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# *KIT* Mutations and Other Genetic Defects in Mastocytosis

## Implications for Disease Pathology and Targeted Therapies

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### KEYWORDS

- Mast cells • Mastocytosis • *KIT* D816V mutation • Mutation burden • ASO-qPCR • ddPCR • Tyrosine kinase inhibitors

### KEY POINTS

- Mastocytosis is a group of rare diseases characterized by abnormal accumulation/proliferation of mast cells (MC) in one or several organs.
- In adults, mastocytosis is mostly systemic (systemic mastocytosis; SM) and chronic, affecting the bone marrow (BM) and other internal organs, with or without skin involvement.
- Acquired *KIT* mutations (mostly *KIT* D816V) are the unique genetic defect found in the vast majority of indolent SM, whereas additional myeloid malignancy-related genetic defect(s) are frequently found in advanced forms of the disease (advanced variants of SM [AdvSM]).

*Continued*

### INTRODUCTION

Mastocytosis is a group of rare diseases characterized by abnormal expansion of neoplastic mast cells (MC) in at least one organ/tissue, mainly skin, bone marrow (BM), and gastrointestinal tract.<sup>1</sup> Mastocytosis can affect both children and adults.

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*Continued*

- Highly sensitive and quantitative techniques such as allele specific-quantitative PCR or droplet digital PCR are recommended to detect and quantify the *KIT* D816V mutant in various tissues, including peripheral blood (PB) and BM, allowing diagnosis, prognostication, and follow-up of patients with SM.
- In SM, including AdvSM, patients may benefit from treatment with *KIT*-targeting tyrosine kinase inhibitors, and the treatment efficacy may be assessed by monitoring *KIT* D816V mutational burden in PB and/or BM.

Most pediatric and adult patients display *KIT* activating mutations in neoplastic MC.<sup>2</sup> The *KIT* D816V mutation is the most frequently detected (>80% of the cases) in systemic mastocytosis (SM), a category of mastocytosis mainly found in adult patients.<sup>2</sup> This mutant can be detected in BM, peripheral blood (PB) and infiltrated tissues with highly sensitive and quantitative techniques, that is, allele specific quantitative-(RT) PCR (ASO-qRT-PCR on mRNA or allele specific-quantitative PCR [ASO-qPCR] on DNA),<sup>3,4</sup> or droplet digital PCR (ddPCR) on DNA.<sup>5</sup> These techniques contribute to diagnosis, prognosis, and follow-up of patients and are thus considered as reference techniques.<sup>6</sup> Although the *KIT* D816V mutant is usually the unique genetic defect found in indolent variant of SM (indolent SM [ISM]), patients with more advanced variants of SM (AdvSM) have often additional genetic defects in other myeloid malignancy-related genes apart *KIT*.<sup>7</sup> These defects, usually associated with worse prognosis,<sup>7</sup> are best evidenced by next-generation sequencing (NGS).<sup>8</sup>

Of note, 2 *KIT*-targeted tyrosine kinase inhibitors (*KIT*-TKIs), namely midostaurin (Rydapt),<sup>9</sup> and avapritinib (Aykakit),<sup>10</sup> are now approved by FDA and European Medicines Agency (EMA) to treat AdvSM. In the era of such *KIT*-TKIs, the monitoring of the *KIT* D816V mutational burden has become one of the most potent markers to evaluate treatment response.

In this review, after a brief overview of the diagnosis and classification of mastocytosis, we will summarize the role of *KIT* and non-*KIT* mutations in the physiopathology of SM. We will then describe the methods currently available not only to detect the *KIT* D816V mutation but also to quantify the mutation burden and to monitor the treatment of AdvSM patients with *KIT*-TKIs. As a conclusion and perspective part, we will point to the question of the definition of molecular responses (MRs) in patients treated with these drugs.

## DIAGNOSTIC CRITERIA AND CLASSIFICATION OF MASTOCYTOSIS

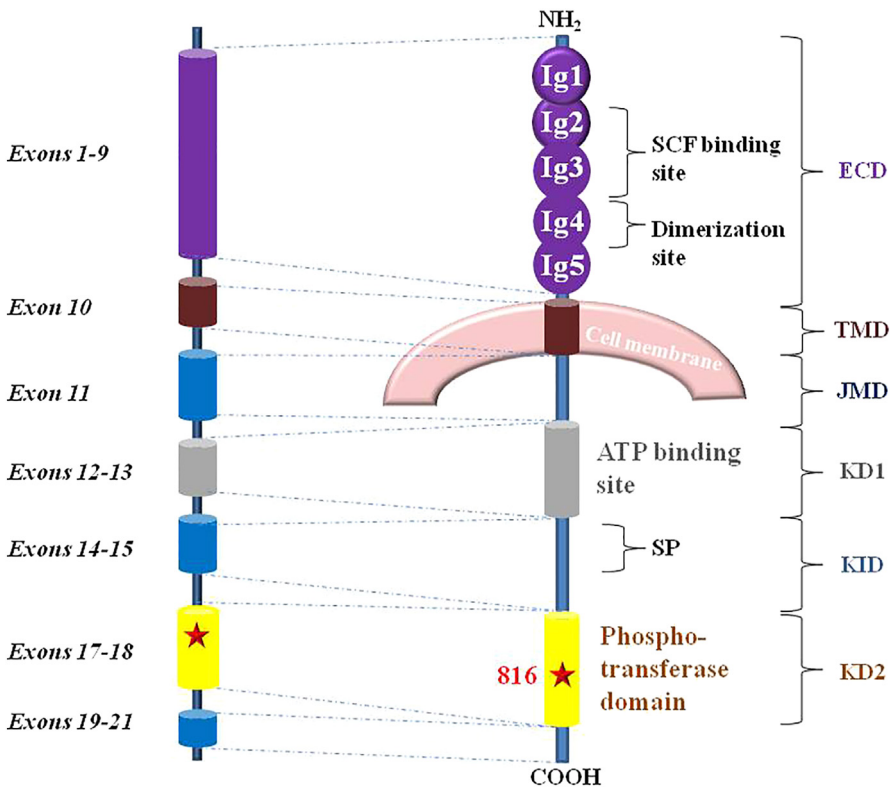
Please refer to the article by Valent and colleagues, “[WHO Classification and Diagnosis of Mastocytosis: Update 2023 and Future Perspectives](#),” in this issue for a detailed background of the most updated classification and diagnostic criteria for mastocytosis. Briefly, mastocytosis is classified into 3 major categories: cutaneous mastocytosis (CM), SM, and a very rare category of localized MC neoplasms, namely MC sarcoma (MCS), a very aggressive localized neoplasm composed of highly atypical MC.<sup>11</sup> The diagnosis of CM in pediatric patients requires the presence of typical skin lesions, positive Darier’s sign, the absence of systemic involvement and, unlike in adults, rarely requires skin or BM biopsy.<sup>12,13</sup> Conversely, in adults, mastocytosis in skin is mostly found as part of SM.<sup>14</sup>

Historical criteria for the diagnosis of SM<sup>15</sup> have been amended recently.<sup>16</sup> Variants of SM are defined according to (1) the presence or absence of high disease burden

(B-findings) and organ involvement (C-findings), (2) the presence or absence of an associated hematologic neoplasm (AHN), and (3) percentage of MC in BM or PB smears.<sup>16–18</sup> Of note, aggressive SM (ASM), mast cell leukemia (MCL), and SM with an associated hematologic neoplasm (SM-AHN) are collectively termed advanced SM (AdvSM).<sup>19</sup> Besides, well-differentiated SM (WDSM), a morphologic variant characterized by compact multifocal infiltrates of round mature MC in BM and constant skin involvement, may occur in any SM type/subtype.<sup>20</sup>

## THE ROLE OF *KIT* MUTATIONS IN THE PATHOPHYSIOLOGY OF MASTOCYTOSIS

In human, the *KIT* gene is located on the long arm of chromosome 4 (4q11–4q13) and contains 21 exons that span overall 80 kb of DNA (Fig. 1).<sup>21</sup> *KIT* encodes a 976 amino acid transmembrane tyrosine kinase receptor, capable of activating mitogenic signals when stimulated by its ligand, the stem cell factor (SCF).<sup>22</sup> *KIT* plays a critical role in



**Fig. 1.** Structure of the human normal *KIT* gene and of the corresponding KIT receptor. The *KIT* gene (left) contains 21 exons and encodes for KIT, the stem cell factor (SCF) receptor (right). The extracellular domain (ECD; in violet) display 5 immunoglobulin (Ig)-like subunits including a ligand binding site for SCF, and a dimerization site. After a TMD (in brown) made by a single helix, the cytoplasmic region contains an auto-inhibitory juxta-membrane domain (JMD), and a kinase domain (KD) split by a large kinase insert domain (KID) into KD1 (ATP-binding site in gray), and KD2 (phosphotransferase domain in yellow; PTD). The "Switch Pocket" (SP) allows the kinase to adopt an active or inactive conformation. The position of the most common *KIT* mutation (D816V) found in >80% of all patients with SM is highlighted by a red star.

the development of MC, melanocytes, hematopoietic stem cells, germ cells, and interstitial cells of Cajal.<sup>23–26</sup> In hematopoietic processes, KIT is involved in the differentiation of myeloid and lymphoid cells from hematopoietic stem cells and is downregulated on mature cells, excepted for MC.<sup>27</sup> The KIT receptor is composed of an extracellular domain (ECD) characterized by 5 Ig-like domains containing a ligand-binding site for SCF and a dimerization site (see Fig. 1). The ECD is linked to a cytoplasmic region by a short transmembrane domain (TMD; see Fig. 1). The cytoplasmic region consists of a juxtamembrane domain (JMD) and a bipartite tyrosine kinase domain where the ATP binding site and the phosphotransferase domain (PTD) are separated by a kinase-insert (see Fig. 1).<sup>21</sup> SCF binding to KIT leads to dimerization and autophosphorylation of the receptor at tyrosine residues serving as docking sites for signal transduction molecules.<sup>28</sup> The transduction process involves multiple signaling pathways such as PI3-kinase, Src family kinase, Ras-Erk, and JAK/STAT, resulting in cell proliferation, survival, and migration.<sup>28</sup>

### ***KIT D816V and Other KIT Mutations in Mastocytosis***

More than 80% of all patients with SM harbor the *KIT* D816V mutation.<sup>29</sup> In ISM and smoldering SM (SSM), this mutation is retrieved in virtually all the patients and is usually the only genetic anomaly detected in neoplastic MC.<sup>29</sup> Other variants at codon 816 have been found occasionally in ISM/SSM.<sup>29</sup> In AdvSM, although the *KIT* D816V mutant is found in greater than 80% of the patients, additional mutations at other *KIT* codons or no *KIT* mutations have been reported, particularly in MCL cases. Indeed, in a recent study, among 85 patients with MCL evaluated for *KIT* structure, *KIT* D816V was found in 73% of the patients, 11% of the individuals had alternative *KIT* mutations, and 17% were *KIT* wild type.<sup>30</sup> In addition, in most patients with WDSM, no *KIT* mutations are found, whereas *KIT* D816V or other *KIT* mutants may be detected in only ~30% of the patients.<sup>20</sup>

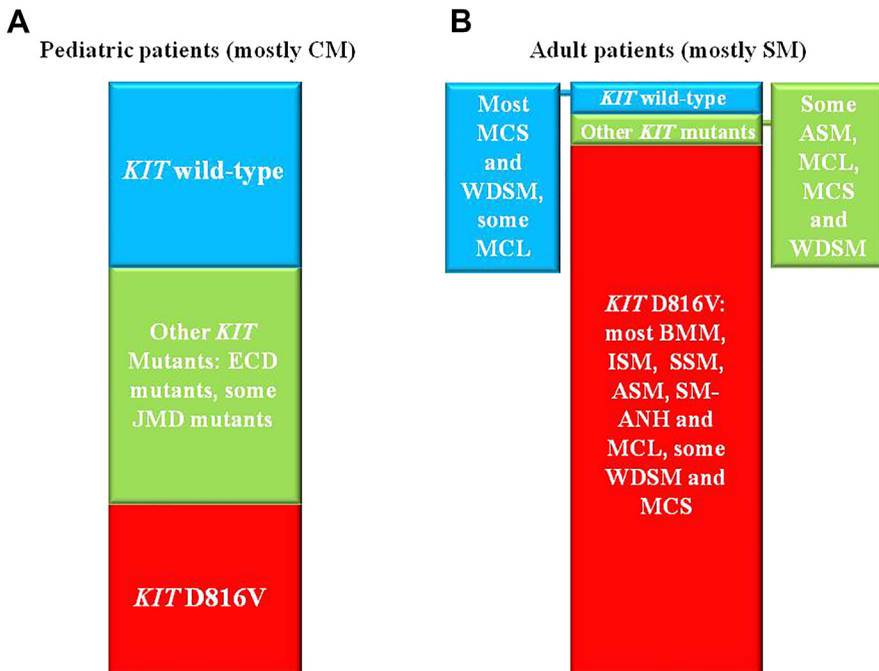
In contrast to adult SM, the *KIT* D816V mutant is found in ~30% of children in skin biopsies.<sup>31</sup> Other *KIT* mutations, mainly located in the ECD of the receptor, are found in ~40% of all cases with childhood CM or SM.<sup>31,32</sup> Thus, ~70% of childhood patients with mastocytosis have *KIT* defects, confirming that pediatric mastocytosis is also a clonal disease similar to SM in adults but with a broader spectrum of *KIT* mutations.<sup>12</sup>

Finally, regarding MCS, in the largest cohort reported to date, *KIT* mutational status was investigated in 14 patients and showed the absence of mutations in 50% of the cases, *KIT* D816V mutation in 21% of the patients and non-816 mutants in the remaining cases.<sup>33</sup>

A comparison of the frequency and nature of the various *KIT* defects found in pediatric versus adult patients with mastocytosis is presented in Fig. 2.

### ***Impact of the KIT D816V Mutant Receptor on Cell Survival and Proliferation***

It is far beyond the scope of this review to detail the signaling pathways aberrantly recruited by the *KIT* D816V mutant receptor. Briefly, the D816V gain of function mutation leads to a conformational change in the PTD, which entails constitutive activation of the receptor, independently of its dimerization.<sup>34</sup> Key downstream signaling pathways aberrantly activated by oncogenic *KIT* D816V mutant comprise, among others, PI3-kinase/protein kinase B (AKT),<sup>35</sup> signal transducer and activator of transcription-5 (STAT-5),<sup>36</sup> nuclear factor-kappa B (NF- $\kappa$ B),<sup>37</sup> mammalian target of rapamycin complex 2,<sup>38</sup> and rotein kinase C-delta (PKC $\delta$ ).<sup>39</sup> In addition, the abnormal accumulation of neoplastic MC in SM could result from the deregulation of proapoptotic and antiapoptotic pathways. There is evidence of overexpression of the antiapoptotic



**Fig. 2.** Differential repartition of *KIT* mutations between pediatric and adult patients and, in adults, between SM variants. In pediatric patients (panel A), the *KIT* D816V mutant is found in ~30% of the cases, whereas ~40% of the patients harbor non-*KIT* D816V mutants, principally located in the ECD of *KIT*. Finally, 30% of the pediatric patients are *KIT* wild-type. By contrast, in adults (panel B), greater than 80% of all patients with SM harbor the *KIT* D816V mutation, as found in BM and/or PB, whereas patients with MCL, MCS, and moreover in the WDSM variant harbor slightly less frequently the *KIT* D816V mutation. ASM, aggressive SM; BM, bone marrow; BMM, bone marrow mastocytosis; ISM, indolent SM; MCL, mast cell leukemia; MCS, mast cell sarcoma; PB, peripheral blood; SM, systemic mastocytosis; SM-AHN, SM with an associated hematologic neoplasm; SSM, smouldering SM; WDSM, well-differentiated SM; WT, wild type.

molecules B-cell leukemia/lymphoma 2 (BCL-2), BCL-xL, and MCL-1 in *KIT* D816V-positive neoplastic MC in SM patients.<sup>40,41</sup> In contrast, a loss of expression of the proapoptotic Bcl-2 interacting mediator of cell death (BIM) molecule is detected in these cells.<sup>42</sup> All these signaling and antiapoptotic pathways aberrantly evoked by the *KIT* D816V mutant receptor may concur to the abnormal accumulation/proliferation/survival of neoplastic MC in mastocytosis.<sup>43</sup>

#### THE ROLE OF NON-*KIT* MUTATIONS IN THE PATHOPHYSIOLOGY AND PROGNOSIS OF DIFFERENT SYSTEMIC MASTOCYTOSIS VARIANTS

Nearly 90% of patients with AdvSM and *KIT* D816V have additional somatic mutations (eg, *JAK2* V617F, *FIPL1-PDGFR*A, *BCR-ABL1*, *TET2*, *SRSF2*, *ASXL1*, *EZH2*, *CBL*, *RUNX1*, *RAS*), most with an SM-AHN.<sup>7</sup> In contrast, these additional mutations are rarely seen in patients with SSM or ISM.<sup>44,45</sup> They can be divided into lesions in disease-specific driver genes such as *JAK2* V617F, *FIPL1-PDGFR*A, or *BCR-ABL1* and lesions in genes not specific for a distinct hematological myeloid neoplasm.

Lesions in disease-related driver genes are found almost exclusively in SM-AHN where they define the nature and variant of the AHN.<sup>46–49</sup> However, other molecular aberrations repeatedly reported in patients with AdvSM, mostly in SM-AHN and to a lesser extent in ASM and MCL,<sup>7,50–55</sup> may affect signaling molecules (eg, *CBL*, *KRAS*, or *NRAS*), transcription factors (eg, *RUNX1*), epigenetic regulators (eg, *ASXL1*, *DNMT3A*, *EZH2*, or *TET2*), splicing factors (eg, *SRSF2*, *SF3B1*, or *U2AF1*) (reviewed in ref.<sup>56</sup>) or the tumor suppressor *SETD2*.<sup>57</sup> Several groups have investigated the prognostic relevance of these additional mutations. The presence and number of mutated genes within the *SRSF2/ASXL1/RUNX1* (S/A/R) panel,<sup>58</sup> the presence of mutations in *ASXL1* and/or *CBL*,<sup>55</sup> and *EZH2* gene mutations in addition to the S/A/R genes panel are associated with inferior survival in AdvSM.<sup>59</sup> In addition, although rarely found, mutations in the S/A/R genes panel together with mutations in *DNMT3A* are associated with poor outcome in patients with ISM and SSM.<sup>45,59</sup> Thus, nowadays, there is a consensus opinion that not only patients with AdvSM but also those with bone marrow mastocytosis (BMM), ISM, and SSM should have a myeloid NGS panel investigation, preferably from BM.<sup>6</sup>

Of note, highlighting the deleterious nature of some of these additional genetic defects has led to their incorporation into new mixed clinical, biochemical, and molecular prognostic scoring systems, such as the Mayo Alliance Prognostic System,<sup>60</sup> the Red Española de Mastocytosis (REMA) score,<sup>59</sup> the Mutation-Adjusted Risk Score,<sup>58</sup> or the Global Prognostic score for SM.<sup>45</sup>

## METHODS TO DETECT (AND QUANTIFY) *KIT* MUTATIONS IN MASTOCYTOSIS

The *KIT* D816V mutant being by far the most frequent *KIT* defect retrieved in the major categories of SM, highly sensitive techniques targeting the detection of this particular mutant are recommended in first line.<sup>6,61</sup> Diagnostic standard is mutation analysis of *KIT* on BM aspirate, and if not available, on BM smear or formalin-fixed paraffin-embedded (FFPE) biopsy sample.<sup>62</sup> However, with the recently developed ASO-qPCR/ddPCR sensitive techniques, the *KIT* D816V mutant can also be detected in PB in most patients with SM, making PB testing the first-line test to be carried out in case of suspicion of SM.<sup>6</sup> Pros and cons of different methods able to detect the D816V mutation in at least 80% of patients with SM; therefore, candidates to be used for routine testing are summarized in [Table 1](#).<sup>63–66</sup>

The most sensitive methods for the identification and quantization (mutational burden) of the *KIT* D816V mutation in different samples, including BM samples, PB and FFPE tissues, are ASO-qPCR and ddPCR. They allow detecting less than 0.01% *KIT* D816V mutation-positive cells.<sup>3</sup> Unfortunately, other D816 mutations cannot be detected by these assays.<sup>5</sup> Interestingly, ASO-qPCR and ddPCR are capable of detecting the *KIT* D816V mutation even in PB in most patients with SM.<sup>3,5,67–69</sup> Thus, the European Competence Network on Mastocytosis (ECNM) recommends *KIT* D816V-specific ASO-qPCR/ddPCR analysis in PB for initial screening in patients with suspected SM, and BM examination, including *KIT* D816V mutation analysis by ASO-qPCR/ddPCR in cases of elevated basal serum tryptase (BST) levels or initial positive screening in PB.<sup>6</sup> However, most *KIT* D816V+ BMM patients and some *KIT* D816V+ ISM patients will test negative in PB (and less frequently in BM) due to low percentage of infiltrating MC. In such cases, fluorescence-activated cell sorting or laser microdissection of MC may help to enhance sensitivity but neither approach is readily feasible in routine diagnostic laboratories.<sup>29</sup> Additionally, infrequent cases of ISM or SSM and some cases of ASM or MCL have another *KIT* mutation at position 816 (D816Y, D816H, and so forth), or in another position, or

Table 1

Overview of the advantages, weaknesses, and sensitivity of the different tests available to detect *KIT* mutations at codon 816

Technique	RT-PCR plus Restriction Fragment Length Polymorphism (RFLP)	Nested RT-PCR Followed by D-HPLC of PCR Amplicons	Peptide Nucleic Acid-Mediated PCR (PNA-PCR)	ASO-qPCR on DNA or RNA/cDNA and ddPCR	NGS and TU-NGS
Advantages	<ul style="list-style-type: none"> <li>• Simple, fast, cost-saving</li> <li>• Reliable</li> <li>• Good sensitivity</li> </ul>	<ul style="list-style-type: none"> <li>• Detects different <i>KIT</i> mutations at position 816</li> </ul>	<ul style="list-style-type: none"> <li>• Allows detection of <i>KIT</i> mutations at position 816 or at adjacent positions</li> <li>• Semiquantitative</li> <li>• Recommended for FFPE tissues</li> </ul>	<ul style="list-style-type: none"> <li>• Simple, fast, cost-saving</li> <li>• Highly sensitive</li> <li>• Quantitative: allows the quantification of the <i>KIT</i> D816V EAB in PB or BM</li> <li>• For ddPCR: works well on FFPE tissues</li> </ul>	<ul style="list-style-type: none"> <li>• Full <i>KIT</i> codons analysis</li> <li>• Quantitative results</li> <li>• Allows detection of non-<i>KIT</i> D816V mutations</li> </ul>
Weaknesses	<ul style="list-style-type: none"> <li>• Detects only <i>KIT</i> D816V mutant</li> <li>• Not quantitative</li> </ul>	<ul style="list-style-type: none"> <li>• Relatively low sensitivity</li> <li>• Not quantitative</li> <li>• Time-consuming</li> <li>• Needs special facilities (HPLC)</li> </ul>	<ul style="list-style-type: none"> <li>• Intermediate sensitivity</li> </ul>	<ul style="list-style-type: none"> <li>• Detects only <i>KIT</i> p.D816V mutant</li> <li>• Needs standardization/harmonization</li> </ul>	<ul style="list-style-type: none"> <li>• Relatively high cost</li> <li>• Relatively time-consuming</li> <li>• Low sensitivity and thus low negative predictive value</li> </ul>
Sensitivity	~ 0.05%	0.5%–1.0%	~ 0.1%	~ 0.01%	~ 0.2% with TU-NGS ~ 1%–5% with classical NGS

*Abbreviations:* ASO-qPCR, allele specific-quantitative polymerase chain reaction; ddPCR, droplet digital PCR; HPLC, high-performance liquid chromatography; NGS, next-generation sequencing; PCR, polymerase chain reaction; RT-PCR, reverse transcription-polymerase chain reaction; TU, targeted ultra-deep.



no *KIT* mutation at all (*KIT* wild type). The analysis of the precise *KIT* structure in such patients has critical therapeutic implications because patients with mutations in the ECD or the JMD of *KIT* or with no *KIT* mutation may respond to imatinib, whereas patients with other D816 variants would require midostaurin-based or avapritinib-based approaches. Thus, in such *KIT* D816V-negative patients, a stepwise approach can be used, at first with peptide nucleic acids (PNA)-mediated PCR to search for other 816 variants, then Sanger sequencing of the entire *KIT* coding region or NGS, keeping in mind the low sensitivity of this technique.<sup>6</sup>

## **QUANTIFICATION OF THE *KIT* D816V MUTATIONAL BURDEN FOR PROGNOSTICATION AND MONITORING OF THERAPEUTIC EFFICACY**

### ***Quantification of the *KIT* D816V Mutational Burden for Prognostication of Systemic Mastocytosis***

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Precise quantification of the *KIT* D816V mutational burden may have prognostic implications. Indeed, *KIT* D816V allele burden correlated significantly with disease subtypes and advancement. *KIT* D816V burden also correlated significantly with BST levels and age. Moreover, a cutoff level of 2% was identified that defined 2 prognostically distinct groups in terms of overall survival (OS).<sup>67</sup> Besides, detection of the *KIT* D816V mutation in hematopoietic cell compartments other than MC (multilineage involvement) has been associated with higher rate of progression from ISM to AdvSM and a worse outcome.<sup>70,71</sup> In line with this data, a value greater than 6% of circulating *KIT* D816V+ cells has been proposed as being suggestive of a multilineage involvement in patients.<sup>68</sup> Thus, the *KIT* D816V mutation burden seems to be a reliable and reproducible prognostic marker of SM,<sup>5,67,72</sup> now incorporated into prognostic scoring systems, such as the REMA score.<sup>59</sup>

### ***Monitoring of the Therapeutic Efficacy of Midostaurin and Avapritinib in Advanced Systemic Mastocytosis***

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Published data suggest that the approved *KIT*-TKIs midostaurin or avapritinib are effective to treat patients with AdvSM.<sup>73,74</sup> The determination of the *KIT* D816V mutational burden in PB and/or in BM may prove useful for monitoring their therapeutic efficacy. Midostaurin is a multikinase inhibitor also active on *KIT* wild type, *KIT* D816V, ECD, and JMD *KIT* mutants. A midostaurin-treated *KIT* D816V+ MCL patient displayed partial remission with a significant decrease in circulating MC and mutation frequency.<sup>75</sup> These encouraging results led to Phase II studies (CPKC412D2213 and CPKC412D2201) in AdvSM, showing an overall response rate of 60% to 69% regardless of *KIT* mutational status, with 38% to 45% of MR.<sup>76,77</sup> Jawhar and colleagues evaluated the impact of molecular markers at baseline and during follow-up in 38 midostaurin-treated patients with AdvSM.<sup>4</sup> Univariate analyses showed that reduction of *KIT* D816V EAB 25% or greater, tryptase 50% or greater, and alkaline phosphatase 50% or greater at 6 months were significantly associated with improved OS, whereas only *KIT* D816V reduction remained an independent on-treatment marker for improved OS by multivariate analysis.<sup>4</sup> More recently, Lübke and colleagues reported a clear superiority of midostaurin over cladribine on several parameters, including *KIT* D816V mutational burden and OS.<sup>73</sup>

Avapritinib, a TKI that targets *KIT* and multiple *KIT* exon 11, 11/17, and 17 mutants, was first evaluated in the phase I EXPLORER study (NCT02561988) on patients with AdvSM.<sup>78</sup> A complete molecular remission (MR) was achieved in 30% of patients, whereas a decrease in *KIT* D816V VAF in PB 50% or greater was obtained in 25 (64%) patients, correlating with decrease in BM MC and in BST levels.<sup>78</sup> Subsequently, interim results from phase 2 ongoing PATHFINDER study (NCT03580655)

reported that among the 32 evaluable patients, 19 (59%) experienced a decrease in the *KIT* D816V VAF in PB 50% or greater, together with profound reductions in other markers of disease burden.<sup>79</sup> A pooled analysis of 53 response-evaluable patients from EXPLORER and PATHFINDER confirmed a marked decrease of *KIT* D816V VAF on treatment with avapritinib.<sup>80–82</sup> It should be noted that the response criteria used in midostaurin and avapritinib studies were not identical.

To conclude, these data confirm the value of highly sensitive and quantitative techniques of determination of *KIT* D816V mutational burden in BM and/or PB to monitor treatment response of patients with AdvSM treated with KIT-TKIs.

## CONCLUDING REMARKS AND OPEN QUESTIONS

A majority of pediatric and adult patients with mastocytosis exhibits *KIT* activating mutations. Although the *KIT* D816V mutation is found in ~ 30% of children, with 40% of pediatric cases bearing other *KIT* mutations, *KIT* D816V is detected in greater than 80% of all adult patients with SM. In ISM, the mutant is considered as the main driver of the disease, whereas most patients with AdvSM present with additional non-*KIT* genetic defects negatively affecting the prognosis. Currently, it is recommended in patients suspected of having SM, as a first step to apply sensitive and quantitative techniques (namely ASO-qPCR and ddPCR) able to detect specifically the *KIT* D816V mutation in various biological samples, including the PB and the BM. These highly sensitive detection techniques are now routinely implemented in most mastocytosis reference centers including ours. In addition, quantification of the *KIT* D816V mutational burden improves also prognostication of patients, leading to the incorporation of this parameter in new prognostic scoring systems. As well, in the era of potent KIT-TKIs such as midostaurin or avapritinib, it is of utmost importance to quantify at regular intervals the *KIT* D816V mutational burden during the treatment, in order to evaluate its efficacy, the aim of such treatment being to obtain a MR. In fact, MR of *KIT* D816V under KIT-TKIs treatment is a new response benchmark and a treatment goal, including in AdvSM. However, the concept of minimal residual disease in AdvSM remains to be established, as well as consensus definition for levels of remission. Other important questions are as follows: (1) will the achievement of MR translate into prolonged progression-free survival and OS and (2) will achievement of MR permit time-limited treatment followed by durable treatment-free remission. Finally, as stated above, a minority of patients with SM may present with non-816V mutations (D816Y, D816H, and so forth), with non-816 mutations (ECD or JMD mutants) or with *KIT* wild type. For such patients, there is still a need to find alternative (tailor-made) techniques able to quantify their response to therapy.

## CLINICS CARE POINTS

- When a patient is suspected of having SM, it is preferable to analyze in a first step both the BST level and the presence of the *KIT* D816V mutation in PB rather than proceeding directly to BM analysis, in order to avoid an excessive use of invasive investigations.
- When searching for the *KIT* D816V mutation in patients suspected of having SM, it is preferable to use highly sensitive and quantitative techniques, such as ASO-qPCR or ddPCR, rather other nonsensitive and nonquantitative techniques.
- In the case of the treatment of a patient with *KIT* D816V+ SM by targeted therapy (Midostaurin or Avapritinib), it is preferable to monitor treatment efficacy by measurement of the *KIT* D816V allele burden rather than by measurement of the BST level, particularly if the SM is accompanied by an associated myeloid neoplasm.

**DISCLOSURE**

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**REFERENCES**

1. Valent P. Mastocytosis: a paradigmatic example of a rare disease with complex biology and pathology. *American journal of cancer research* 2013;3(2):159–72.
2. Arock M, Hoermann G, Sotlar K, et al. Clinical impact and proposed application of molecular markers, genetic variants, and cytogenetic analysis in mast cell neoplasms: Status 2022. *J Allergy Clin Immunol* 2022;149(6):1855–65.
3. Kristensen T, Vestergaard H, Moller MB. Improved detection of the KIT D816V mutation in patients with systemic mastocytosis using a quantitative and highly sensitive real-time qPCR assay. *J Mol Diagn* 2011;13(2):180–8.
4. Jawhar M, Schwaab J, Naumann N, et al. Response and progression on midostaurin in advanced systemic mastocytosis: KIT D816V and other molecular markers. *Clinical Trial, Phase II. Blood* 2017;130(2):137–45.
5. Greiner G, Gurbisz M, Ratzinger F, et al. Digital PCR: A Sensitive and Precise Method for KIT D816V Quantification in Mastocytosis. *Clin Chem* 2018;64(3):547–55.
6. Hoermann G, Sotlar K, Jawhar M, et al. Standards of Genetic Testing in the Diagnosis and Prognostication of Systemic Mastocytosis in 2022: Recommendations of the EU-US Cooperative Group. *J Allergy Clin Immunol Pract* 2022;10(8):1953–63.
7. Schwaab J, Schnittger S, Sotlar K, et al. Comprehensive mutational profiling in advanced systemic mastocytosis. *Blood* 2013;122(14):2460–6.
8. Nicolosi M, Patriarca A, Andorno A, et al. Precision Medicine in Systemic Mastocytosis. *Medicina (Kaunas, Lithuania)* 2021;57(11):1135.
9. Kim ES. Midostaurin: First Global Approval. *Review. Drugs* 2017;77(11):1251–9.
10. Dhillon S. Avapritinib: First Approval. *Drugs* 2020;80(4):433–9.
11. Valent P, Arock M, Akin C, et al. Recent Developments in the Field of Mast Cell Disorders: Classification, Prognostication, and Management. *J Allergy Clin Immunol Pract* 2022;10(8):2052–5.
12. Lange M, Hartmann K, Carter MC, et al. Molecular Background, Clinical Features and Management of Pediatric Mastocytosis: Status 2021. *Int J Mol Sci* 2021;22(5):2586.
13. Sandru F, Petca RC, Costescu M, et al. Cutaneous Mastocytosis in Childhood—Update from the Literature. *J Clin Med* 2021;10(7):1474.
14. Fuchs D, Kilbertus A, Kofler K, et al. Scoring the Risk of Having Systemic Mastocytosis in Adult Patients with Mastocytosis in the Skin. *J Allergy Clin Immunol Pract* 2021;9(4):1705–1712 e4.
15. Valent P, Horny HP, Escribano L, et al. Diagnostic criteria and classification of mastocytosis: a consensus proposal. *Leuk Res* 2001;25(7):603–25.
16. Valent P, Akin C, Hartmann K, et al. Updated Diagnostic Criteria and Classification of Mast Cell Disorders: A Consensus Proposal. *HemaSphere*. 2021;5(11):e646.
17. Khoury JD, Solary E, Abla O, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. *Leukemia* 2022;36(7):1703–19.

18. Zanotti R, Bonifacio M, Lucchini G, et al. Refined diagnostic criteria for bone marrow mastocytosis: a proposal of the European competence network on mastocytosis. *Leukemia* 2022;36(2):516–24.
19. Ustun C, Arock M, Kluin-Nelemans HC, et al. Advanced systemic mastocytosis: from molecular and genetic progress to clinical practice. *Haematologica* 2016; 101(10):1133–43.
20. Alvarez-Twose I, Jara-Acevedo M, Morgado JM, et al. Clinical, immunophenotypic, and molecular characteristics of well-differentiated systemic mastocytosis. *J Allergy Clin Immunol* 2016;137(1):168–78.
21. Giebel LB, Strunk KM, Holmes SA, et al. Organization and nucleotide sequence of the human KIT (mast/stem cell growth factor receptor) proto-oncogene. *Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S. Oncogene* 1992;7(11):2207–17.
22. Qiu FH, Ray P, Brown K, et al. Primary structure of c-kit: relationship with the CSF-1/PDGF receptor kinase family—oncogenic activation of v-kit involves deletion of extracellular domain and C terminus. *Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S. EMBO J* 1988;7(4):1003–11.
23. Broxmeyer HE, Maze R, Miyazawa K, et al. The kit receptor and its ligand, steel factor, as regulators of hemopoiesis. *Cancer Cells* 1991;3(12):480–7.
24. Lyman SD, Williams DE. Biological activities and potential therapeutic uses of steel factor. A new growth factor active on multiple hematopoietic lineages. *Am J Pediatr Hematol Oncol* 1992;14(1):1–7.
25. Ward SM, Burns AJ, Torihashi S, et al. Impaired development of interstitial cells and intestinal electrical rhythmicity in steel mutants. *Am J Physiol* 1995;269(6 Pt 1):C1577–85.
26. Costa JJ, Demetri GD, Harrist TJ, et al. Recombinant human stem cell factor (kit ligand) promotes human mast cell and melanocyte hyperplasia and functional activation in vivo. *J Exp Med* 1996;183(6):2681–6.
27. Ashman LK. The biology of stem cell factor and its receptor C-kit. *Int J Biochem Cell Biol* 1999;31(10):1037–51.
28. Roskoski R Jr. Signaling by Kit protein-tyrosine kinase—the stem cell factor receptor. *Review. Biochem Biophys Res Commun* 2005;337(1):1–13.
29. Garcia-Montero AC, Jara-Acevedo M, Teodosio C, et al. KIT mutation in mast cells and other bone marrow hematopoietic cell lineages in systemic mast cell disorders: a prospective study of the Spanish Network on Mastocytosis (REMA) in a series of 113 patients. *Research Support, Non-U.S. Gov't. Blood* 2006;108(7):2366–72.
30. Kennedy VE, Perkins C, Reiter A, et al. Mast Cell Leukemia: Clinical and Molecular Features and Survival Outcomes of Patients in the ECNM Registry. *Blood advances* 2022;7(9):1713–24.
31. Bodemer C, Hermine O, Palmérini F, et al. Pediatric mastocytosis is a clonal disease associated with D816V and other activating c-KIT mutations. *J Invest Dermatol* 2010;130(3):804–15.
32. Yang Y, Letard S, Borge L, et al. Pediatric mastocytosis-associated KIT extracellular domain mutations exhibit different functional and signaling properties compared with KIT-phosphotransferase domain mutations. *Blood* 2010;116(7): 1114–23.
33. Monnier J, Georgin-Lavialle S, Canioni D, et al. Mast cell sarcoma: new cases and literature review. *Review. Oncotarget* 2016;7(40):66299–309.

34. Rajan V, Prykhozij SV, Pandey A, et al. KIT D816V is dimerization-independent and activates downstream pathways frequently perturbed in mastocytosis. *Br J Haematol* 2022. <https://doi.org/10.1111/bjh.18116>.
35. Chian R, Young S, Danilkovitch-Miagkova A, et al. Phosphatidylinositol 3 kinase contributes to the transformation of hematopoietic cells by the D816V c-Kit mutant. *Blood* 2001;98(5):1365–73.
36. Baumgartner C, Cerny-Reiterer S, Sonneck K, et al. Expression of activated STAT5 in neoplastic mast cells in systemic mastocytosis: subcellular distribution and role of the transforming oncoprotein KIT D816V. *Research Support, Non-U.S. Gov't. Am J Pathol* 2009;175(6):2416–29.
37. Tanaka A, Konno M, Muto S, et al. A novel NF-kappaB inhibitor, IMD-0354, suppresses neoplastic proliferation of human mast cells with constitutively activated c-kit receptors. *Blood* 2005;105(6):2324–31.
38. Smrz D, Kim MS, Zhang S, et al. mTORC1 and mTORC2 differentially regulate homeostasis of neoplastic and non-neoplastic human mast cells. *Research Support, N.I.H., Extramural Research Support, N.I.H., Intramural. Blood* 2011; 118(26):6803–13.
39. Tobio A, Alfonso A, Botana LM. Cross-talks between c-Kit and PKC isoforms in HMC-1(560) and HMC-1(560,816) cells. Different role of PKCdelta in each cellular line. *Cell Immunol* 2015;293(2):104–12.
40. Hartmann K, Artuc M, Baldus SE, et al. Expression of Bcl-2 and Bcl-xL in cutaneous and bone marrow lesions of mastocytosis. *Research Support, Non-U.S. Gov't. Am J Pathol* 2003;163(3):819–26.
41. Aichberger KJ, Mayerhofer M, Gleixner KV, et al. Identification of MCL1 as a novel target in neoplastic mast cells in systemic mastocytosis: inhibition of mast cell survival by MCL1 antisense oligonucleotides and synergism with PKC412. *Blood* 2007;109(7):3031–41.
42. Aichberger KJ, Gleixner KV, Mirkina I, et al. Identification of proapoptotic Bim as a tumor suppressor in neoplastic mast cells: role of KIT D816V and effects of various targeted drugs. *Blood* 2009;114(26):5342–51.
43. Bibi S, Langenfeld F, Jeanningros S, et al. Molecular defects in mastocytosis: KIT and beyond KIT. *Immunol Allergy Clin* 2014;34(2):239–62.
44. Munoz-Gonzalez JI, Jara-Acevedo M, Alvarez-Twose I, et al. Impact of somatic and germline mutations on the outcome of systemic mastocytosis. *Research Support, Non-U.S. Gov't. Blood advances* 2018;2(21):2814–28.
45. Muñoz-González JI, Álvarez-Twose I, Jara-Acevedo M, et al. Proposed global prognostic score for systemic mastocytosis: a retrospective prognostic modelling study. *The Lancet Haematology* 2021;8(3):e194–204, d.
46. Sotlar K, Bache A, Stellmacher F, et al. Systemic mastocytosis associated with chronic idiopathic myelofibrosis: a distinct subtype of systemic mastocytosis associated with a [corrected] clonal hematological non-mast [corrected] cell lineage disorder carrying the activating point mutations KITD816V and JAK2V617F. *J Mol Diagn* 2008;10(1):58–66.
47. Dobrea C, Ciochinaru M, Găman A, et al. Systemic mastocytosis associated with essential thrombocythemia. *Rom J Morphol Embryol* 2012;53(1):197–202.
48. Schwaab J, Umbach R, Metzgeroth G, et al. KIT D816V and JAK2 V617F mutations are seen recurrently in hypereosinophilia of unknown significance. *Am J Hematol* 2015;90(9):774–7.
49. Naumann N, Lübke J, Shomali W, et al. Clinical and histopathological features of myeloid neoplasms with concurrent Janus kinase 2 (JAK2) V617F and KIT

- proto-oncogene, receptor tyrosine kinase (KIT) D816V mutations. *Br J Haematol* 2021;194(2):344–54.
50. Traina F, Visconte V, Jankowska AM, et al. Single nucleotide polymorphism array lesions, TET2, DNMT3A, ASXL1 and CBL mutations are present in systemic mastocytosis. *PLoS One* 2012;7(8):e43090.
  51. Damaj G, Joris M, Chandesris O, et al. ASXL1 but not TET2 mutations adversely impact overall survival of patients suffering systemic mastocytosis with associated clonal hematologic non-mast-cell diseases. *PLoS One* 2014;9(1):e85362.
  52. Hanssens K, Brenet F, Agopian J, et al. SRSF2-p95 hotspot mutation is highly associated with advanced forms of mastocytosis and mutations in epigenetic regulator genes. *Haematologica* 2014;99(5):830–5.
  53. Jawhar M, Schwaab J, Schnittger S, et al. Molecular profiling of myeloid progenitor cells in multi-mutated advanced systemic mastocytosis identifies KIT D816V as a distinct and late event. *Leukemia* 2015;29(5):1115–22.
  54. Jawhar M, Schwaab J, Schnittger S, et al. Additional mutations in SRSF2, ASXL1 and/or RUNX1 identify a high-risk group of patients with KIT D816V(+) advanced systemic mastocytosis. *Leukemia* 2016;30(1):136–43.
  55. Pardanani A, Lasho T, Elala Y, et al. Next-generation sequencing in systemic mastocytosis: Derivation of a mutation-augmented clinical prognostic model for survival. *Am J Hematol* 2016;91(9):888–93.
  56. González-López O, Muñoz-González JI, Orfao A, et al. Comprehensive Analysis of Acquired Genetic Variants and Their Prognostic Impact in Systemic Mastocytosis. *Cancers* 2022;14(10):2487–511.
  57. Martinelli G, Mancini M, De Benedittis C, et al. SETD2 and histone H3 lysine 36 methylation deficiency in advanced systemic mastocytosis. *Leukemia* 2018;32(1):139–48.
  58. Jawhar M, Schwaab J, Alvarez-Twose I, et al. MARS: Mutation-Adjusted Risk Score for Advanced Systemic Mastocytosis. Multicenter Study Research Support, Non-U.S. Gov't Validation Study. *J Clin Oncol* 2019;37(31):2846–56.
  59. Muñoz-González JI, Álvarez-Twose I, Jara-Acevedo M, et al. Frequency and prognostic impact of KIT and other genetic variants in indolent systemic mastocytosis. *Blood* 2019;134(5):456–68.
  60. Pardanani A, Shah S, Mannelli F, et al. Mayo alliance prognostic system for mastocytosis: clinical and hybrid clinical-molecular models. Research Support, Non-U.S. Gov't. *Blood advances* 2018;2(21):2964–72.
  61. Gowney JD, Clark JJ, Adelsperger J, et al. Activation mutations of human c-KIT resistant to imatinib mesylate are sensitive to the tyrosine kinase inhibitor PKC412. Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S. *Blood* 2005;106(2):721–4.
  62. Sotlar K. c-kit mutational analysis in paraffin material. *Methods Mol Biol* 2013;999:59–78.
  63. Akin C, Kirshenbaum AS, Semere T, et al. Analysis of the surface expression of c-kit and occurrence of the c-kit Asp816Val activating mutation in T cells, B cells, and myelomonocytic cells in patients with mastocytosis. *Exp Hematol* 2000;28(2):140–7.
  64. De Matteis G, Zanotti R, Colarossi S, et al. The impact of sensitive KIT D816V detection on recognition of indolent Systemic Mastocytosis. *Leuk Res* 2015;39(3):273–8.
  65. Sotlar K, Escribano L, Landt O, et al. One-step detection of c-kit point mutations using peptide nucleic acid-mediated polymerase chain reaction clamping and hybridization probes. *Am J Pathol* 2003;162(3):737–46.

66. Kristensen T, Broesby-Olsen S, Vestergaard H, et al. Targeted ultradeep next-generation sequencing as a method for KIT D816V mutation analysis in mastocytosis. *Eur J Haematol* 2016;96(4):381–8.
67. Hoermann G, Gleixner KV, Dinu GE, et al. The KIT D816V allele burden predicts survival in patients with mastocytosis and correlates with the WHO type of the disease. *Allergy* 2014;69(6):810–3.
68. Jara-Acevedo M, Teodosio C, Sanchez-Munoz L, et al. Detection of the KIT D816V mutation in peripheral blood of systemic mastocytosis: diagnostic implications. *Mod Pathol* 2015;28(8):1138–49.
69. Kristensen T, Broesby-Olsen S, Vestergaard H, et al. Towards rational diagnostics in mastocytosis: clinical validation of sensitive KIT D816V mutation analysis of unfractionated whole-blood. *Leuk Lymphoma* 2019;60(1):268–70.
70. Escribano L, Alvarez-Twose I, Sanchez-Munoz L, et al. Prognosis in adult indolent systemic mastocytosis: a long-term study of the Spanish Network on Mastocytosis in a series of 145 patients. *J Allergy Clin Immunol* 2009;124(3):514–21.
71. Navarro-Navarro P, Álvarez-Twose I, Pérez-Pons A, et al. KITD816V mutation in blood for the diagnostic screening of systemic mastocytosis and mast cell activation syndromes. *Allergy* 2022;16.
72. Greiner G, Gurbisz M, Ratzinger F, et al. Molecular quantification of tissue disease burden is a new biomarker and independent predictor of survival in mastocytosis. *Haematologica* 2020;105(2):366–74.
73. Lübke J, Schwaab J, Naumann N, et al. Superior Efficacy of Midostaurin Over Cladribine in Advanced Systemic Mastocytosis: A Registry-Based Analysis. *J Clin Oncol* 2022;40(16):1783–94.
74. Reiter A, Gotlib J, Álvarez-Twose I, et al. Efficacy of avapritinib versus best available therapy in the treatment of advanced systemic mastocytosis. *Leukemia* 2022;36(8):2108–20.
75. Gotlib J, Berube C, Growney JD, et al. Activity of the tyrosine kinase inhibitor PKC412 in a patient with mast cell leukemia with the D816V KIT mutation. Case Reports Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S. *Blood* 2005;106(8):2865–70.
76. Gotlib J, DeAngelo DJ, George TI, et al. KIT Inhibitor Midostaurin Exhibits a High Rate of Clinically Meaningful and Durable Responses in Advanced Systemic Mastocytosis: Report of a Fully Accrued Phase II Trial. *Blood* 2010;116(21):316.
77. Gotlib J, Kluijn-Nelemans HC, George TI, et al. Efficacy and Safety of Midostaurin in Advanced Systemic Mastocytosis. *N Engl J Med* 2016;374(26):2530–41.
78. DeAngelo DJ, Radia DH, George TI, et al. Safety and efficacy of avapritinib in advanced systemic mastocytosis: the phase 1 EXPLORER trial. *Nat Med* 2021;27(12):2183–91.
79. Gotlib J, Reiter A, Radia DH, et al. Efficacy and safety of avapritinib in advanced systemic mastocytosis: interim analysis of the phase 2 PATHFINDER trial. *Nat Med* 2021;27(12):2192–9.
80. Gotlib J, Reiter A, DeAngelo DJ. Avapritinib for Advanced Systemic Mastocytosis. *Blood* 2022;140(15):1667–73.
81. Arber DA, Orazi A, Hasserjian RP, et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. *Blood* 2022;140(11):1200–28.
82. Valent P, Sotlar K, Sperr WR, et al. Refined diagnostic criteria and classification of mast cell leukemia (MCL) and myelomastocytic leukemia (MML): a consensus proposal. Review. *Ann Oncol* 2014;25(9):1691–700.