Prevalence of the *KIT* D816V Mutation in Peripheral Blood of Patients With Evidence of Systemic Mast Cell Activation: Results of the Prospective, Multi-centered, Global PROSPECTOR Clinical Trial

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Background

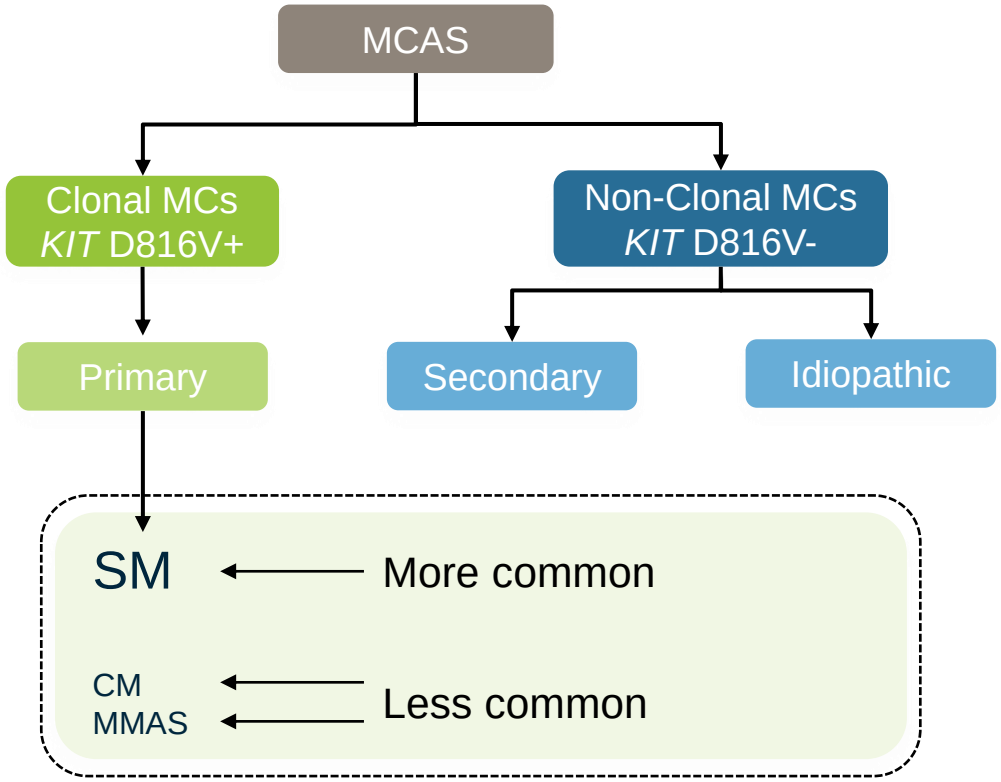
- Systemic mast cell activation (MCA) involves ≥2 organ systems; classified as clonal or non-clonal based on *KIT* D816V mutation status[1–3]
- Systemic mastocytosis (SM) is a clonal mast cell disease driven by *KIT* D816V in ~95% of cases[4,5]
  - Hallmark symptoms that should lead to increased suspicion include: cutaneous mastocytosis; anaphylaxis with hypotension/syncope; and either of these with the involvement of another organ system, including GI
  - SM in some cases is associated with hereditary alpha-tryptasemia (HaT): increased *TPSAB1* gene copy number and increased serum tryptase levels that can potentially worsen the severity of mast cell activation conditions
- The prevalence of *KIT* D816V-driven clonal mast cells and mast cell disease (SM) in patients with systemic mast cell activation symptoms is not known

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

GI, gastrointestinal; HaT, hereditary alpha-tryptasemia; MCA, mast cell activation; SM, systemic mastocytosis; *TPSAB1*, tryptase Alpha/Beta 1.

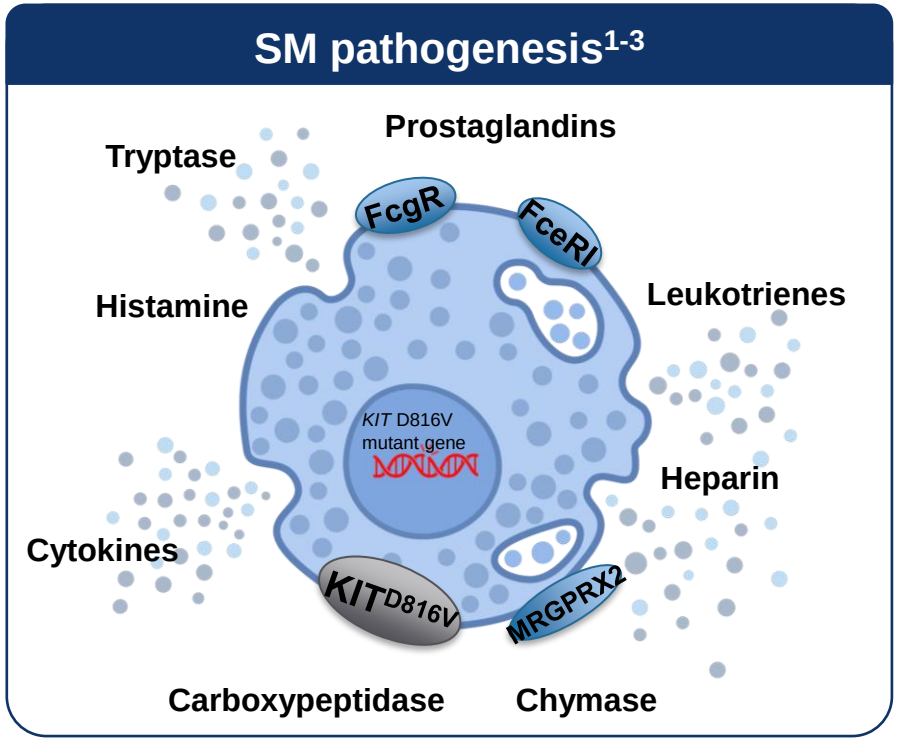
1. Jackson CW et al. *Int J Mol Sci*. 2021;22(20); 2. González-de-Olano D et al. *Front Immunol*. 2017;8:792; 3. Akin C et al. *J Allergy Clin Immunol*. 2017;140:349–355;
4. Garcia-Montero AC et al. *Blood*. 2006;108:2366–2372; 5. Kristensen T et al. *J Mol Diagn*. 2011;13:180–188.

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



Mast cell activators
Allergens, bacteria, cytokines, drugs, fungi, peptides, toxins, and viruses[1]

Activation leads to degranulation and secretion of vasoactive and pro-inflammatory mediators[1]



CM, cutaneous mastocytosis; MC, mast cell; MCA, mast cell activation; MCAS, mast cell activation syndromes; MMAS, monoclonal MCAS; SM, systemic mastocytosis.

1. Theoharides TC et al. *N Engl J Med*. 2015;373(2):163-172; 2. Pardanani A. *Am J Hematol*. 2023;98(7):1097-1116; 3. Metcalfe DD et al. Chapter 1. Overview of mast cells in human biology. In Akin C, ed. *Mastocytosis: A Comprehensive Guide*. Cham, Switzerland: Springer Nature; 2020.

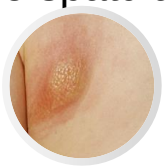
Hallmark symptoms may warrant investigation of systemic mastocytosis

Anaphylaxis

- **Anaphylaxis** with hypotension and syncope can occur[1]
- **50% of adult patients with SM** experience recurrent or unexplained anaphylaxis[2,3]

Skin

- **Maculopapular lesions** with **Darier's sign** is a highly specific diagnostic feature[2]
- Wheal-and-flare reaction is elicited by stroking lesion with a tongue spatula[2,a]



Gastrointestinal

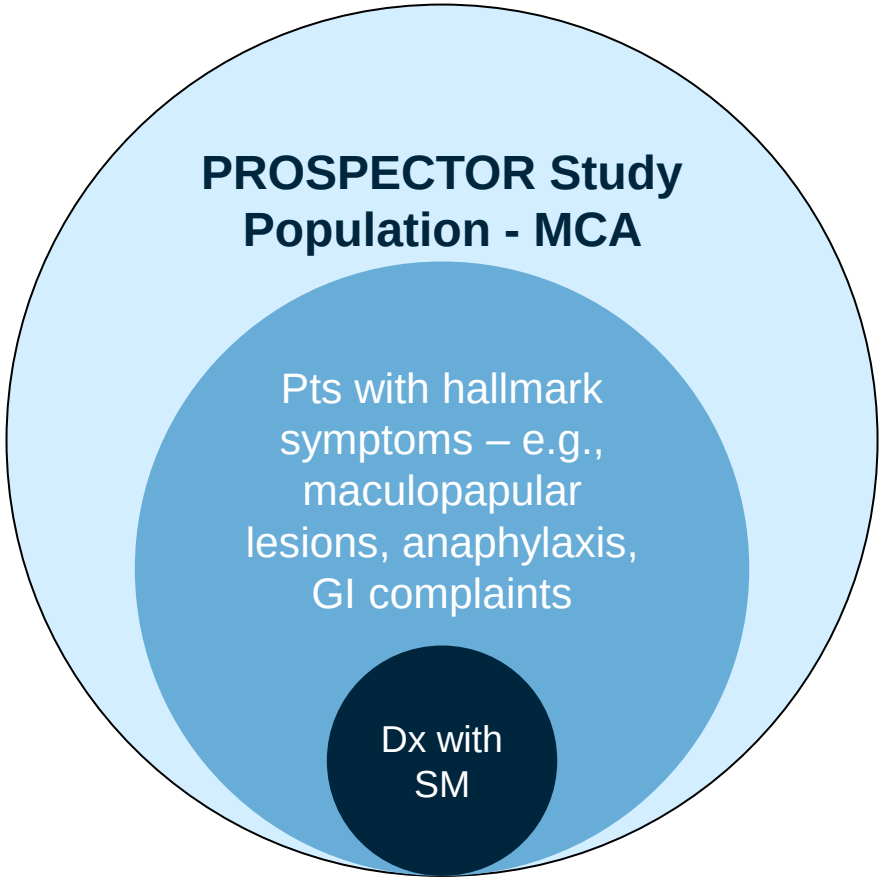
- Many patients report **nausea, vomiting and/or diarrhea**[1,4]
- Symptoms can be unpredictable and severe[1,4]

Per WHO guidelines, it is recommended to test for serum tryptase and *KIT* D816V at the first sign of the disease[5]

SM, systemic mastocytosis; WHO, World Heath Organization.

1. Hartmann K, Escribano L, Grattan C, et al. *J Allergy Clin Immunol*. 2016;137(1):35-45; 2. Pardanani A. *J Hematol*. 2019;94(3):363-377; 3. Gilreath JA, Tchertanov L, Deininger MW. *Clin Pharmacol*. 2019;11:77-92; 4. Slee VM, Zack RM, et al. *Immunol Allergy Clin North Am*. 2018;38(3):505-525; 5. Pardanani A. *Am J Hematol*. 2021;96:508–525.

The prevalence of KIT D816V-driven clonal MC disease in patients with broad systemic MCA is not precisely known



PROSPECTOR 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 and Messmer grading ≥II) due to Hymenoptera sting
3. Severe anaphylaxis (Ring and 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

Key exclusion criteria:

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

[a]Involvement is characterized by skin (pruritus, uritcaria, 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; GI, gastrointestinal; ISM, indolent systemic mastocytosis; MC, mast cell; MCA, mast cell activation; MCL, mast cell leukemia; SM-AHN, systemic mastocytosis with an associated hematologic neoplasm; SSM, smoldering systemic mastocytosis; WHO, World Health Organization.

PROSPECTOR study design

Prospective, multicenter (USA & EU), non-interventional *KIT* D816V screening study

Patients with suspected mast cell activation
N=381

Informed consent, medical history

<u>Collect samples – Single visit:</u>
Peripheral blood (PB)
(D816V by ddPCR [LoD 0.03%]; basal serum tryptase; ARUP Labs)

Buccal Swab (*TPSAB1* GCN; Gene by Gene)

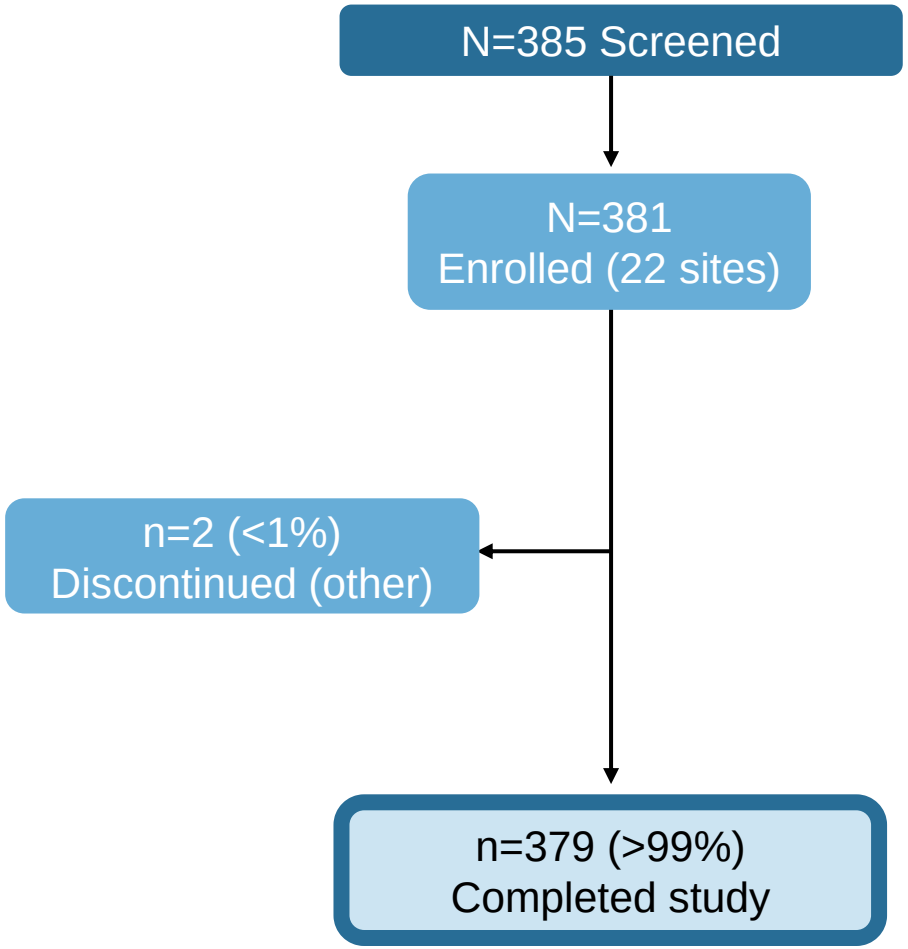
Primary endpoint: Proportion of patients with *KIT* D816V mutation in PB

Secondary endpoints

- *KIT* D816V variant allele fraction in PB
- Prevalence of HaT, defined as the proportion of patients with an increased *TPSAB1* GCN encoding alpha-tryptase
- Relationship between *KIT* D816V in PB and REMA score, other MCA clinical parameters, or HaT diagnosis

ddPCR, droplet digital polymerase chain reaction; ARUP, Associated Regional and University Pathologists; EU, European Union; GCN, gene copy number; HaT, hereditary alpha-tryptasemia; LoD, limit of detection; MCA, mast cell activation; PB, peripheral blood; REMA, Red Española de Mastocitosis (The Clinical Reference Center for the Spanish Network on Mastocytosis); *TPSAB1*, tryptase alpha/beta 1; USA, United States of America.

Disposition and demographics



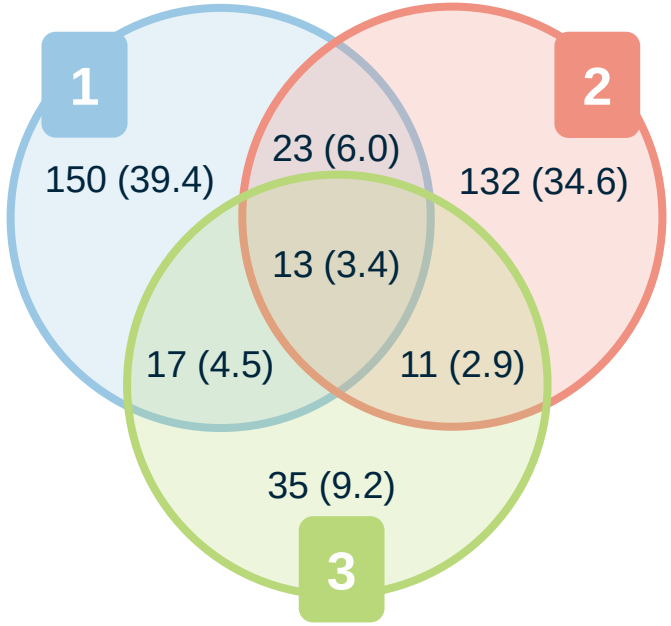
Parameters	All enrolled patients (N=381)
Age, years	
Mean (SD)	53.7 (14.85)
Median (min, max)	56.0 (18, 92)
Gender, n (%)	
Female	227 (59.6)
Male	154 (40.4)
Race, n (%)	
White	295 (77.4)
Other[a]	8 (2.1)
Not reported	64 (16.8)
Unknown	14 (3.7)
Region, n (%)	
EU + UK	294 (77.2)
US	87 (22.8)

[a]Other n (%) includes Asian 1 (<1), Black or African American 2 (<1), and Multiple 2 (<1).

Enrolled patients met ≥1 of the 3 inclusion criteria for systemic mast cell activation

Met key inclusion criteria 1 to 3	All enrolled patients (N=381) n (%)
1	150 (39.4)
2	132 (34.6)
3	35 (9.2)
1 + 2	23 (6.0)
1 + 3	17 (4.5)
2 + 3	11 (2.9)
1 + 2 + 3	**13 (3.4)**
Total	**381 (100)**

All enrolled patients (N=381), n (%)



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[a]Involvement is characterized by skin (pruritus, uritcaria, 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).

KIT D816V mutation was detected in 4% of patients with systemic mast cell activation symptoms

KIT D816V mutation was detected in 15 patients in PB
(primary endpoint; 4%, 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

15% of patients (2/13) who met all 3 inclusion criteria were *KIT* D816V positive

- 138 patients (36.2%) tested positive for HaT

[a]4.1%, n=369 with mutation absence/presence confirmed.
HaT, hereditary alpha-tryptasemia; PB, peripheral blood; SD, standard deviation; VAF, variant allele fraction.

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

Criterion 1	Criterion 2	Criterion 3	*KIT* D816V positive, n/N (%)
Yes	Yes	Yes	2/13 (15)
No	Yes	Yes	1/11 (9)
No	No	Yes	2/35 (6)
Yes	No	Yes	1/17 (6)
No	Yes	No	5/132 (4)
Yes	No	No	4/150 (3)
Yes	Yes	No	0

- Overall, **8/15 (67%)** patients positive for *KIT* D816V met criterion 2
  - 7/8 (88%) had ≥ grade 3 Ring and Messmer anaphylaxis
- 93% of patients positive for *KIT* D816V experienced anaphylaxis

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The majority of patients had basal serum tryptase ≤20 ng/mL

Basal serum tryptase, ng/mL	All enrolled patients (N=381)
n	365
Mean (SD)	12.9 (13.98)
Median (min, max)	10 (2, 200)

- Of the 15 patients positive for *KIT* D816V, 12 (80%) had basal serum tryptase ≤20 ng/mL and 1 patient had HaT
- Among patients with HaT (n=138, 36.2%), none had basal serum tryptase <8 ng/mL
- Elevated basal serum tryptase in the absence of HaT is suggestive of clonal MCA
  - 11/38 patients (29%) with basal serum tryptase >11.4 ng/mL and without HaT were positive for *KIT* D816V

Basal serum tryptase group, n (%)	N=381
0 to 20 ng/mL	309 (81.1)
>20 ng/mL	56 (14.7)
Missing	16 (4.2)
Basal serum tryptase group for patients with *KIT* D816V mutation, n (%)	**n=15**
0 to 20 ng/mL	12 (80.0)
>20 ng/mL	3 (20.0)
Missing	0
Basal serum tryptase group for patients with HaT, n (%)	**n=138**
0 to 20 ng/mL	82 (59.4)
>20 ng/mL	46 (33.3)
Missing	10 (7.2)

HaT, hereditary alpha-tryptasemia; MCA, mast cell activation

Conclusions

- PROSPECTOR is the first prospective, global, multicenter study to evaluate prevalence of *KIT* D816V and HaT in patients with systemic mast cell activation in a population enriched for HaT
- *KIT* D816V in PB was detected in 4% of patients (15 of 381 screened) by ddPCR (LoD 0.03%)
  - Screening PB of patients with suspected MCA for *KIT* D816V mutation enriches detection (>400×) of clonal MC disease versus general SM prevalence of 1:10,000
  - 15% of patients (2/13) who met all 3 inclusion criteria were *KIT* D816V positive
- Data is supportive of ECNM/AIM guidance for high-sensitivity screening for *KIT* D816V as a first step in diagnosis of SM
  - Consider repeat assessment in bone marrow if negative for *KIT* D816V mutation in PB despite clinical symptoms of SM
- Additional studies leveraging enrichment strategies and/or higher-sensitivity assays may be required to more accurately detect the *KIT* D816V mutation in patients with general MCA symptoms

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

ddPCR; digital droplet polymerase chain reaction; HaT, hereditary alpha-tryptasemia; LoD, limit of detection; MCA, mast cell activation; PB, peripheral blood; SM, systemic mastocytosis.

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