KIT Mutations and Other Genetic Defects in Mastocytosis: Implications for Disease Pathology and Targeted Therapies

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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]).

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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.[1] Mastocytosis can affect both children and adults.

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- 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.[2] 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.[2] 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),[3,4] or droplet digital PCR (ddPCR) on DNA.[5] These techniques contribute to diagnosis, prognosis, and follow-up of patients and are thus considered as reference techniques.[6] 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*.[7] These defects, usually associated with worse prognosis,[7] are best evidenced by next-generation sequencing (NGS).[8]

Of note, 2 KIT-targeted tyrosine kinase inhibitors (KIT-TKIs), namely midostaurin (Rydapt),[9] and avapritinib (Ayvakit),[10] are now approved by FDA and European Mediines 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.[11] 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.[12,13] Conversely, in adults, mastocytosis in skin is mostly found as part of SM.[14]

Historical criteria for the diagnosis of SM[15] have been amended recently.[16] 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.[16–18] Of note, aggressive SM (ASM), mast cell leukemia (MCL), and SM with an associated hematologic neoplasm (SM-AHN) are collectively termed advanced SM (AdvSM).[19] 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.[20]

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).[21] *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).[22] KIT plays a critical role in

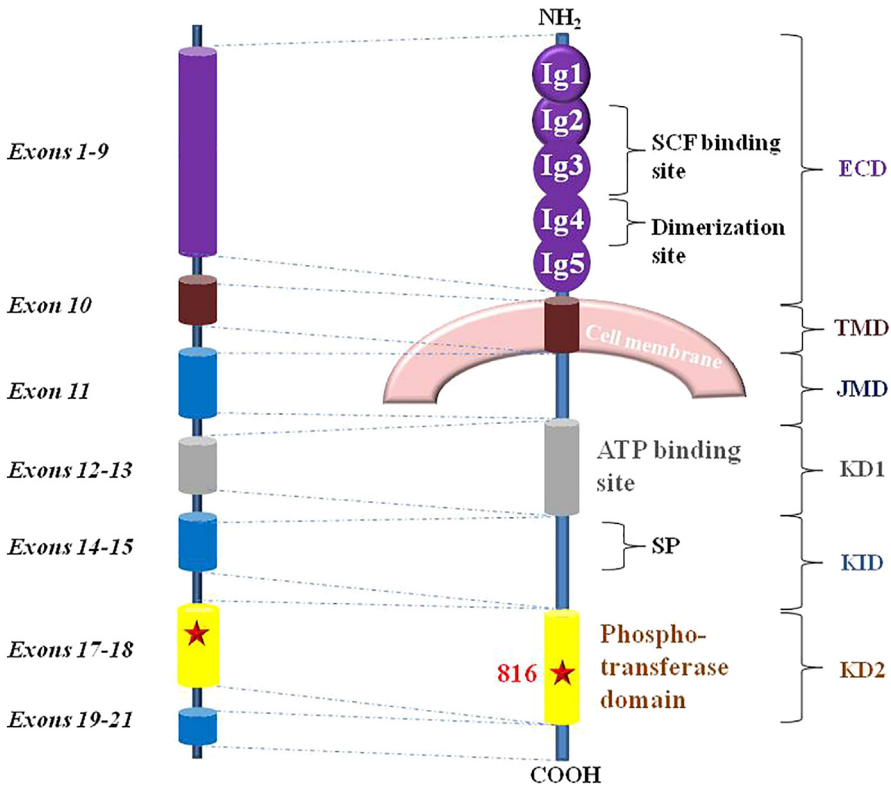

Fig. 1. Structure of the human normal *KIT* gene and of the corresponding KIT receptor. The *KIT* gene (left) contains 21 exons and encodes for KIT, the stem cell factor (SCF) receptor (right). The extracellular domain (ECD; in violet) display 5 immunoglobulin (Ig)-like subunits including a ligand binding 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.[23–26] 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.[27] 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**).[21] SCF binding to KIT leads to dimerization and autophosphorylation of the receptor at tyrosine residues serving as docking sites for signal transduction molecules.[28] 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.[28]

KIT D816V and Other KIT Mutations in Mastocytosis

More than 80% of all patients with SM harbor the *KIT* D816V mutation.[29] 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.[29] Other variants at codon 816 have been found occasionally in ISM/SSM.[29] 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.[30] 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.[20]

In contrast to adult SM, the *KIT* D816V mutant is found in ~30% of children in skin biopsies.[31] Other *KIT* mutations, mainly located in the ECD of the receptor, are found in ~40% of all cases with childhood CM or SM.[31,32] 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.[12]

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.[33]

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.[34] Key downstream signaling pathways aberrantly activated by oncogenic KIT D816V mutant comprise, among others, PI3-kinase/protein kinase B (AKT),[35] signal transducer and activator of transcription-5 (STAT-5),[36] nuclear factor-kappa B (NF-kB),[37] mammalian target of rapamycin complex 2,[38] and rotein kinase C-delta (PKCδ).[39] 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

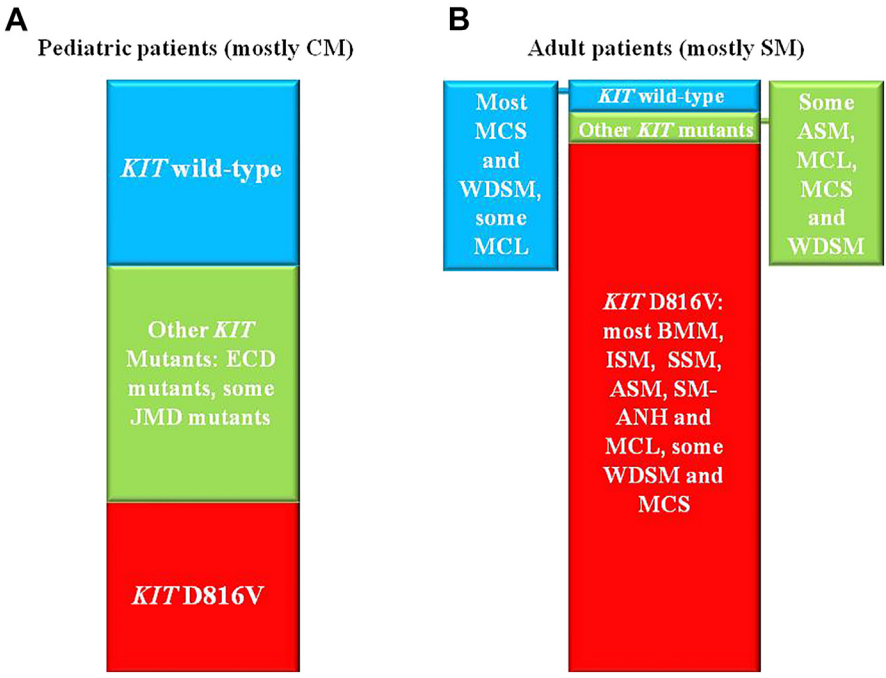

Fig. 2. Differential repartition of *KIT* mutations between pediatric and adult patients and, in adults, between SM variants. In pediatric patients (panel *A*), the *KIT* D816V mutant is found in ~30% of the cases, whereas ~40% of the patients harbor non-*KIT* D816V mutants, principally located in the 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.[40,41] In contrast, a loss of expression of the pro-apoptotic Bcl-2 interacting mediator of cell death (BIM) molecule is detected in these cells.[42] All these signaling and antiapoptotic pathways aberrantly evoked by the KIT D81V mutant receptor may concur to the abnormal accumulation/proliferation/survival of neoplastic MC in mastocytosis.[43]

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-PDGFRA*, *BCR-ABL1*, *TET2*, *SRSF2*, *ASXL1*, *EZH2*, *CBL*, *RUNX1*, *RAS*), most with an SM-AHN.[7] In contrast, these additional mutations are rarely seen in patients with SSM or ISM.[44,45] They can be divided into lesions in disease-specific driver genes such as *JAK2* V617F, *FIPL1-PDGFRA*, 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.[46–49] However, other molecular aberrations repeatedly reported in patients with AdvSM, mostly in SM-AHN and to a lesser extent in ASM and MCL,[7,50–55] 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.[56]) or the tumor suppressor *SETD2*.[57] 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,[58] the presence of mutations in *ASXL1* and/or *CBL*,[55] and *EZH2* gene mutations in addition to the S/A/R genes panel are associated with inferior survival in AdvSM.[59] 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.[45,59] 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.[6]

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,[60] the Red Española de Mastocitosis (REMA) score,[59] the Mutation-Adjusted Risk Score,[58] or the Global Prognostic score for SM.[45]

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.[6,61] 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.[62] 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.[6] 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**.[63–66]

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.[3] Unfortunately, other D816 mutations cannot be detected by these assays.[5] Interestingly, ASO-qPCR and ddPCR are capable of detecting the *KIT* D816V mutation even in PB in most patients with SM.[3,5,67–69] 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.[6] 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.[29] 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	• Simple, fast, cost-saving • Reliable • Good sensitivity	• Detects different KIT mutations at position 816	• Allows detection of KIT mutations at position 816 or at adjacent positions • Semiquantitative • Recommended for FFPE tissues	• Simple, fast, cost-saving • Highly sensitive • Quantitative: allows the quantification of the KIT D816V EAB in PB or BM • For ddPCR: works well on FFPE tissues	• Full KIT codons analysis • Quantitative results • Allows detection of non-KIT D816V mutations
Weaknesses	• Detects only KIT D816V mutant • Not quantitative	• Relatively low sensitivity • Not quantitative • Time-consuming • Needs special facilities (HPLC)	• Intermediate sensitivity	• Detects only KIT p.D816V mutant • Needs standardization/ harmonization	• Relatively high cost • Relatively time-consuming • Low sensitivity and thus low negative predictive value
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 ultradeep.

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.[6]

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

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).[67] 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.[70,71] 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.[68] Thus, the *KIT* D816V mutation burden seems to be a reliable and reproducible prognostic marker of SM,[5,67,72] now incorporated into prognostic scoring systems, such as the REMA score.[59]

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

Published data suggest that the approved KIT-TKIs midostaurin or avapritinib are effective to treat patients with AdvSM.[73,74] 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.[75] 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.[76,77] Jawhar and colleagues evaluated the impact of molecular markers at baseline and during follow-up in 38 midostaurin-treated patients with AdvSM.[4] 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.[4] More recently, Lübke and colleagues reported a clear superiority of midostaurin over cladribine on several parameters, including *KIT* D816V mutational burden and OS.[73]

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.[78] 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.[78] 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.[79] A pooled analysis of 53 response-evaluable patients from EXPLORER and PATHFINDER confirmed a marked decrease of *KIT* D816V VAF on treatment with avapritinib.[80–82] 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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