrently diagnosed SM-AHN (Supplementary Table 1). The *KIT*^{D816V} mutation was detected in the first NGS test for all of these patients, and these initial time points were nearly always ($n = 17$, 85%) obtained within 6 months of the SM-AHN diagnosis. In all but one case, at least one additional pathogenic non-*KIT* mutation was also identified in the first NGS test (Fig. 2). In both PB and BM specimens, the *KIT*^{D816} variant allele frequency (VAF) was observed at lower or equivalent (within 5%) levels compared with the non-*KIT* mutations (Supplementary Table 1).

BM biopsies from the two remaining patients with a chronic myeloid neoplasm and *KIT*^{D816V} mutation (MDS/MPN-U and MDS-excess blasts-1) lacked clinicopathologic evidence of SM following immunohistologic investigation and expert review. The *KIT*^{D816V} mutation was detected in the first NGS test for one of these patients. For the other patient, the *KIT*^{D816V} mutation was not present at the time of the initial NGS test (which identified *IDH2*^{R140Q} [45.4%], *ASXL1*^{G642fs*} [46.7%], *CSF3R*^{T618I} [6.3%], and *NRAS*^{G12D} [29.4%] driver mutations) but emerged later, following treatment with a hypomethylating agent and an IDH inhibitor, as the dominant clone (subsequent NGS test; *KIT*^{D816V} [29.2%], *IDH2*^{R140Q} [1%], *ASXL1*^{G642fs*} [10%]).

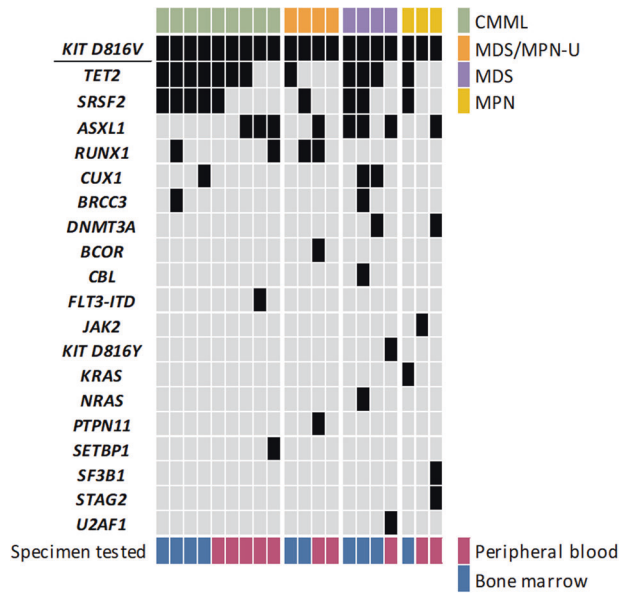

Fig. 2 Comutation plot of all pathogenic mutations by next-generation sequencing from 20 patients in whom a *KIT*^{D816V} variant was detected, at closest timepoint to initial diagnosis of systemic mastocytosis with associated hematological neoplasm (SM-AHN), where AHN = chronic myelomonocytic leukemia (CMML); myelodysplastic syndrome/myeloproliferative neoplasm, unclassifiable (MDS/MPN-U); myelodysplastic syndrome (MDS); myeloproliferative neoplasm (MPN). Each column represents a single patient.

Histopathologic investigation of 30 randomly-selected MDS or CMML cases without a pathogenic *KIT* mutation (p.D816V or otherwise) showed no evidence of concurrent SM (data not shown).

KIT^{D816V} mutations in acute myeloid leukemia

Eighteen patients in the *KIT*^{D816V} cohort were diagnosed with AML at some point during their disease course; diagnoses included AML-MRC ($n = 9$), AML with mutated *NPM1* ($n = 5$), AML, not otherwise specified (AML, NOS, $n = 2$), CBF-AML ($n = 1$), and therapy-related AML ($n = 1$) (Fig. 1a, Supplementary Table 1). Of the nine patients ultimately diagnosed with AML-MRC, the diagnosis of AML-MRC was based on a prior history of MDS ($n = 3$) or MDS/MPN ($n = 3$), AML-MRC-defining karyotype ($n = 1$) or based only on the presence of AML-MRC-defining morphologic dysplasia ($n = 2$). Of these nine patients, seven (78%) had known ($n = 3$) or were concurrently diagnosed with ($n = 4$) SM at the time of AML-MRC diagnosis. In contrast, only one (11%) of nine patients diagnosed with non-MRC AML had evidence of SM ($p = 0.0152$).

Of the seven patients with SM-AHN where the AHN component was AML-MRC, three had NGS performed at multiple time points including before and after development of AML-MRC. In these three patients, the *KIT*^{D816V} mutation decreased in VAF or became undetectable at the time of AML diagnosis, whereas the other driver mutation VAFs remained stable (Supplementary Fig. 1). In two of these cases, additional driver mutations in genes encoding epigenetic modifiers and signaling proteins were detected at AML diagnosis. Three additional patients with SM-AHN where the AHN component was AML-MRC had only one NGS timepoint. Detailed NGS data with VAF were only available for two of them, and in both cases, *KIT*^{D816V} was present at a lower VAF compared with other non-*KIT* mutations (Supplementary Table 1), suggesting that the *KIT*^{D816V} was present in a subclone. The seventh patient in this group had gastrointestinal mast cell infiltrates without malabsorption (ISM) in addition to maculopapular cutaneous mastocytosis (MPCM) but did not exhibit BM involvement by SM at any point in the disease course. The *KIT*^{D816V} mutation, which had been previously detected by PB qualitative allele-specific polymerase chain reaction (PCR) at time of MPCM diagnosis, was detected by PB

NGS at a lower VAF (3%) compared with an $IDH2^{R140Q}$ mutation (36%) at the time of AML-MRC diagnosis; that *KIT* mutation was not detected in subsequent marrow samples showing persistent AML-MRC and $IDH2^{R140Q}$.

The two AML-MRC patients without SM exhibited scattered abnormal mast cells with CD25 co-expression, but the overall findings failed to definitively meet current WHO diagnostic criteria for SM (Supplementary Table 1). Pretransformation molecular data is only available for one of these patients, and in that case, the KIT^{D816V} mutation was not present at the time of the initial diagnosis of MDS and was only detected in a secondary AML that arose following allogeneic transplant.

Of the remaining nine patients with AML (non-MRC), eight did not have any clinicopathologic evidence of SM. In three patients (two AML with mutated *NPM1*, one AML-CBF), the KIT^{D816V} mutation was present at the time of AML diagnosis. In one patient (AML, NOS), the KIT^{D816V} mutation was detected subsequent to the initial diagnosis and then persisted until the time of death. In four cases (two AML with mutated *NPM1*, one AML, NOS, one therapy-related AML), the KIT^{D816V} mutation was only detected in the postallogeneic transplant setting; in these patients, death occurred within 4 months of detection of the mutation. Scattered abnormal mast cells with CD25 co-expression were noted in the biopsy from the patient with therapy-related AML, but the overall findings were insufficient for a current WHO diagnosis of SM (Supplementary Table 1). The final patient in this group, with a history of ASM, developed AML with mutated *NPM1* during SM-directed therapy; at the time of AML diagnosis, there was no evidence of SM in the BM and the *KIT* mutation was no longer detectable.

Systemic mastocytosis without an associated hematological neoplasm

The remaining 12 patients within the KIT^{D816V} cohort were diagnosed with SM only, without histologic evidence of an AHN following expert review. Eight of these 12 cases had no additional mutations detected by clinical NGS sequencing. In the remaining four cases, including two cases of mast cell leukemia, additional pathogenic mutations in *DNMT3A*, *TET2*, and/or *ATM* were detected (data not shown).

Non-D816V *KIT* mutations

Twelve patients from the cohort produced samples containing one or more pathogenic non-D816V *KIT* mutations without a concurrent KIT^{D816V} mutation. One patient was diagnosed with mast cell leukemia (KIT^{D816Y}). The other 11 patients included five CBF-AML with t(8;21) [p.418_419insFF ($n = 1$); p.V559D and p.D816Y ($n = 1$); p.Y823C ($n = 1$); p.D816Y ($n = 1$); p.D816Y and p.D816H ($n = 1$)]; two CBF-AML with inv(16) or t(16;16) [p.417_419delTYDinsI ($n = 1$); p.419delD ($n = 1$)]; two AML, NOS [p.D816Y ($n = 1$); p.D820Y ($n = 1$)]; one AML-MRC (p.419delD); and one acute promyelocytic leukemia (p.N822K) (Fig. 1b, Supplementary Table 2). Only two of these patients had clinicopathologic evidence of SM, both of whom had CBF-AML (p.418_419insFF; p.D816Y and p.V559D); the patient with p.418_419insFF exhibited well-differentiated SM without aberrant CD25 expression in the mast cell aggregates [28]. One patient with AML, NOS had scattered abnormal mast cells with CD25 co-expression, not fulfilling current WHO criteria for SM (Supplementary Table 1). All *KIT* mutations detected within the non-D816V *KIT* mutation cohort were present at the first NGS timepoint. At least one additional non-*KIT* pathogenic mutation was identified in samples from 8 of these 12 patients (Supplementary Fig. 2). The maximal non-D816V *KIT* VAF ranged from subclonal to within 10% of the levels of other non-*KIT* mutations.

Unsuspected SM

For the majority of patients with SM described in this study, the diagnosis of SM was already known or suspected before the corresponding *KIT* mutation result was available, based on clinicopathologic findings. However, for nearly one-third (9 of 30) of SM-AHN patients in this study, there was no clinical or pathologic suspicion of a systemic mast cell disorder, and a diagnosis of SM-AHN was not rendered upon initial pathologic review (Table 1). In seven of these cases, a mast cell infiltrate was not initially appreciated on hematoxylin and eosin (H&E)-stained sections and was instead revealed by IHC performed during case workup or as part of this study (Fig. 3). The average degree of marrow involvement by lesional mast cells in these patients was 10% of the cellularity (range, <5–30%). In two additional cases, the mast cell infiltrate was only identified on deeper tissue sections (Fig. 4).

Review of the clinical records of these patients revealed one patient with a prior history of MPCM; this patient had KIT^{D816V} detected by PB digital droplet PCR at the time of MPCM diagnosis but did not undergo BM biopsy with NGS until 13 months later for cytopenias. Splenomegaly was present in 7 of 9 patients, elevated tryptase was present in all tested patients, and four patients had "C" findings [29]. Despite the associated myeloid neoplasm, karyotype was normal in 7 of 8 tested patients. All cases except one exhibited additional non-*KIT* driver mutations by NGS testing. The KIT^{D816V} VAF ranged from substantially lower to equivalent to the VAFs of other non-*KIT* mutations, implying that the *KIT* mutated clone may be either subclonal or dominant.

Table 1 Clinicopathologic characteristics of patients with unsuspected systemic mastocytosis.

Associated hematologic neoplasm (original diagnosis)	Age	Gender	Myeloid neoplasm preceded SM diagnosis	Splenomegaly	Tryptase level (ng/mL)	Cutaneous lesions	C findings	Mast cell infiltrate	Prior sequencing	Mutational profile on next-generation sequencing	Karyotype
MDS-MLD and plasma cell neoplasm	73	M	No	Yes	94.1	No	Ascites, portal hypertension, weight loss	20%; not initially appreciated on H&E	No	*ASXL1* p.G642fs*(53.1%) *TET2* p.E547* (46.4%) *CUX1* p.W824* (46.3%) *TET2* p.H1380Y (45.7%) *CBL* p.K382R (31.8%) *SRSF2* p.P95L (23.7%) *BRCC3* p.L80fs* (6.8%) ***KIT* p.D816V (5.8%)** *NRAS* p.G12R (5.4%)	46,XY[20]
CMML-0	72	M	No	Yes	88	No	Ascites, portal hypertension, weight loss	5%; not present on initial H&E level	No	*ASXL1* p.G642fs* (70.6%) *TET2* p.Q866* (45.4%) ***KIT* p.D816V (41.8%)**	46,XY[20]
CMML-1	82	F	Yes	Yes	29	No	No	<5%; not initially appreciated on H&E	No	*ASXL1* p.W796* (48.8%) ***KIT* p.D816V (48.8%)** *FLT3-ITD*	46,XX[cp20]
MDS/MPN-U	76	M	No	Yes	181	No	No	<5%; not initially appreciated on H&E	No	*ASXL1* p.R417* (47.7%) *RUNX1* p.R201Q (47.5%) *BCOR* p.A552fs* (40%) ***KIT* p.D816V (22.4%)** *BCOR* p.A1240fs* (16.4%) *PTPN11* p.N308D (8.6%) BCOR p.*I730fs** (7%)	46,XY[20]
MDS/MPN-U	86	F	No	Yes	ND	No	ND	5%; not initially appreciated on H&E	No	***KIT* p.D816V (52.9%)**	46,XX[20]
MDS-SLD	68	F	No	Yes	28.8	MPCM, 13 months prior	Ascites, osteosclerotic lesions	10%; not present on initial H&E level	*TET2* p.F653fs* (23.5%) *TET2* p.C1271fs*92 (26.9%) in peripheral blood 32 months prior ddPCR *KIT* p.D816V testing positive in peripheral blood 13 months prior	*TET2* p.F653fs* (43%) *TET2* p.C1271fs*92 (38.9%) *SRSF2* p.P95H (9.4%) *ASXL1* p.D1078fs* (6.8%) ***KIT* p.D816V (3.7%)**	46,XX[20]
MPN-U	89	F	Leukocytosis ×12 years before marrow evaluation	No	ND	No	Weight loss	5%; not initially appreciated on H&E	No	*ASXL1* p.G642* (47.6%) *SF3B1* p.E592K (47.6%) *STAG2* p.R1242* (45.3%) ***KIT* p.D816V (44.8%)** *DNMT3A* p.F731V (2.9%)	ND
AML-MRC	71	M	No	No	149	No	No	10%; not initially appreciated on H&E	No	*TET2* p.F1104fs* (95.7%) *U2AF1* p.S34F (48.7%) *DNMT3A* p.G543C (43.6%) *GATA2* p.G320D (36.7%) *CEBPA* p.N293D (26.8%) ***KIT* p.D816V (9.2%)**	46,XY,del(8)(q11q13)[cp3]/46,XY[cp17]
AML-MRC	79	M	Yes (MDS)	Yes	ND	No	No	30%; not initially appreciated on H&E	No	*ASXL1* p.G646Wfs* (28%) *RUNX1* p.D269 [splice] (10%) ***KIT* p.D816V (4%)**	46,XY[20]

ND no data, *MPCM* maculopapular cutaneous mastocytosis, *MDS* myelodysplastic syndrome, *MLD* multilineage dysplasia, *CMML* chronic myelomonocytic leukemia, *MDS/MPN-U* myelodysplastic/myeloproliferative neoplasm, unclassifiable, *SLD* single lineage dysplasia, *MPN-U* myeloproliferative neoplasm, unclassifiable, *AML* acute myeloid leukemia, *MRC* myelodysplasia-related changes, *SM* systemic mastocytosis, *H&E* hematoxylin and eosin

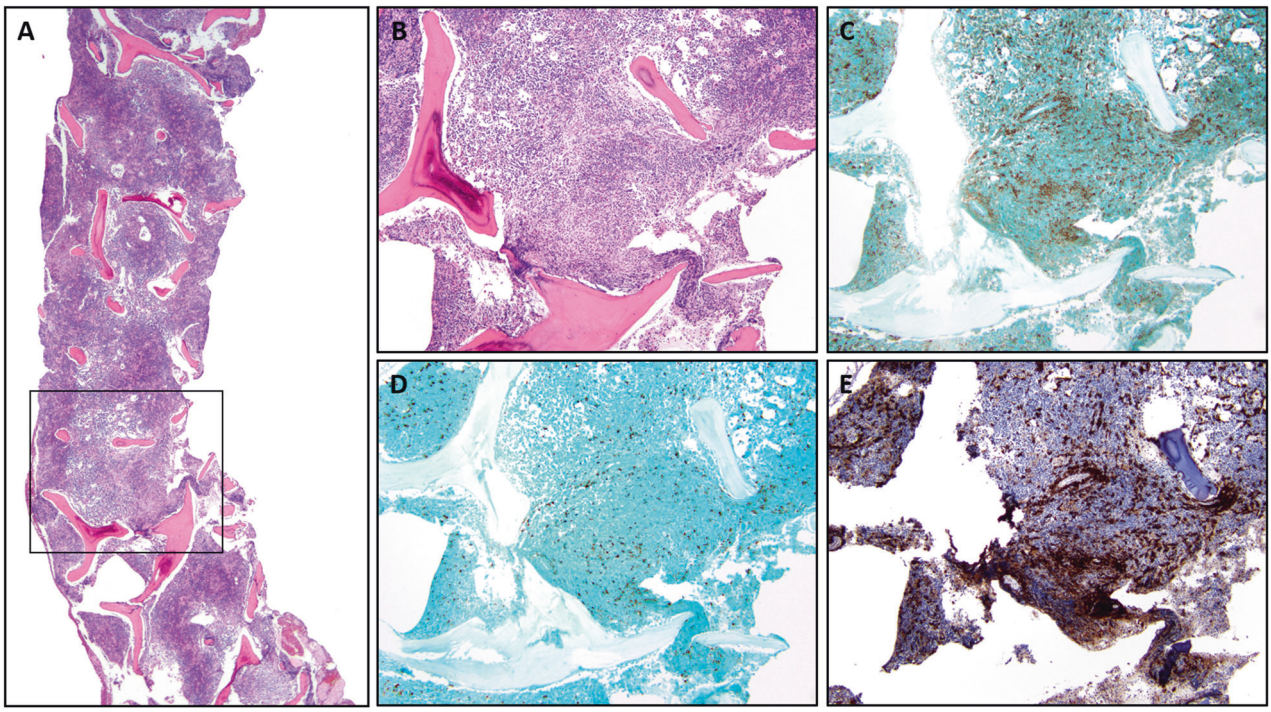

Fig. 3 Bone marrow biopsy from a patient with chronic myelomonocytic leukemia and systemic mastocytosis. a The abnormal mast cells were not appreciated on initial evaluation, given extensive involvement by chronic myelomonocytic leukemia (hematoxylin and eosin (H&E), 20×). **b**–**e** Additional immunostaining revealed large clusters and loose aggregates of spindled, CD25-positive mast cells (**b** H&E; **c** CD117; **d** mast cell tryptase; **e** CD25; all 200×). Some degranulated forms are not highlighted by mast cell tryptase (**d**).

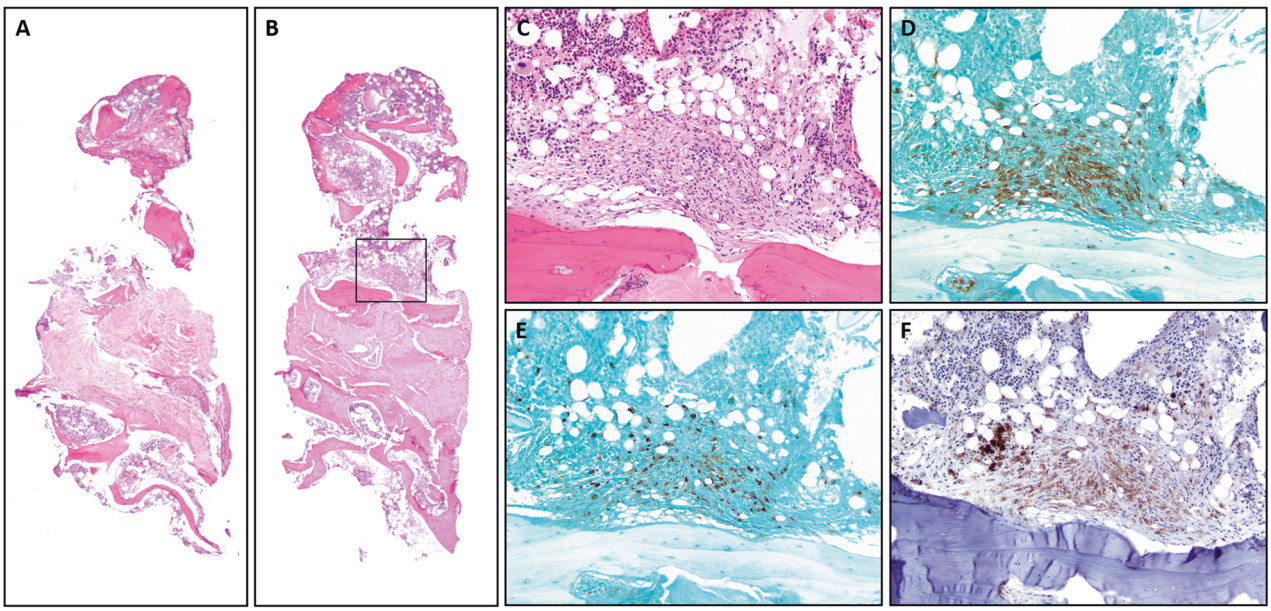

Fig. 4 Bone marrow biopsy from a patient with myelodysplastic syndrome and systemic mastocytosis. a The abnormal mast cells were not present on initial levels of this bone marrow biopsy (hematoxylin and eosin (H&E), 20×). **b**, **c** Deeper levels revealed an abnormal paratrabecular aggregate of spindled cells associated with a lymphoplasmacytic inflammatory infiltrate (H&E; **b** 20×; **c** 200×). **d**–**f** Additional immunostaining revealed aberrant CD25 expression in the mast cell aggregates (**d** CD117; **e** mast cell tryptase; **f** CD25; all 200×). Some degranulated forms are not highlighted by mast cell tryptase (**e**).

Discussion

SM-AHN can be challenging to recognize and establish as a diagnosis due to variability in clinical presentation and histologic appearance, which collectively spans the entire spectrum of hematologic neoplasia. Moreover, many clinical and laboratory findings of SM (e.g., splenomegaly and elevated serum tryptase level) and certain associated morphologic features (e.g., BM fibrosis) also occur frequently in non-mast cell hematologic disease. Thus, in the absence of clinical or pathologic suspicion, SM may remain unrecognized in patients with other hematologic neoplasms.

KIT mutations have long been associated with AML, particularly CBF-AML, where they have important prognostic and therapeutic implications. While KIT^{D816V}-mutated CBF-AML is usually not associated with SM, non-CBF-AML with KIT^{D816V} was recently reported to correlate strongly with the presence of concurrent SM [20]. However, the implication of detecting a *KIT* mutation in the setting of other myeloid neoplasms, especially those with a chronic course, has not been thoroughly investigated. We took advantage of the inclusion of *KIT* in our clinical NGS panels to systematically ask whether the presence of a *KIT* mutation (p.D816V or otherwise) in myeloid neoplasms correlated with concurrent SM.

Of the 22 chronic myeloid neoplasms (MDS, MPN, or MDS/MPN) with a KIT^{D816V} mutation detected by our clinical NGS panels over an ~8-year period, 20 (91%) exhibited concurrent SM on BM biopsy examination. In contrast, thorough pathologic investigation of 30 randomly-selected MDS or CMML cases without a pathogenic *KIT* mutation (p.D816V or otherwise) showed no evidence of concurrent SM. These results indicate that the detection of KIT^{D816V} by NGS in patients with chronic myeloid neoplasms is a powerful predictor of SM-AHN.

In all 20 patients with SM-AHN where the AHN component was a chronic myeloid neoplasm in our cohort, the KIT^{D816V} mutation was present at the time of initial NGS testing at subclonal or equivalent levels to other pathogenic driver mutations. Notably, the DNA source for 10 of these 20 initial NGS tests was PB, suggesting that the KIT^{D816V} mutation was present in circulating myeloid cells. Indeed, prior studies have demonstrated that at least a subset of AHN cells also share the KIT^{D816V} mutation with the neoplastic mast cells. In one study of 48 patients with SM-AHN, the KIT^{D816V} mutation was found in the AHN cells of nearly all patients with CMML (89%), although less frequently in the AHN cells of MPN (20%) or AML (30%) [30]. Given the presence of *KIT* mutations within the AHN component, it is not possible to use the *KIT* VAF to predict SM burden in SM-AHN. Prior investigations have shown that the KIT^{D816V} mutation is a temporally late (subclonal) event in clonal evolution following mutations in *TET2*, *SRSF2,* and *ASXL1*. Additional studies of granulocyte-macrophage colony-forming progenitor cell units from patients with SM-AHN suggest that the KIT^{D816V} mutation is also an ontologically late event in many cases, occurring only in more differentiated cells driving the SM phenotype [31]. The KIT^{D816V} mutation was invariably present at the first NGS timepoint in the current study, along with *TET2*, *SRSF2*, and/or *ASXL1* mutations detected in 18 of 20 cases (90%). Due to the lack of comprehensive serial samples, we are unable to properly assess the timing of *KIT* mutation acquisition in this study; however, VAF data from our cohort appears to support the subclonal and therefore temporally later acquisition of *KIT* mutations in SM-AHN.

In our cohort of 18 patients with KIT^{D816V}-mutated AML, 17 (94%) were non-CBF forms of AML. Fritsche-Polanz et al. identified SM in 8 of 101 patients with AML, of which seven had a KIT^{D816V} mutation. Interestingly, 6 of the 7 patients with a KIT^{D816V} mutation had an antecedent history of either MDS or CMML [32]. Similarly, in our KIT^{D816V}-mutated AML cohort, SM was much more commonly identified in cases of AML-MRC (7 of 9, including four with known preceding MDS or MPD/MPN; 78%) compared with non-MRC AML (1 of 9, 11%; $p = 0.0152$). Another study investigated patients with KIT^{D816V}-mutated non-CBF AML and SM [20]. Of 40 such patients, the majority (24; 60%) were diagnosed with AML-MRC following a prior SM-AHN, where the AHN was MDS/MPN-U, CMML, MDS, or MPN. In addition, 11 of the 40 patients (28%) were diagnosed with de novo AML. In our cohort, no patients with de novo AML ($n = 7$; four AML with mutated *NPM1*, two AML-NOS, and one CBF-AML) or therapy-related AML ($n = 1$) and a KIT^{D816V} mutation were diagnosed with SM. Interestingly, in 4 of these 8 patients, the *KIT* mutation only appeared in the setting of posttransplant relapse with a survival of less than 4 months in all patients following detection of the mutation. Similarly, one of the AML-MRC patients without SM acquired the KIT^{D816V} mutation at relapse after allogeneic transplantation, with patient death 3 months later; in all other AML-MRC cases, the *KIT* mutation was present at the time of initial diagnosis. Taken together, the findings suggest that KIT^{D816V} is far more frequently associated with SM in the setting of AML-MRC than other non-CBF-AML subtypes, where acquisition of KIT^{D816V} appears to often be a late event associated with relapsed AML without concurrent SM.

The diagnostic criteria for SM in the setting of an AHN require that either the major criterion (≥15 mast cells in aggregates) and at least one minor criterion (>25% spindled/atypical mast cells; presence of *KIT* codon 816 mutation; aberrant co-expression of CD25 by mast cells), or at least three minor criteria, are met [27]. Of note, persistently elevated serum total tryptase is not a valid minor criterion in the setting of an AHN. Cases diagnosed based solely on

minor criteria typically show a diffuse interstitial pattern and are challenging to recognize in BM biopsies stained with H&E alone [33–35]. Although Giemsa staining can be a helpful adjunct to H&E staining for recognition of mast cells in routine cases (and is performed routinely on all BM cases at our institutions), neoplastic mast cells may be difficult to identify on Giemsa stain due to degranulation. In the course of our study, we identified four cases with subtle (well <5% of the cellularity in most cases) abnormal populations of interstitially-distributed mast cells that showed CD25 co-expression and at least subset (~5–10%) spindled morphology, but not definitively >25% of BM mast cells, on the BM core (and not apparent on the aspirate smears). This included one case of AML, NOS with a KIT^{D820Y} mutation, two cases of AML-MRC with KIT^{D816V} mutation, and one case of therapy-related AML with a KIT^{D816V} mutation. None of these patients exhibited splenomegaly or "C" findings, and tryptase was not measured in any case. The significance of these abnormal mast cell populations is uncertain but suggests a propensity for *KIT* mutations to drive occasionally more subtle SM-like phenotypic changes that fall short of current WHO diagnostic criteria for SM-AHN.

Notably, inclusion criteria for this study required evaluation on an NGS platform, as opposed to single-gene *KIT* assays such as allele-specific PCR or digital droplet PCR. Indeed, the reason that there are relatively few patients with SM alone in this NGS-based cohort is that, since 2016, patients at our institutions who are suspected or known to have SM without suspicion for AHN typically undergo PB analysis for the KIT^{D816V} mutation by ultrasensitive digital droplet PCR rather than NGS [36]. Although a definitive conclusion is beyond the scope of this study, our findings suggest that detection of KIT^{D816V} in the PB using a less sensitive testing technique (i.e., NGS) should raise suspicion for an underlying SM-AHN, as it may reflect the presence of circulating myeloid cells bearing pathogenic *KIT* mutations. On the other hand, had a more sensitive testing technique been applied to our target population, it is conceivable that we may have detected additional patients with lower levels of SM involvement, or encountered a threshold KIT^{D816V} VAF below which the association between KIT^{D816V} and concurrent SM was no longer apparent, either due to the detection of minor/transient subclones or the inability of low-level SM-like disease to meet current diagnostic criteria.

In this study, we identified nine patients (seven with a chronic myeloid neoplasm; two with AML-MRC) in whom the SM component of the SM-AHN diagnosis was not recognized upon initial BM evaluation. The challenge of identifying low-level or patchy involvement by the SM component in SM-AHN is well-established [14], but in many of our cases the SM component was not suspected even after both the clinician and pathologist became aware of the KIT^{D816V} mutation. This is perhaps not surprising, as *KIT* mutations have long been identified in CBF-AML without necessarily being indicative of associated SM. However, in the setting of MDS, MPN, MDS/MPN, or AML-MRC, our findings indicate that detection of the KIT^{D816V} should further prompt investigation for SM, even in the absence of overt histologic evidence. Given the availability of therapies that specifically target the SM component of SM-AHN [37–39], establishing this diagnosis has substantial clinical significance, particularly in patients more with aggressive forms of SM.

In summary, our data indicate that identification of KIT^{D816V} in patients diagnosed with chronic myeloid neoplasms or AML-MRC should prompt additional testing for underlying SM. In non-CBF, non-MRC AML, acquisition of the KIT^{D816V} mutation appears to often be a late event, occurring in the posttransplant setting, and is not frequently associated with SM. However, as the co-occurrence of SM in cases of KIT^{D816V}-mutated, non-CBF de novo AML has also been reported [20], careful clinicopathologic evaluation for SM is also indicated in this setting. Although the numbers are small, SM was not common in patients with non-D816V *KIT* mutations in our cohort. Nearly all (7 of 8) CBF-AML cases bearing a *KIT* mutation in our cohort had non-D816V *KIT* mutations in either exons 8 or 17, similar to prior studies [40, 41], and only two (both non-D816V KIT mutated) were associated with SM. One possible explanation is that the capacity of non-D816V *KIT* mutations to induce mast cell differentiation and modulate the phenotype of affected clones is simply lower than that of KIT^{D816V}. Further studies are needed to investigate this possibility.

Author contributions JWC, RCL, and EAM were responsible for conception and design of the study. JWC, RPH, GSP, and EAM were responsible for acquisition of data. JWC, RPH, ASK, JCA, JLH, DPS, RCL, DJD, and EAM interpreted and analyzed data. JWC and EAM wrote the paper. All authors critically revised the article and approved the final version.

Compliance with ethical standards

Conflict of interest DJD has served as a consultant for Amgen, Autolus, Blueprint, Celgene, Incyte, Forty-Seven, Jazz, Novartis, Pfizer, and Takeda Pharmaceuticals and has received research funding from Abbvie, Glycomimetics, Novartis, and Blueprint Pharmaceuticals. All other authors declare that they have no conflict of interest.

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