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Identifying KIT *D816V Mutation in Patients With Evidence of Systemic Mast Cell Activation (MCA) and Enriched for Hereditary Alpha-tryptasemia (HaT): Results From the PROSPECTOR Clinical Trial*

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Disclosures

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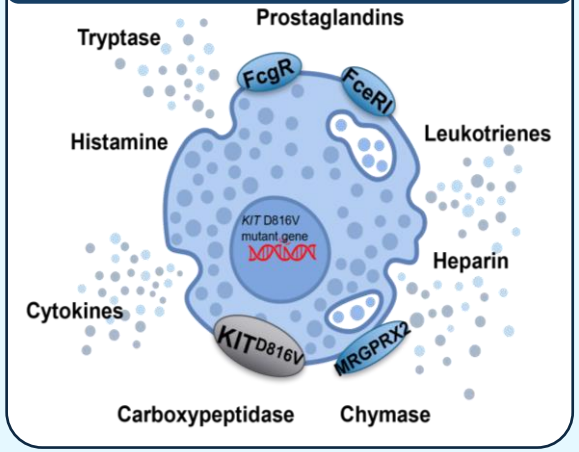
Systemic MCA involves ≥2 organ systems, classified as clonal or non-clonal based on *KIT* D816V mutation status

- Systemic mast cell activation (MCA) is characterized by abnormal release of mast cell (MC) mediators[1–3]
- Systemic mastocytosis (SM) is a clonal mast cell disease driven by *KIT* D816V in ~95% of cases[4,5]
- The prevalence of *KIT* D816V–driven clonal mast cell disease in patients with MCA symptoms is not known

Mast cell activators
Allergens, bacteria, cytokines, drugs, fungi, peptides, toxins, and viruses[6]

Activation leads to degranulation and secretion of vasoactive and pro-inflammatory mediators[6]

SM pathogenesis[6–8]



- SM in some cases is associated with hereditary alpha-tryptasemia (HaT), a genetic condition defined by an increased copy number for the *TPSAB1* gene, which encodes the α tryptase enzyme; this leads to elevated serum tryptase levels produced by MCs that can potentially worsen the severity of MCA conditions[9–11]

FceR1, high-affinity IgE receptor; FcgR, high-affinity IgG receptor; MCA, mast cell activation; MRGPRX2, Mas-related G-protein coupled receptor member X2; *TPSAB1*, tryptase alpha/beta 1.

1. Jackson CW et al. *Int J Mol Sci*. 2021;22:11270; 2. González-de-Olano D et al. *Front Immunol*. 2017;8:792; 3. Akin C et al. *J Allergy Clin Immunol*. 2017;140:349–355; 4. Garcia-Montero AC et al. *Blood*. 2006;108:2366–2372; 5. Kristensen T et al. *J Mol Diagn*. 2011;13:180–188; 6. Theoharides TC et al. *N Engl J Med*. 2015;373(2):163-172; 7. Pardanani A. *Am J Hematol*. 2023;98(7):1097-1116; 8. Metcalfe DD et al. Overview of mast cells in human biology. In: Akin, C (eds) Mastocytosis. Springer, Cham. 2020; doi.org/10.1007/978-3-030-27820-5_1; 9. Lyons JJ et al. *J Allergy Clin Immunol*. 2021;14:622–632; 10. Glover SC et al. *Ann Allergy Asthma Immunol*. 2022;128:460–461; 11. Bonadonna P et al. *Curr Opin Allergy Clin Immunol*. 2022;22:277–282.

KIT D816V and HaT screening are warranted to differentiate between overlapping symptoms of systemic MCA and SM

- Activating *KIT* mutations and elevated basal serum tryptase (BST) are minor diagnostic criteria of SM[1–3,a]
- Per WHO guidelines, it is recommended to test for *KIT* D816V and serum tryptase at the first sign of disease[2,4]

Hallmark symptoms of SM that should lead to increased suspicion include

Skin

- **Maculopapular lesions** with **Darier's sign** is a highly specific diagnostic feature[5]
- Wheal-and-flare reaction is elicited by stroking the lesion with a tongue spatula[5]

Anaphylaxis

- **Anaphylaxis** with hypotension and syncope can occur[6]
- **50% of adult patients with SM** experience recurrent or unexplained anaphylaxis[4,5]

Gastrointestinal

- Many patients report **nausea, vomiting, and/or diarrhea**[6,7]
- Symptoms can be unpredictable and severe[6,7]

Bone

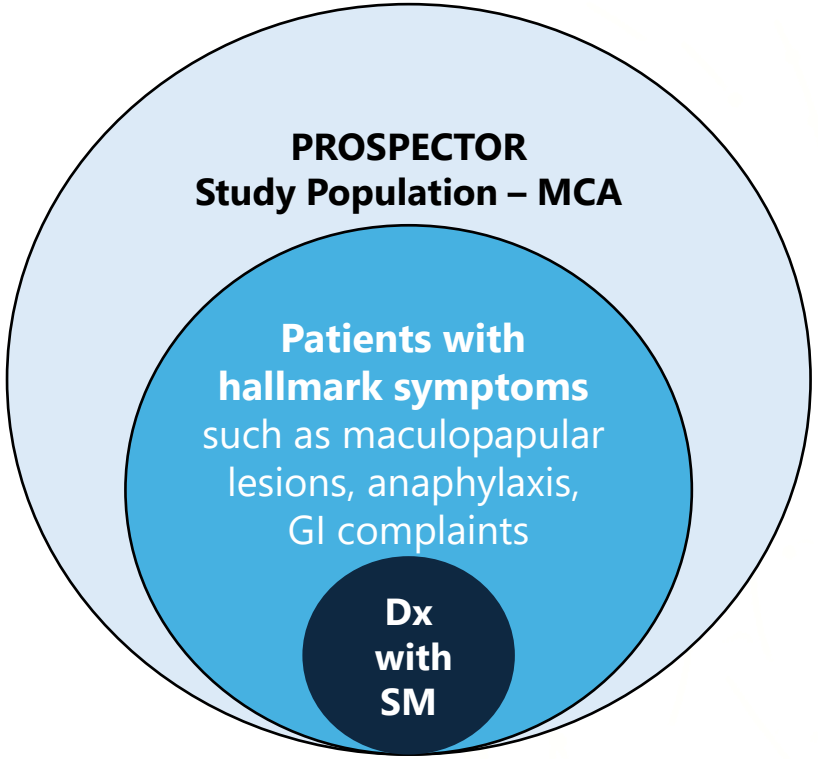
- **Osteoporosis occurs in 38%** of patients with ISM[8]; **osteosclerosis occurs in 2–19%**[9] of patients and is associated with advanced disease[8]
- Unexplained osteoporosis, especially in young males, should raise suspicion for SM[10]

[a]BST >20 ng/mL is a minor criterion; BST levels should be adjusted in the case of HaT.

HaT, hereditary alpha-tryptasemia; ISM, indolent systemic mastocytosis; MCA, mast cell activation; SM, systemic mastocytosis; WHO, World Health Organization.

1. © International Agency for Research on Cancer. Verstovek S et al. 2023. Systemic Mastocytosis. https://tumourclassification.iarc.who.int/chaptercontent/63/20. Beta online ahead of print. Accessed April 2, 2024; 2. Khoury JD et al. *Leukemia*. 2022;36:1703–1719; 3. Arber DA et al. *Blood*. 2022;140:1200–1228; 4. Pardanani A. *Am J Hematol*. 2023;98:1097–1116; 5. Hartmann K et al. *J Allergy Clin Immunol*. 2016;137:35–45; 6. Gilreath JA et al. *Clin Pharmacol*. 2019;11:77–92; 7. Jennings SV et al. *Immunol Allergy Clin North Am*. 2018;38:505–525; 8. Riffel P et al. *J Cancer Res Clin Oncol*. 2020;146:945–951; 9. Wang M et al. *Endocrinol Diabetes Metab Case Rep*. 2023;2023:22-0408; 10. Abramowitz JD et al. *Endocr Pract*. 2012;18:158–161.

PROSPECTOR: The first prospective, multicenter screening study evaluating the prevalence of peripheral blood *KIT* D816V mutation and HaT in patients with evidence of systemic MCA



PROSPECTOR[1] Key Inclusion Criteria:

Adults presenting with at least one of the three criteria below as evidence of systemic MCA

1. Involvement of ≥2 organ systems[a] (cardiovascular involvement necessary) and basal serum tryptase levels ≥8 ng/mL
2. Severe anaphylaxis (Ring-Messmer grade ≥II) due to Hymenoptera sting
3. Severe anaphylaxis (Ring-Messmer grade ≥II) with cardiovascular involvement and event-related tryptase elevation fitting the formula 20% of baseline plus 2 ng/mL evaluated in ≥1 event

Key exclusion criteria:

Patients previously diagnosed with any of the following WHO subclassifications: CM only, ISM, SSM, SM-AHN, ASM, MCL, MC sarcoma

[a]Involvement is characterized by skin (pruritus, urticaria, flushing and angioedema), cardiovascular (tachycardia, syncope, and hypotension), gastrointestinal (diarrhea, nausea, vomiting, and gastrointestinal cramping) or respiratory/naso-ocular (wheezing, conjunctival injection, and nasal stuffiness).

ASM, aggressive systemic mastocytosis; CM, cutaneous mastocytosis; Dx, diagnosis; GI, gastrointestinal; HaT, hereditary alpha-tryptasemia; ISM, indolent systemic mastocytosis; MC, mast cell; MCA, mast cell activation; MCL, mast cell leukemia; SM, systemic mastocytosis; SM-AHN, systemic mastocytosis with an associated hematologic neoplasm; SSM, smoldering systemic mastocytosis; WHO, World Health Organization.

1. (PROSPECTOR) Screening Study Evaluating the Prevalence of the *KIT* D816V Mutation in Patients With Systemic Mast Cell Activation. NCT04811365. https://classic.clinicaltrials.gov/ct2/show/NCT04811365. Accessed April 18, 2024.

PROSPECTOR study design

Prospective, multicenter (US & EU), noninterventional *KIT* D816V screening study (NCT04811365)[1]

Patients with suspected mast cell activation:
N=381 enrolled; N=379 completed the study[2]

- Median age was 56 years[2]
- 60% were female and 77% were white[2]
- Patients were enrolled across 22 sites in the EU & UK (77%) and US (23%)[2]

Informed consent, medical history

Collect samples – single visit:

- Peripheral blood (PB) (*KIT* D816V by ddPCR [LoD 0.03%]; basal serum tryptase; ARUP Labs)
- Buccal swab (*TPSAB1* GCN; Gene by Gene)

Primary endpoint:
Proportion of patients with *KIT* D816V mutation in PB[1]

Key secondary endpoints[1]:

- *KIT* D816V variant allele fraction in PB
- Prevalence of HaT, defined as the proportion of patients with an increased *TPSAB1* GCN encoding alpha-tryptase

ARUP, Associated Regional and University Pathologists; ddPCR, droplet digital polymerase chain reaction; EU, European Union; GCN, gene copy number; HaT, hereditary alpha-tryptasemia; LoD, limit of detection; *TPSAB1*, tryptase alpha/beta 1; UK, United Kingdom; US, United States.

1. (PROSPECTOR) Screening Study Evaluating the Prevalence of the *KIT* D816V Mutation in Patients With Systemic Mast Cell Activation. NCT04811365. https://classic.clinicaltrials.gov/ct2/show/NCT04811365. Accessed April 18, 2024; 2. Hartmann K et al. Presented at AAAAI 2024. Presentation 739.

KIT D816V mutation detected in 4% of patients with systemic MCA – 400× enriched compared with the general population

Primary endpoint:
***KIT* D816V mutation was detected in PB of 15 (4%) patients with systemic MCA symptoms[1]**
(N=381 enrolled[a])

	All enrolled patients (N=381)
***KIT* D816V mutation, n (%)**	
Detected	**15 (4)**
Not detected	354 (92.9)
Unknown	12 (3.1)
Total	381 (100)
***KIT* D816V VAF, %**	
N	369
Mean (SD)	0.2 (2.27)
Range, min–max	0–37

1:10,000 patients diagnosed with SM in the general population[2]

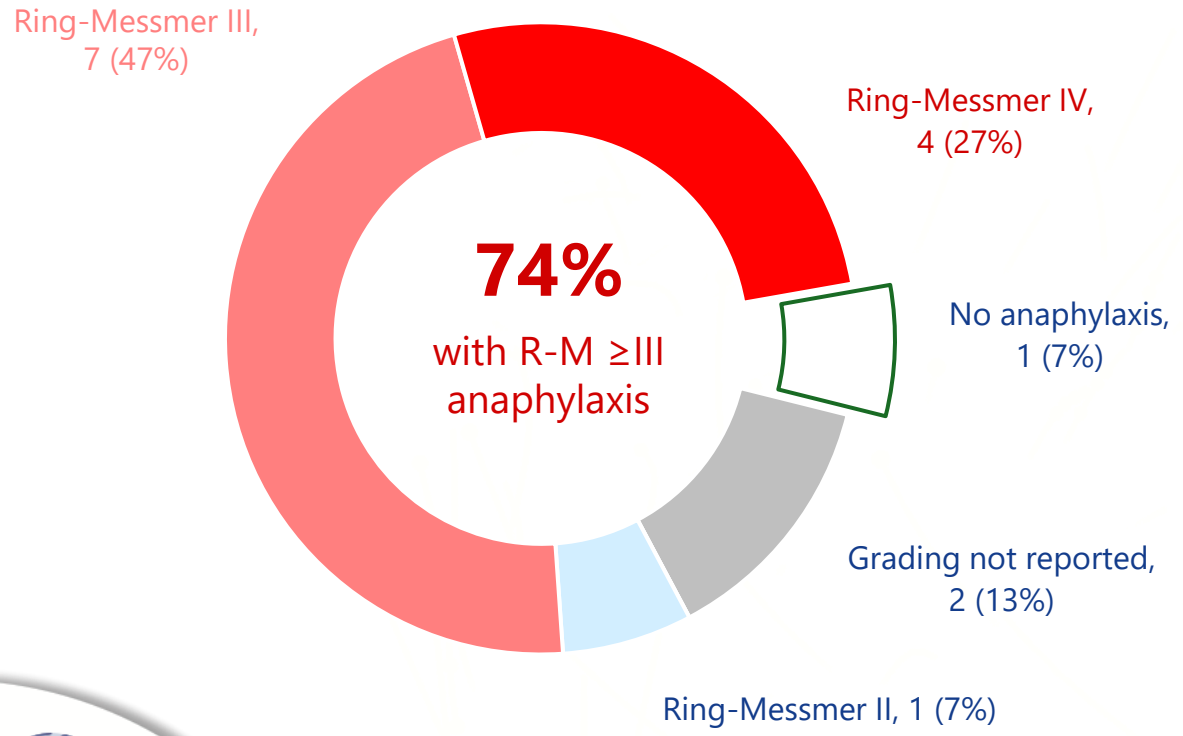
ENRICHED >400×

1:25 patients with systemic MCA symptoms had underlying clonal MC disease

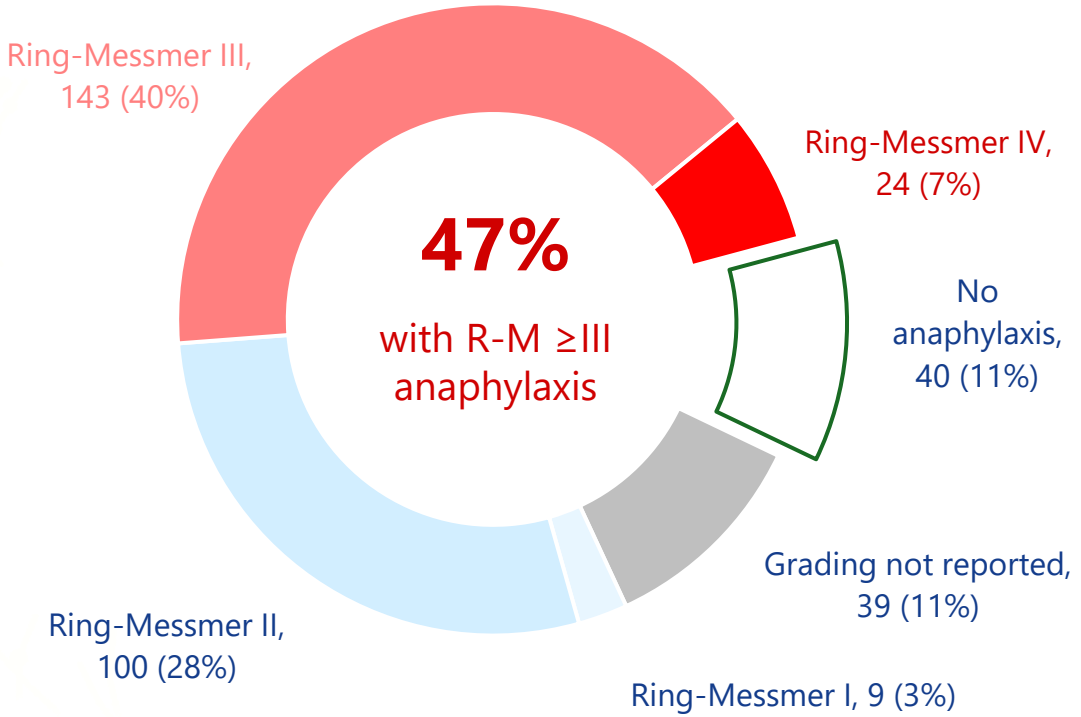
[a]4.1%, n=369 with mutation absence/presence confirmed; limit of detection = 0.03%.
MC, mast cell; MCA, mast cell activation; PB, peripheral blood; SM, systemic mastocytosis; VAF, variant allele fraction.
1. Hartmann K et al. Presented at AAAAI 2024. Presentation739; 2. Brockow K. *Immunology Allergy Clin North Am.* 2014;34(2):283–295.

Detection of *KIT* D816V was higher in patients who experienced severe anaphylaxis

Patients with detectable *KIT* D816V in peripheral blood (n=15)[a]



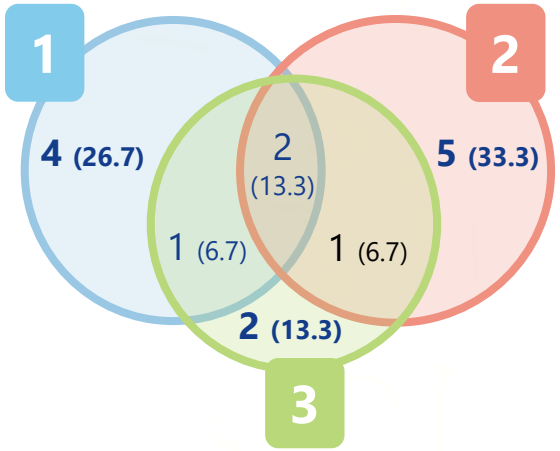
Patients *without* detectable *KIT* D816V in peripheral blood (n=355)



[a]Sum of percentages may not total to 100 due to rounding.
R-M, Ring-Messmer anaphylaxis grading scale.

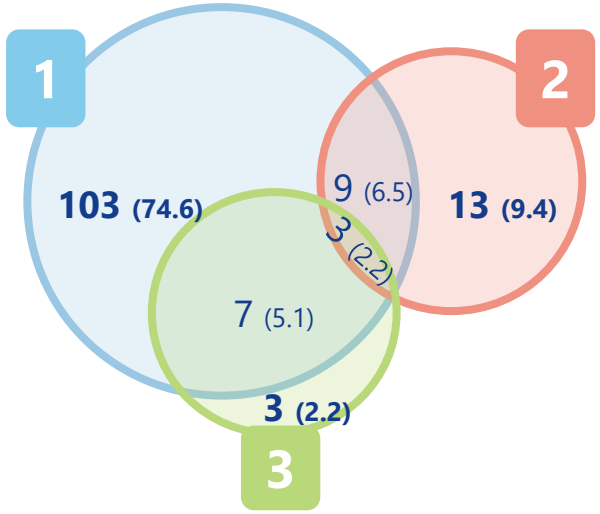
KIT D816V was detected proportionately across inclusion criteria

15 patients (4%) tested positive for *KIT* D816V[1]



- **8/15 (53%)** patients positive for *KIT* D816V met criterion 2 (Hymenoptera sting)
  - **7/8 (88%)** experienced R-M ≥III anaphylaxis

138 patients (36.2%) tested positive for HaT



- **48/138 (35%) patients with HaT** experienced R-M ≥III anaphylaxis[a]
- **131/242 (54%) patients without HaT** experienced R-M ≥III anaphylaxis

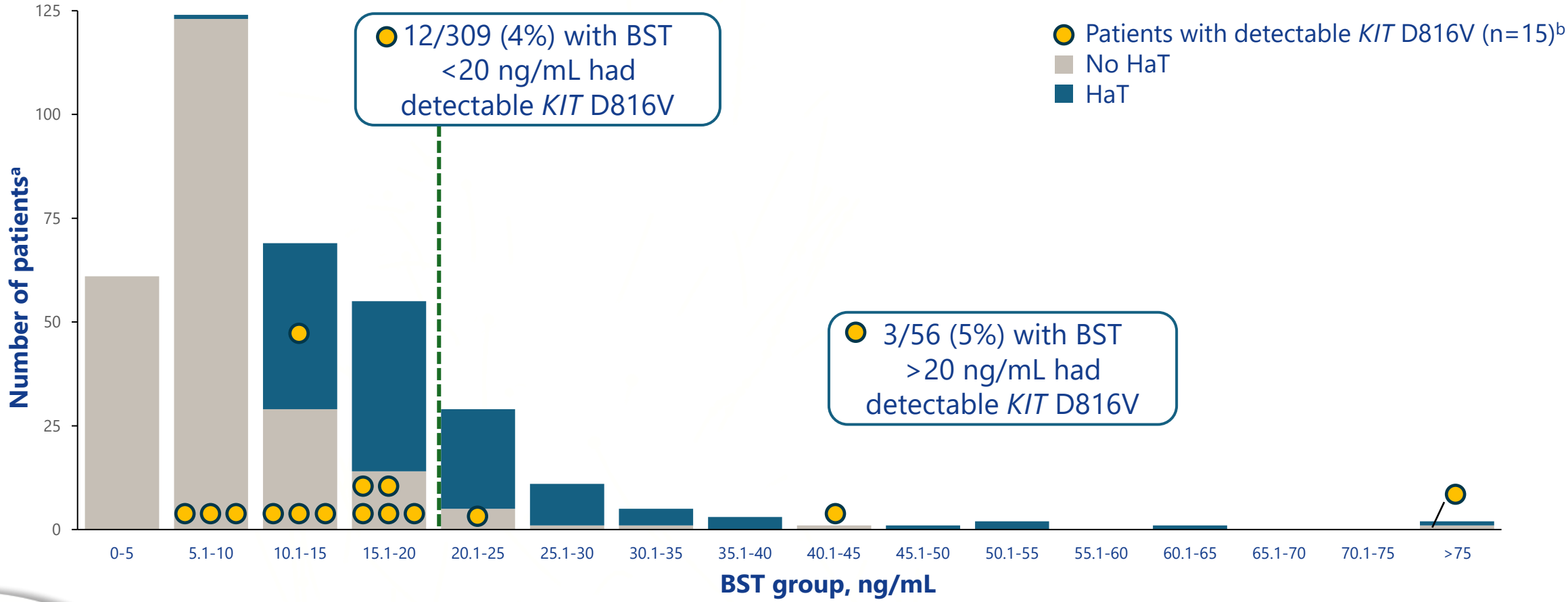
1 Involvement of ≥2 organ systems[b] (cardiovascular involvement necessary) and basal serum tryptase levels ≥8 ng/mL

2 Severe anaphylaxis (Ring-Messmer grading ≥II) due to Hymenoptera sting

3 Severe anaphylaxis (Ring-Messmer grading ≥II) with cardiovascular involvement and event-related tryptase elevation fitting the formula 20% of baseline plus 2 ng/mL evaluated in ≥1 event

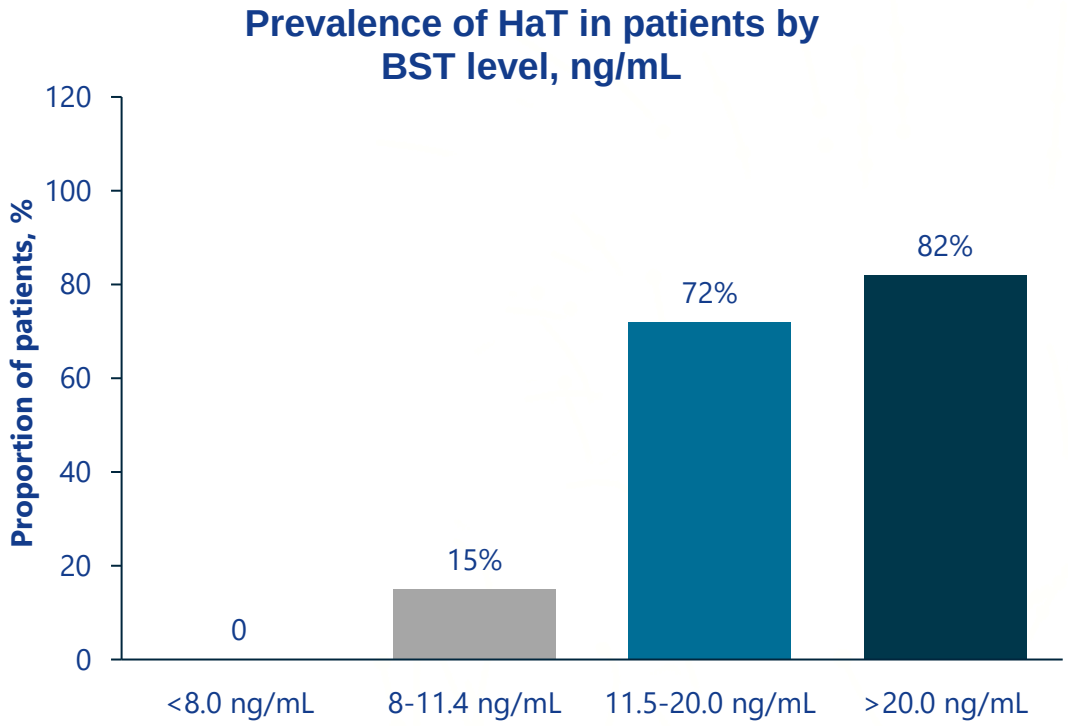
[a]One patient with HaT had detectable *KIT* D816V in peripheral blood (limit of detection >0.03%). Patient experienced anaphylaxis Ring-Messmer grading 3 and had a REMA score of -4.
[b]Involvement is characterized by skin (pruritus, urticaria, flushing, and angioedema), cardiovascular (tachycardia, syncope, and hypotension), gastrointestinal (diarrhea, nausea, vomiting, and gastrointestinal cramping), or respiratory/naso-ocular (wheezing, conjunctival injection, and nasal stuffiness).
HaT, hereditary alpha-tryptasemia; REMA, Red Española de Mastocitosis (The Clinical Reference Center for the Spanish Network on Mastocytosis); R-M, Ring-Messmer anaphylaxis grading scale.
1. Hartmann K et al. Presented at AAAAI 2024. Presentation 739.

80% of patients positive for *KIT* D816V had BST <20 ng/mL

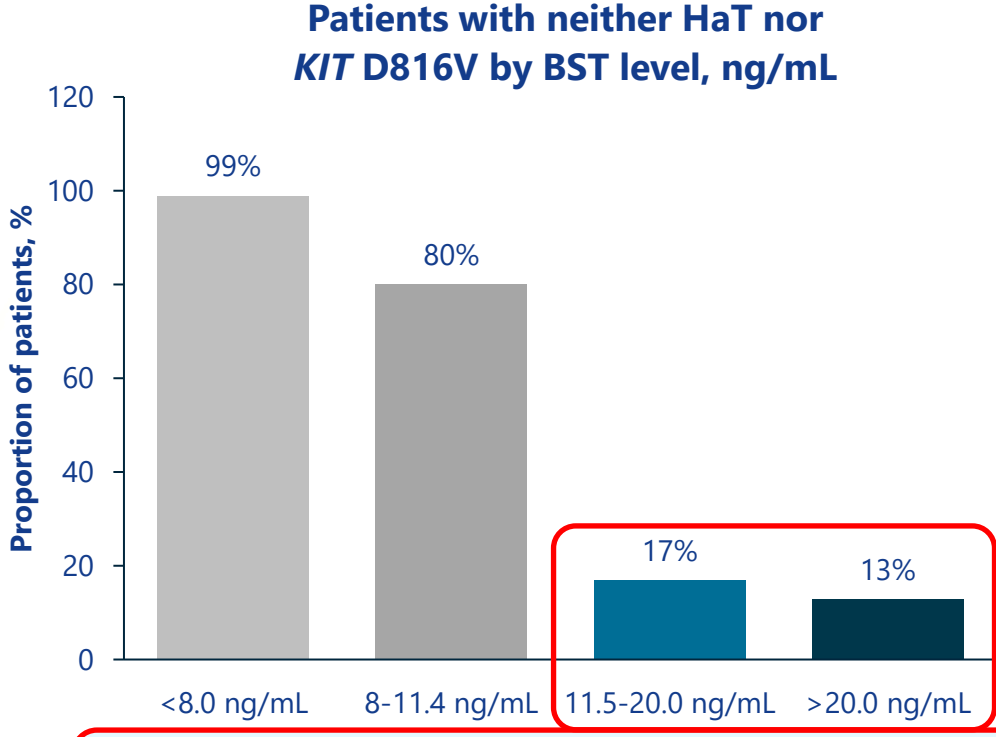


[a]*KIT* D816V detection in patients with systemic MCA by BST level (n/N=365/381); patients missing a BST value (n=16).
[b]1/15 (7%) patients positive for *KIT* D816V had HaT (BST = 11.9 ng/mL; *TPSAB1* genotype 2β3α).
BST, basal serum tryptase; HaT, hereditary alpha-tryptasemia; MCA, mast cell activation; *TPSAB1*, tryptase alpha/beta 1.

Proportion of patients with HaT increased with BST levels; no patient with BST <8 ng/mL had HaT



- Proportion of patients with HaT increased with BST levels
  - **119/158 (75%)** patients with BST >11.4 ng/mL had HaT
  - **9/61 (15%)** patients with BST 8-11.4 ng/mL had HaT
- **46/56 (82%)** patients with BST >20.0 ng/mL had HaT without detectable *KIT* D816V



- **26/37 (70%)** patients with BST >11.4 ng/mL and no HaT had no detectable *KIT* D816V mutation in PB
  - **In those with additional local evaluation** (e.g., bone marrow biopsy), investigators reported **14/18 (78%) with clonal MC disease (SM=10; MMAS=4)**[a]

[a]The remaining 4 patients were negative for *KIT* D816V upon repeat local testing, and all 4 declined bone marrow testing.
BST, basal serum tryptase; HaT, hereditary alpha-tryptasemia; MC, mast cell; MMAS, monoclonal mast cell activation syndrome; PB, peripheral blood; SM, systemic mastocytosis.

Conclusions

- In the PROSPECTOR trial, *KIT* D816V in PB was detected in 4% of patients with suspected MCA (15 of 381 screened) by ddPCR (LoD 0.03%); detection of clonal MC disease was enriched by >400× versus general SM prevalence of 1:10,000
  - Detection of *KIT* D816V was higher in patients who experienced severe anaphylaxis
- *KIT* D816V in PB was detected irrespective of BST levels
  - 80% of patients positive for *KIT* D816V had BST <20 ng/mL (threshold for minor diagnostic criteria); 20% of patients positive for *KIT* D816V had BST <11.4 ng/mL
- Interpretation of BST is closely connected to HaT status; proportion of patients with HaT increased with BST levels
  - 75% of patients with BST >11.4 ng/mL had HaT
- Data are supportive of ECNM/AIM guidance for high-sensitivity screening for *KIT* D816V as a first step in diagnosis of SM[1]
  - Consider repeat assessment in bone marrow if negative for *KIT* D816V mutation in PB despite clinical symptoms of SM or unexplained BST elevation >11.4 ng/mL
- Clonal MC disease was detected in 78% of patients with BST >11.4 ng/mL who were negative for both HaT and *KIT* D816V in PB, suggesting additional studies leveraging enrichment strategies and/or higher-sensitivity assays may be required

Patients with MCA with signs or symptoms of systemic involvement regardless of BST should initially be screened for *KIT* D816V with a high-sensitivity assay (LoD 0.03%) followed by a full evaluation for SM

AIM, American Initiative in Mast Cell Diseases; BST, basal serum tryptase; ddPCR; digital droplet polymerase chain reaction; ECNM, European Competence Network on Mastocytosis; HaT, hereditary alpha-tryptasemia; LoD, limit of detection; MC, mast cell; MCA, mast cell activation; PB, peripheral blood; SM, systemic mastocytosis.

1. Valent P et al. *J Allergy Clin Immunol: In Practice*. 2022;10:P1999–P2012.E6

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